A-CELL

A case study-based approach
to integrating QbD principles in
Cell-based Therapy CMC programs

Alliance *for* Regenerative Medicine

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

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CHAPTER 1

Introduction

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Introductory Remarks

The cell and gene therapy (CGT) field is rapidly developing due to its promise to provide significant long-term health benefits to people suffering from a wide range of diseases, from ophthalmological disorders to cancer. Similar to other forms of new advanced therapies, CGT products face the challenge of streamlined, cost effective manufacturing due to a lack of standardized methodologies and training. Following success of A-Mab[1] and recently A-Gene,[2] the Alliance for Regenerative Medicine (ARM) has decided to continue similar efforts applied to cell-based therapies.

In previous years, the broader life sciences industry has encountered systemic barriers to the continued development of promising technologies. In both the monoclonal antibody industry and the vaccine development world, the whirl of energy around scientific discovery was stalled by the realities of manufacturing. While a small team of well-trained experts can produce high-quality batches of drug product for use in process development and early clinical trials, it is inevitable that this process will need to be scaled up dramatically, and the process transferred to other parties for commercial production. To help lower the barrier to this necessary tech transfer, and to better prepare new entrants to the industry, the leading developers of monoclonal antibodies and vaccines have produced a consolidated set of recommendations for implementation of Quality by Design (QbD). A-Mab and A-Vax, respective to each industry, have played a significant role in elevating best practices within their industries, and have been effective in continued workforce development efforts. More recently, the Parenteral Drug Association (PDA) also published a similar effort on manufacturing control strategies for a hypothetical chondrocyte product, A-Cet.[3] Borrowing from these models, the members of ARM have worked to produce a similar document for use by the burgeoning cell therapy sector. This follows the success of ARM's A-Gene project, completed in June of 2021 and now posted on ARM's website, freely available to the regenerative medicine community.[4]

This effort to catalog expertise in cell therapy development occurred in parallel with the approval of some cell-based therapies, which have shown the value and clinical relevance of such programs. As the field continues to develop, it has been recognized that the future of cost-effective cell-based therapy relies on implementation of best practices, development of specialized technologies, and uniformity of methods. Given the wide scope of innovation underway in each of these areas of focus, ARM and the A-Cell team sought to bring in as much thought leadership as possible to ensure that what was recorded was truly a representation of best practices in the industry. Reflecting on this, A-Cell is a truly collaborative effort that has been crafted with contributions from more than 50 industry experts from more than 30 leading therapeutics developers and service/technology providers.

A-Cell is not intended to represent a standard to be rigidly applied. It is a hypothetical case study of the development of a cell-based therapy product. Therefore, it is a snapshot in time of current best practices in a rapidly evolving field. The data cited in this document are non-proprietary, and are intended for illustrative purposes only. While we have attempted to be as comprehensive as possible, and have subjected the document to rigorous review, it is not a "recipe book" for cell-based therapy manufacture. Importantly, A-Cell is not an example of a mock regulatory submission, nor should it be interpreted as regulatory advice, or cited as regulatory guidance.

Introduction to Cell Therapy

Cell therapy is a type of therapeutic where live cells are delivered into patients to treat a disease. There are generally two approaches of cell therapies: (1) autologous, where the cells originate from the patient themselves, and (2) allogeneic, where the cells originate from a healthy donor.

The most common type of cell therapy is blood transfusion, and the transfusion of red blood cells, white blood cells, and platelets from a donor. Another common cell therapy is the transplantation of hematopoietic stem cells to create bone marrow. There is currently no formal classification system for cell therapies. One way to classify is by potency: pluripotent cells can transform into any cell type in the body and multipotent cells can transform into other cell types, however, multipotent cells are more limited than pluripotent cells. Differentiated or primary cells are of a fixed type. Some examples of common cell therapies are:

- **Immune cell therapy.** These therapies use cells from our immune system, such as T cells and natural killer (NK) cells to recognize and eliminate infected or damaged cells due to diseases. These immune cells can be removed from the body, isolated from a mixed cell population, modified, and then expanded before being returned to the body. Examples of immune cell therapies include Chimeric Antigen Receptor (CAR) T cell therapy, Tumor-Infiltrating Lymphocyte (TIL) therapy, engineered T Cell Receptor (TCR) therapy, and NK cell therapy.
- **Hematopoietic stem cells (HSCs) therapy.** HSCs are multipotent blood stem cells that give rise to all types of blood cells. HSCs can be found in adult bone marrow, peripheral blood, and umbilical cord blood.
- **Mesenchymal stem/stromal cells (MSCs) therapy.** MSCs are multipotent cells present in multiple tissues including umbilical cord, bone marrow, and fat tissue, which can differentiate to bone, cartilage, muscle, and adipocytes (fat cells).
- **Embryonic stem cells (ESCs).** ESCs are pluripotent stem cells derived from embryos that retain the ability to self-renew and to form any cell in the body. While versatile, ESCs often raise ethical concerns due to the use of embryos in the development of therapeutics.
- **Induced pluripotent stem cells (iPSCs) therapy.** iPSCs are pluripotent cells that can be generated directly from a somatic cell by transforming the adult cell with a cocktail of genes usually delivered via a viral vector. These cells offer the advantage of pluripotency but without the ethical concerns of embryonic stem cells. iPSCs may also be derived from the patient and thus avoid the problem of immune rejection.

Document Scope

To make A-Cell an effective resource and reflective of the ongoing innovation in the sector, we choose to focus on immune cell therapy as the case study. This is based on strong performance and the field's enthusiasm of the cell-based immuno-oncology sector, as shown in the strong financing in H1 2021 (raising $6.6B–for the first time outraising gene therapy companies), and a major increase (>400%) in industry-sponsored clinical trials worldwide in the past 5 years,[5] accelerating scientific and clinical advances and bringing life-changing therapies to patients worldwide.

To further maximize the utility of A-Cell, we felt it was necessary to focus on one specific use case. We decided to further focus this document on CAR-T cell therapy, given the recent approvals and the number of available products in the market. Although CAR-T therapy is regulated as an *ex vivo* gene therapy, the manufacturing of this cell-based modality shares a lot of the same paradigm as that of other cell therapies, thus it is the focus of this document. Most recently, the U.S. FDA approved CARVYKTI™, the sixth CAR-T therapy in the U.S., and the second targeting BCMA, for treatment of multiple myeloma. The trial reported a 98% overall response rate and an 78% stringent complete response rate.[6] This trend is expected to continue as developers pursue different approaches for innovative CAR-T products to treat a broader range of liquid and solid tumors. In addition, more data from CD19 CAR-T therapies are available to suggest that CAR-T therapies may be used as an earlier line of treatment. The primary case study example presented in this document is a lentiviral vector-modified

CAR-T, as lentivirus is the predominantly used vector for *ex vivo* gene modification of cell therapies.[7]

In CAR-T cell therapy, the patient's own immune cells (T-cells) are modified to express a receptor on their surface that recognizes structures (antigens) on the surface of malignant cells. Once the receptor binds to a tumor antigen, the T-cell is stimulated to attack the malignant cells. So far, CAR-T therapies are only approved for hematologic liquid tumors such as B-cell lymphoma and relapsed or refractory multiple myeloma. While there are a number of approved products out there showing positive outcomes (complete or partial response) in adults and pediatric cases, a lot of learnings are still being collected to assess the long-term success of these treatments, and new strategies are being employed to manage the adverse events that have been observed following treatment, such as cytokine release syndrome and neurotoxicity.

While this document uses a hypothetical CAR-T product as an example to illustrate various manufacturing and CMC principles, many of the concepts presented here are applicable and beneficial to a broader profile of cell therapy products.

Definitions

The following terms are concepts that are common in cell therapy development, and particularly CAR-T therapies that will appear throughout the document.

Key terminology	Definition
Quality by Design (QbD)	A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.[8]
Quality Target Product Profile (QTPP)	A prospective summary of the quality characteristics of a drug product (DP) that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy.[8]
Design Space	The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality.[8] Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval.[8]
Critical Quality Attribute (CQA)	Physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality.[8] CQAs serve as the basis to identify critical process parameters (CPPs) and facilitate development of the design space.[9]
Critical Process Parameter (CPP)	A process parameter whose variability has an impact on a CQA and therefore should be monitored or controlled to ensure the process produces the desired quality.[8]
Control Strategy	A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.[10]

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Definitions; *continued from previous page*

Risk assessment	Science-based process that can aid in identifying which material attributes and process parameters potentially have an effect on product CQAs. Risk assessment consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.[11]
Design of Experiment (DOE)	A structured, organized method for determining the relationship between factors affecting a process and the output of that process.[8] DOE is used to gain knowledge and estimate the best operating conditions of a system, process, or product.
Ancillary materials	Materials that come into contact with the cellular therapeutic product during the manufacturing process, but are not intended to be in the final product, which can include culture media, growth factors, and other biological and non-biological components.[12]
Adventitious agents	Contaminating microorganisms of the cell culture or source materials including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, Rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents, and viruses that may have been unintentionally introduced into the manufacturing process of a biological product.[13]
Current Good Manufacturing Practice (CGMP)	A system that assures proper design, monitoring, and control of manufacturing processes and facilities to ensure a drug product (DP) meets the quality standards.[14] This is done through establishing strong quality management systems, obtaining appropriate quality raw materials, establishing robust operating procedures, detecting and investigating product quality deviations, and maintaining reliable testing laboratories.
Chimeric Antigen Receptor (CAR)	A synthetic receptor designed to bind to certain proteins on cancer cells. The CAR is then added to T cells to help the T cells find and kill cancer cells that have the specific protein that the receptor is designed to bind.[15]
Cytokine Release Syndrome (CRS)	Systemic inflammatory response that is an adverse effect resulting from rapid immune activation induced by CAR-T therapies. The clinical signs of CRS correlate with T cell activation and release of high levels of inflammatory cytokines including Interleukin 6 (IL-6).[16]
Immune effector cell-associated neurotoxicity syndrome (ICANS)	Common complication of CAR-T therapies in the form of acute neurologic signs / symptoms (headache, confusion, delirium, language disturbance, seizures, acute cerebral edema). It is associated with high levels of cytokines in the blood and cerebrospinal fluid.[17]
CD19 (Cluster of Differentiation 19; B-lymphocyte antigen CD19)	A member of the immunoglobulin superfamily of proteins expressed on the surface of B cells, where it functions as a critical component of the B cell receptor signaling complex.[18] CD19 is an ideal target for CAR-directed therapies because it is expressed on most B cell malignancies—chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), and many non-Hodgkin's lymphomas (NHLs), it is not expressed on hematopoietic stem cells, and elimination of all CD19+ B cells in the body is a manageable on-target treatment effect.[19] The most mature CAR-T cell therapies to date are directed against CD19.
B-cell maturation antigen (BCMA)	A member of the tumor necrosis factor receptor superfamily that is highly expressed on mature B lymphocytes, with minimal expression on hematopoietic stem cells or nonhematopoietic tissue.[20] BCMA has become a novel treatment target for multiple myeloma (MM) due to its highly selective expression in malignant plasma cells.[21]

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CHAPTER 2

Regulatory Considerations, Comparability, and Standards in Cell-Based Therapies

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Introductory Remarks

Cell and gene therapies (CGTs) are transformative therapies addressing a wide range of conditions such as cancer and genetic and infectious diseases. These modalities are still in their early days, as reflected by the small number of therapies that have been approved and commercialized for patient use. Currently, there are pathways for regulatory review and approval for these therapies, which this chapter aims to cover.

For the purpose of the discussion in this chapter, it is critical to understand the classifications of these advanced therapies, which can be categorized into three main types:

- **Gene therapy medicines:** these contain genes that lead to a therapeutic, prophylactic, or diagnostic effect. They work by inserting recombinant genes into the body to treat a variety of diseases, including genetic disorders, cancer, or long-term diseases. Included in this category of medicines are:
  - *In vivo* gene therapy, where the gene is administered directly to the patient. AAV-based gene therapy is an example of this therapy, which is the focus of A-Gene.[1] Gene editing can also be performed directly *in vivo*.
  - *Ex vivo* gene therapy, where the targeted cells, which may be from healthy donors or removed from the patients, are genetically modified (e.g., viral, non-viral and/or gene editing-based) *in vitro* before they are returned to the patient's body. For instance, autologous CAR-T therapies, which is the focus of A-Cell, fall into this category.

- **Cell therapy medicines:** these contain cells or tissues that have been manipulated (however not genetically modified, otherwise they are considered gene therapies) to change their biological characteristics, or cells or tissues not intended to be used for the same essential functions in the body. They can be used to cure, diagnose, or prevent diseases.

- **Tissue-engineered medicines:** these contain cells or tissues that have been modified so they can be used to repair, regenerate, or replace human tissue, which may also contain a device component, such as a scaffold.

In the EU, these products belong to the overarching classification of Advanced Therapy Medicinal Products (ATMPs).

As prescribed in Chapter 1, this document focuses on autologous CAR-T case study, which is classified as an *ex vivo* gene therapy and therefore regulated as a gene therapy.[2] However, since this modality is cell-based, and therefore shares a lot of the same regulatory considerations as other cell therapies, the term "cell-based therapy" is used throughout this document where such inclusiveness is appropriate, which also distinguishes this document from A-Gene.

The approval of autologous CAR-T products has shaped the regulatory thinking of these advanced therapies compared to other biologics. While this chapter uses autologous CAR-T as an example to illustrate various CMC principles, many of the concepts presented here are applicable and beneficial to a broader profile of cell-based products–both genetically modified and unmodified (e.g., mesenchymal stem/stromal cells (MSCs), natural killer (NK) cells, induced Pluripotent Stem Cell (iPSC)-derived products), and cell-based combination products. As the field continues to pursue allogeneic approaches, the regulatory landscape will take shape to accommodate its specific considerations or requirements.

The authors acknowledge that regulations and standards are constantly evolving and that this chapter is written to capture a snapshot in time of current regulatory considerations and standard activities. Where the principles apply to both *in vivo* gene therapies and cell-based therapies, cross-references to the relevant A-Gene sections are made throughout this chapter.

Regulatory Considerations

Currently, there are pathways for regulatory review and approval for cell and gene therapies in numerous major markets; three of the most advanced include: United States (US), Europe, and Japan. This section of the chapter covers the regulatory frameworks in these three major markets, regulatory interactions including accelerated regulatory pathways, information to be included in the electronic Common Technical Document (eCTD), and submission content.

REGULATORY FRAMEWORK

The novel and diverse nature of cell-based therapies has resulted in evolving regulatory frameworks to support these products in the three major markets. As regulatory agencies have increased experience with this family of products, they will further define guidance to facilitate therapeutic development to address patients' unmet needs.

United States

In the US, regenerative medicine therapies are regulated by the FDA Office of Tissue and Advanced Therapies (OTAT) within the Center for Biologics Evaluation and Research (CBER). OTAT engages in extensive pre-submission communication with sponsors/applicants and conducts regulatory review of applications for investigational use and marketing approval, operating within the Prescription Drug User Fee Act (PDUFA) timeframes. OTAT also participates in the wider scientific community by reviewing manuscripts and grant proposals, editing, and participating in and organizing scientific conferences. Applications for cellular and gene therapy products are first reviewed and evaluated by the Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) within OTAT. OTAT is comprised of 5 divisions in addition to the Office of the Director:[3]

- Division of Cellular and Gene Therapies (DCGT)
- Division of Clinical Evaluation and Pharmacology/Toxicology (DCEPT)
- Division of Human Tissues (DHT)
- Division of Plasma Protein Therapeutics (DPPT)
- Division of Regulatory Project Management (DRPM)

DCGT, DCEPT, and DPPT constitute the main review disciplines of the regulatory review process: Product (specifically DCGT and DPPT), Preclinical (pharmacology/toxicology), and Clinical (specifically DCEPT). References for the regulatory process of OTAT include the following sources:

- Cellular and Gene Therapy Guidance Documents
- CBER-CDRH Cross-Center Guidance Documents
- Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC)
- Cellular and Gene Therapy Products

It is likely that OTAT will obtain independent expert advice via an Advisory Committee meeting for marketing applications for first-in-class cell-based therapies. The CTGTAC reviews and evaluates available data relating to the safety, efficacy, and appropriate use of human cells (https://www.fda.gov/advisory-committees/blood-vaccines-and-other-biologics/cellular-tissue-and-gene-therapies-advisory-committee).

OTAT has specific goals with respect to chemistry, manufacturing, and controls (CMC), preclinical and clinical investigations, and safety issues related to human tissues. For CMC, the objectives of OTAT are aimed at enhancing quality, consistency, and performance of advanced therapeutics through development of strategies and methods for improved raw materials sourcing, manufacturing, and product characterization (including test methods, standards, identification of critical quality attributes (CQAs), and pursuit of related biological investigations). For preclinical and clinical investigations, the goals of OTAT are aimed at enhancing safety and effectiveness of advanced therapeutics through establishing *in silico, in vitro*, and *in vivo* preclinical models and conducting analyses to increase understanding of clinical trial design issues and patient characteristics that determine outcomes. For safety issues related to human tissues, OTAT aims at enhancing safety and effectiveness of donor screening tests by contributing to research on the development of devices and technologies used in sourcing, manufacturing, processing, and testing of tissues and advanced therapeutics.

It should be noted that cell and gene therapy products are regulated by both the Public Health Service Act with respect to compliance with Good Tissue Practice (Donor testing and screening; section 361) and with respect to requirements for a Biologic License (section 351) and regulation as drugs under the Food, Drug and Cosmetic Act.

Additional resources: CBER grants

CBER awards grants to support research projects aimed at studying and recommending improvements for the advanced manufacturing of biological products, including the investigation and development of innovative monitoring and control techniques. The funded research addresses knowledge and experience gaps identified for emerging manufacturing and testing technologies and

supports the development and adoption of such technologies in the biological product sector.

In 2018, as part of its commitment to achieving the goals of the 21st Century Cures Act, a law enacted in 2016 that allocates $500 million over 9 years to help accelerate medical product development and bring new innovations and advances to patients who need them faster and more efficiently, CBER awarded nearly $3 million in grants to support research at five institutions aimed at developing more innovative, consistent, and dependable manufacturing of biological products. The agency continues to support efforts to develop the standards and policies needed to foster the effective and efficient development and adoption of new manufacturing platforms.

Particular consideration is given towards issuing grants to study continuous manufacturing of drugs and biological products (and similar innovative monitoring and control techniques) as a counterpoint to "batch" technology, a process that involves many stops and starts in a series of manufacturing steps. The aim is to address inefficiencies, delays, and risks of introducing human error into manufacturing processes that such breaks inherent to batch technology can cause. From the perspective of the FDA and CBER, continuous manufacturing has significant potential to improve the agility, flexibility, cost, and robustness in the development of manufacturing processes for a wide variety of therapeutic products. Thus, expert FDA staff issue grants to enhance knowledge of novel continuous manufacturing technologies and develop scientific standards and policies for articulating how the FDA will evaluate these technologies as part of regulatory submissions for new products.

The main grant application portal can be found at https://www.grants.gov/. Grants awarded by the FDA (including those awarded specifically by CBER) are listed, along with contact information for the grant management specialists who are administrating the grants and specific instructions for each grant proposal. Applicants prepare and submit their applications through the https://www.grants.gov/ workspace and track their submissions through the FDA eRA Commons system.

Europe

The regulatory agency governing advanced therapies medicinal product (ATMP) marketing applications assessment in the European Union and European Economic Area (EEA; the EU plus Lichtenstein, Iceland, and Norway) is the European Medicines Agency (EMA), and within the Agency, the Committee for Medicinal Products for Human Use (CHMP) and the Committee for Advanced Therapy (CAT) are the main committees evaluating these products. Others within the agency are also involved in the review of applications, such as the Committee for Orphan Medicinal Products (COMP; for ODD), the Pediatric Committee (PDCO; for PIP), the Pharmacovigilance Risk Assessment Committee (PRAC; for MAA), or working parties, including the Scientific Advice Working Party (SAWP; for Scientific Advices). ATMP products are regulated by EU Regulation (EC) No. 1394/2007.

The EU, unlike the US, does not have an entirely centralized system of approvals for market authorization, and sponsors may seek market authorization for some medicinal products within individual EU member states. However, all ATMPs must pursue approval under the EMA's centralized procedure because the evaluation of these products is considered to require broad and diverse scientific expertise from across all states under EMA jurisdiction. In addition, the scientific and technical requirements needed to demonstrate the quality, safety, and efficacy of ATMPs are particularly unique as compared to other medicinal products.

If the ATMP will be used on a non-routine basis within a hospital environment and under the responsibility of a medical practitioner, the ATMP may qualify to be regulated under the "Hospital Exemption" scheme. It will not be considered as a "medicinal product" and not regulated under the scope of EU Directive 2001/83/EC on medicinal products for human use. The preparation and manufacturing of such medicinal products will instead be regulated at the national level in the individual member states.

In addition, and as with any other medicinal products, if the ATMP is intended for treatment of a rare condition, it may be eligible for the orphan drug designation.

Of note, in the EU, in addition to the EMA, all EU member states have national competent authorities. Germany, for example, has two agencies: one dedicated to ATMPs and biologicals (the Paul Ehrlich Institute, PEI) and another for all other products (the BfARM).

In addition, since 1 January 2021 and following the UK's decision to leave the EU, the Medicines and Healthcare products Regulatory Agency (MHRA) is the UK's standalone medicines and medical devices regulator.

ATMP classification

In order to get confirmation of the classification of a medicinal product, developers of cell-based therapy can request a formal ATMP classification from the CAT (requests for ATMP classification should be sent to advancedtherapies@ema.europa.eu). The procedure allows them to receive confirmation that their medicine based on genes, cells, or tissues meets the scientific criteria that define ATMPs, and is of particular interest for borderline products. The CAT will deliver its scientific recommendation after consultation with the European Commission within 60 days of receiving the request. Cell-based medicinal products can be classified as somatic cell therapy medicinal product (sCTMP) or tissue-engineered product (TEP), according to the primary mode of action. While sCTMP action is through pharmacological activity (e.g., release of neutrophic factors), TEP is intended to regenerate, repair or replace a human tissue. Regarding medicinal product containing genetically modified cells, they can be classified either as gene therapy medicinal products (GTMP) if the target genetic sequence provides the therapeutic effect, or as sCTMP if the target genetic sequence has another purpose (for example a manufacturing purpose for the generation of induced pluripotent stem cells). Gene modified cellular products like CAR-T cell therapies are classified as gene therapy medicinal product (GTMP). Positioning a product in the ATMP category gives developers an early opportunity to engage with regulators, clarifies the applicability of downstream regulatory processes, and opens the door to incentives designed specifically for ATMPs.

Japan

Japan has two main regulatory authorities that are independent agencies with distinct roles during the drug approval process: the Ministry of Health, Labor, and Welfare (MHLW) is responsible for publishing regulatory guidelines, managing advisory committees, and providing final authorizations for applications; and the Pharmaceuticals and Medical Devices Agency (PMDA), which is responsible for regulatory and scientific review (i.e., Office of Cellular and Tissue-based Products), and Good Manufacturing Practice (GMP).

Following a major reform in 2013, Japan created two regulatory paths to support and expedite the approval of regenerative medicines. In the 'drug' track, regenerative medicine products (RMPs) sponsored by companies are regulated by the Pharmaceuticals and Medical Devices (PMD) Act, in which, after demonstration of safety and presumed clinical benefit, PMDA issues a time-limited market approval under the condition that the clinical benefit is demonstrated within seven years. As a result, the product becomes available for clinical use, and data collected from that period can be used for final confirmation of safety and efficacy.

The 'clinic' track is regulated by the Regenerative Medicine (RM) Act, and relates to regenerative products that are used in clinical research (other than clinical trials) in an exploratory way, usually by physicians, medical institutions, or outside companies with appropriate licenses. Clinical research under the RM Act has different research and regulatory standards than clinical trials under the PMD Act, and is usually a cheaper and a less stringent way to evaluate efficacy of a GTP product, particularly one that is not ready for clinical trials. If the data collected during this track are positive, a sponsor may use the data to inform and initiate an application for a clinical trial under the PMD Act track.

In addition to the three regions, other global regulatory agencies are listed in Table 2-1.

HEALTH AUTHORITIES AND SPONSOR MEETINGS

Development of innovative investigational products such as cell-based therapies often introduce unique challenges due to unknown safety profiles, complex manufacturing technologies, incorporation of innovative devices, and the use of cutting-edge testing methodologies. In recognition of the complex nature of cell-based therapy products, most health authorities (HAs) have introduced informal consultations to allow sponsors to obtain feedback early in the product development (in addition to the conventional meetings).

Table 2-1: *List of global regulatory agencies*

Country	Regulatory Agency	Website link
United States	Food and Drug Administration (FDA)	https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products
Canada	Health Canada	https://www.canada.ca/en/health-canada/services/drugs-health-products/biologics-radiopharmaceuticals-genetic-therapies/applications-submissions.html
European Economic Area (European Union + Iceland, Lichtenstein and Norway)	European Medicines Agency (EMA)	https://www.ema.europa.eu/en
China	National Medical Products Administration (NMPA)	https://www.emergobyul.com/resources/china/china-food-drug-administration
Japan	Pharmaceuticals and Medical Devices Agency PMDA	https://www.pmda.go.jp/english/
Korea	Pharmaceutical Affairs Act (PAA) Ministry of Food and Drug Safety (MFDS)	https://www.mfds.go.kr/eng/index.do
India	Central Drugs Standard Control Organization (CDSCO)	https://cdsco.gov.in/opencms/opencms/en/Home
Australia	Therapeutic Goods Administration (TGA)	https://www.tga.gov.au
United Kingdom	Medicines and Healthcare products Regulatory Agency (MHRA)	https://www.gov.uk/government/organisations/medicines-and-healthcare-products-regulatory-agency

FDA (US)

Meetings between FDA and sponsors occur at critical junctures during the life cycle of product development and are aimed at minimizing wasteful expenditures of time and resources. These interactions can be in the form of informal and formal meetings, which are discussed in subsequent sections.

Informal meeting: INTERACT

Sponsors applying to the FDA can obtain a preliminary consultation with the FDA through the INitial Targeted Engagement for Regulatory Advice on CBER producTs (INTERACT) meeting prior to a pre-IND meeting (in effect replacing the pre-pre-IND meeting).[4] INTERACT meetings are proposed to become part of the formal PDUFA meeting according to the PDUFA VII Commitment Letter.[5] The INTERACT meeting, available only for cell and gene therapy (CGT) products, gives the sponsors the opportunity to access the FDA much earlier and seek advice regarding CMC, nonclinical, and clinical areas, particularly if innovative manufacturing or testing is used. Actual topics of discussion are at the discretion of FDA; therefore sponsors are encouraged to reach out to FDA on whether they will receive clinical input. This meeting is non-binding in nature, which means that a sponsor is not bound to pursue a particular regulatory pathway.

The INTERACT meeting can: 1) assist sponsors conducting early product characterization and preclinical proof-of-concept studies; 2) initiate discussion for new delivery devices; 3) inform sponsors about overall early-phase clinical trial design elements; and 4) identify critical issues or deficiencies for sponsors to address in the development of innovative products.

An INTERACT meeting is not intended to take the place of a pre-IND meeting, which occurs prior to the submission of an IND to discuss the scope and design of planned initial studies, design of animal studies needed

to support human clinical testing, and the format for the IND. Conversely, an INTERACT meeting also is not a venue to provide advice to sponsors who have yet to initiate any product development activities. Prior to requesting an INTERACT meeting, a sponsor needs to have selected a specific investigational product or a product-derivation strategy to evaluate in a clinical study.

Optimizing the strategic value for the time of the meeting relative to product development might be the sponsor's greatest challenge when seeking an INTERACT meeting. Anecdotal evidence suggests that the meeting might be declined if it is requested too early in the process at a point when a product candidate has not been established, or when it is considered too late, after a Tox program has already been developed/implemented.

Informal meeting: CATT

The CBER Advanced Technologies Team (CATT) was established to create an interactive platform for prospective developers to seek advice and feedback from CBER staff regarding advanced manufacturing and testing technologies that fall under the scope of CBER regulation. In addition, these technologies may have regulatory implications or have a significant impact on product development and CMC. Meetings with CATT can be requested during the preclinical stage; however, inquiries can also be made to discuss new technologies that are not part of a specific project.

The CATT will facilitate discussion of inquiries or responses to meeting requests pertaining to advanced manufacturing technologies that are intended to be implemented in the development of products regulated by CBER. Inquiries or meeting requests submitted to the CATT should focus on novel technologies that can have a significant impact on product development, manufacturing process, and control strategies, and may also have regulatory implications. This includes manufacturing and analytical methods for those products or classes of products for which the center has limited experience with the manufacturing or development process. Details on the process for submitting inquiries/meeting requests to CATT can be found here: https://www.fda.gov/vaccines-blood-biologics/industry-biologics/cber-advanced-technologies-team-catt

Formal meetings

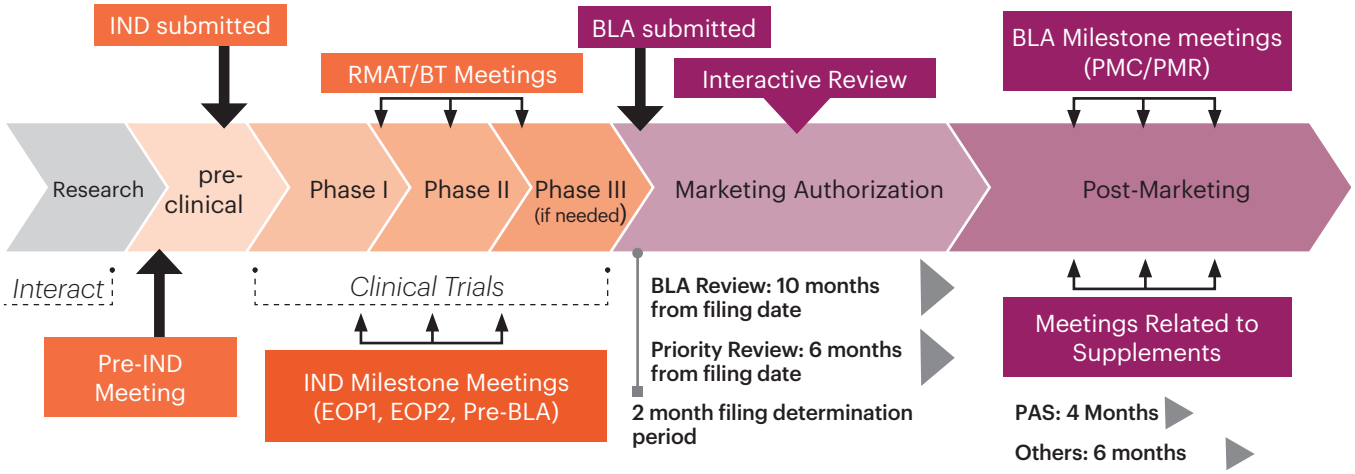
Formal milestone meetings under PDUFA include pre-IND, end-of-phase 1 (EOP1), EOP2, and pre-Biologics License Application (pre-BLA) meetings. For most cell and gene therapy manufacturers, the product undergoes revision and evolution as more data are gathered and deeper product knowledge is gained throughout product development. For example, changes to the manufacturing process can occur, which may potentially impact the product's critical characteristics and therefore its clinical outcomes. It is therefore important for manufacturers to perform risk assessment, report these changes, and seek advice from health authorities during the product life cycle to support a streamlined pathway to commercialization. Further discussion on managing changes during manufacturing is provided in the Comparability section.

During the life cycle of drug development, sponsors may seek advice from the FDA with regard to several topics, including (but not limited to) the following: regulatory concerns, clinical/statistical concerns, safety concerns, product quality concerns, clinical pharmacokinetics and pharmacology, nonclinical pharmacokinetics and pharmacology, and pediatrics. When soliciting feedback, the FDA advises sponsors to keep in mind that FDA policy positions are typically documented and described in FDA guidances, Manual of Policies and Procedures (MAPPs), and Standard Operating Policy and Procedures (SOPPs). Complex scientific and technical drug development questions should be directed to the FDA regulatory project manager (RPM). General questions that cannot be answered by using existing resources can be directed to an FDA RPM or to the designated enhanced communication staff within each FDA center. The FDA will either respond to the questions or redirect the sponsor to an alternative pathway for receiving a response (e.g., other FDA subject matter experts or the formal meeting request process).

Details on the available meetings between the FDA and sponsors are described in the following sections and summarized in Figure 2-1. This figure simply provides a chronological guide; it is not meant to reflect actual timescale.

The FDA offers four types of meetings with requesters seeking advice relating to the development and review of INDs and biologics, and drug or biological product marketing applications under reauthorizations of the PDUFA: Type A, Type B, Type B (end of phase [EOP]), and Type C.[6]

Figure 2-1: *Interactions with FDA for Regenerative Medicine*



- **Type A meetings** are necessary for an otherwise stalled product development program to proceed or to address an important safety issue. It is important to point out that Type A meetings are only granted for stalled product development due to an action taken by the FDA, and not for an issue from the developer side. Topics that are often covered by Type A meetings include dispute resolution as described in 21 CFR 10.75, 312.48, and 314.103, clinical holds, receipt of an FDA Nonagreement Special Protocol Assessment letter, FDA regulatory action other than an approval (must be requested within 3 months of action), and FDA issuance of a refuse-to-file letter.
- **Type B meetings** cover pre-investigational new drug applications (pre-INDs), pre-biologics license applications (pre-BLAs), pre-emergency use authorization, FDA regulatory actions other than approval (must be requested within 3 months of action), risk evaluation and mitigation strategies, post-marketing requirements outside the context of the review of a marketing application, and development programs for products granted breakthrough therapy designation (BTD) and/or RMAT designation status.
- **Type B Comprehensive Multidisciplinary RMAT meeting**[7] is a comprehensive discussion of the drug development program, including planned clinical trials and plans for expediting the manufacturing development strategy. The meeting must be held within 6 months of designation granted and follows the same PDUFA timelines as for any Type B meeting. The objective of the meeting includes agreement on Phase III clinical trial (or pivotal trial that provides primary evidence of clinical efficacy for BLA), potential surrogate or intermediate endpoints in support of accelerated approval, agreement clinical development plan, and plans for expediting the manufacturing development strategy.
- **Type B (EOP) meetings** include certain end-of phase I meetings of products considered for marketing approval under 21 CFR part 312, subpart E, or 21 CFR part 314, subpart H, or similar products and end-of-Phase II or pre-Phase III meetings (21 CFR 312.47).
- **Type C meetings** are any meeting other than Type A, Type B, or Type B (EOP) meeting regarding the development and review of a product, including meetings to discuss adequacy of facility design and establishment issues, and to facilitate early consultations on the use of a biomarker as a new surrogate endpoint that has never been previously used as the primary basis for product approval in the proposed context of use.

There are three meeting formats: face to face, teleconference/videoconference, and written response only (WRO). In face-to-face meetings, the majority of attendees participate in person at the FDA. WRO responses are sent to requesters in lieu of meetings conducted in one of the other two formats. Most meetings shifted to WRO format as a significantly reduced number of face-to-face meetings were being granted, and FDA adjusts their resources for efficiency. The FDA does permit follow-up questions/clarifications to its WRO feedback in many cases.

Table 2-2: *Summary of PDUFA meeting management procedural goals*

Meeting Type	FDA Response to Request	FDA Receipt of Meeting Package	FDA Preliminary Responses to Requester (if applicable)	Requester Response to FDA Preliminary Responses (if applicable)	FDA Scheduled Meeting Date (days from receipt of request)	FDA Meeting Minutes to Requester (if applicable)
A	14 days	With meeting request	No later than 2 days before meeting	--	Within 30 days	30 days after meeting
B	21 days	No later than 30 days before meeting	No later than 2 days before meeting	--	Within 60 days	30 days after meeting
B (EOP)*	14 days	No later than 50 days before meeting**	No later than 5 days before meeting	No later than 3 days after receipt of preliminary responses	Within 70 days	30 days after meeting
C	21 days	No later than 47 days before meeting***	No later than 5 days before meeting	No later than 3 days after receipt of preliminary responses	Within 75 days	30 days after meeting

* EOP=end of phase

** If the scheduled date of a Type B (EOP) meeting is earlier than 70 days from FDA receipt of the meeting request, the requester's meeting package will be due no sooner than 6 calendar days after FDA response time for issuing the letter granting the meeting.

*** If the scheduled date of a Type C meeting is earlier than 75 days from FDA receipt of the meeting request, the meeting package will be due no sooner than 7 calendar days after the FDA response time for issuing the letter granting the meeting. Note that for Type C meetings that are requested as early consultations on the use of a new surrogate endpoint to be used as the primary basis for product approval in a proposed context of use, the meeting package is due at the time of the meeting request.

Pre-IND meetings

Pre-IND meetings are Type B meetings and are meant to initiate dialogue regarding product development in its early stages, with the aim of understanding the mechanism of actions of the drug and possible study designs. They can prevent clinical hold issues from arising and aid sponsors in developing a complete IND. Pre-IND meetings are for cell-based products that are too mature for an INTERACT program and permit a multi-disciplinary assessment of CMC, pharmacology/toxicology, clinical, and other regulatory topics for a future IND sponsor to prepare for that IND.

Clinical Meetings — End of Phase

End of Phase (EOP) meetings serve to evaluate the next clinical phase plan and protocols, the adequacy of current studies and plans to assess safety and efficacy, and the adequacy of manufacturing and testing plans to support the next clinical phase studies.

EOP1 meetings are meant to review and reach agreement on the design of Phase II controlled clinical trials and to discuss issues related to the proposed drug development program. Because of limited resources, the FDA has traditionally encouraged sponsors to request an EOP1 meeting only for drugs intended to treat life-threatening and severely debilitating illnesses, particularly situations where approval based on Phase II trials or accelerated approval may be appropriate. Thus, EOP1 meetings may be particularly useful for sponsors developing cell therapy products.

EOP2 meetings serve to evaluate the Phase III plan and protocols, the adequacy of current studies and plans to assess safety and efficacy, evaluate the human factors validation plan, and identify any additional information necessary to support a marketing application for the uses under investigation, allowing for preparation for commercial manufacturing. The FDA encourages sponsors to request an EOP2 meeting.

All EOP meetings are Type B meetings and subject to different timelines as summarized in Table 2-2. From a CMC perspective, by the end of the Phase II clinical studies,

the sponsor should have a very robust knowledge of the manufacturing process and have started preparing for the Phase III clinical materials that will be representative of the commercial product. For cell-based therapy products, this timeline is not straightforward. As previously discussed, the clinical results in cell-based therapy products is often ahead of the CMC development. Clinical Phase II (or pivotal) and Phase III (if needed) timelines are condensed and, as a result, the CMC development must be expedited.

In preparation for the EOP2 meeting, the sponsor should take the opportunity to seek advice from the FDA that the current data package, in addition to the potential planned studies, will be sufficient for a BLA submission.[8] It is recommended that sponsors request a CMC-focused EOP2 meeting to ensure that there is sufficient time dedicated to CMC discussions, while the pre-BLA meeting can focus on other regulatory matters. Typical topics discussed during the EOP2 meetings include, but are not limited to: release specifications and justifications, overall control strategy with definitions of critical quality attributes (CQAs) and critical process parameters (CPPs), manufacturing process and analytical assay validation plans, and stability data to support product storage and shelf-life. At such a meeting, sponsors should also discuss readiness/plans for the device (used for product administration) and/or companion diagnostics that will be part of the marketed product.

Pre-BLA meetings are meant to familiarize FDA reviewers with the format and content of the planned marketing application, including labeling and risk management activities, presentation and organization of data, dataset structure, acceptability of data for submission, and the projected submission date of the marketing application. They are also intended to uncover major issues, identify studies intended to establish the drug's safety and efficacy, discuss the status of pediatric studies, and discuss statistical analysis methods and results. The FDA encourages sponsors to request pre-BLA meetings for all planned marketing applications.

EMA and EU national agencies (Europe)

Like the FDA, the EMA and the EU national competent authorities (NCA) offer a number of opportunities for sponsors to discuss scientific, technical, and regulatory topics with the agency(ies) at any stage of the development. The choice of reaching out to national agencies or to EMA is dictated by the stage of development, the questions to be raised, and more importantly, the overall objective of the advice (specific clinical study, overall development, etc.), and should be determined on a case-by-case basis. Of note, national agency experts serve as members of the EMA's scientific committees, working parties, or in assessment teams. A list of national competent authorities can be found here: https://www.ema.europa.eu/en/partners-networks/eu-partners/eu-member-states/national-competent-authorities-human#list-of-national-competent-authorities-in-the-eea-section.

Though meetings with the EMA provide opportunities for developers in industry to discuss product development strategies with the regulators and clarify interpretations of the regulations, there are, in general, relatively few guidance documents to help companies prepare for meetings with the EMA compared with the FDA. For developers who do not have the experience of meeting the EMA or submitting a briefing document, there is the possibility to submit pre-submission meeting requests to clarify the content of the document (no scientific assessment performed at this stage).

Innovation Task Force and Innovation Network

In an effort to provide sponsors of emerging therapies and technologies early access to the EMA and to ensure coordination across all moving parts of the regulatory process, the EMA offers the support of the Innovation Task Force (ITF).[9,10,11] ITF is comprised of experts from scientific, regulatory, and legal sectors, and core members, who are appointed based on competence, areas of interest, and commitment to participate in meetings relating to their specialized area. ITF briefing meetings are informal and non-binding and are intended to offer early feedback on innovative medicinal products and technologies and their impact on regulatory and scientific proceedings. Such meetings are intended to complement and reinforce existing formal procedures (e.g., CHMP scientific advice, ATMP classification and certification, and designation of orphan medicines) and prepare the sponsor for such procedures should they be necessary along the regulatory track for the development program. The meetings are

free of charge and last 1.5 hours. After the meeting, the sponsor will provide meeting minutes to the ITF.

In addition, since 2015, the ITF has been supported by the Innovation Network. The Innovation Network is comprised of innovation offices from national competent authorities (NCAs) across the EU. The aim of the network is to improve regulatory support for medicine developers at national and EU levels and make this more appealing to innovators.[12]

Scientific advice and protocol assistance from the EMA

Early engagement with the EMA is a key driver of faster, and more often, successful registration. The EMA provides developers scientific advice and protocol assistance on the most appropriate way to generate robust evidence on a medicinal product's benefits and risks.[13] Sponsors can request meetings with the EMA for overall advice on the topics of choice, and there is no limit to the number of scientific advice meetings (or protocol assistance as it is called for products with orphan drug status) that can be requested during the development of a given cell therapy.

EMA scientific advice and protocol assistance are delivered by CHMP on the recommendation of the SAWP; for cell-based medicinal products, the two rapporteurs assigned to the procedure and in charge of preparing the recommendations are appointed from the CAT. This advice is meant to support the timely and sound development of high-quality, effective, and safe medicines, and may be sought at any stage of a product's development program. To request a scientific advice (or protocol assistance), developers must submit a briefing document on the IRIS portal hosted by the EMA, accompanied by information to be filled in online.

Developers may ask questions relating to such topics as manufacturing, chemical, pharmaceutical, and biological aspects of product development, clinical and nonclinical aspects of development, and data analysis and statistics. In addition, for products that have received the orphan drug designation, developers may inquire about the demonstration of significant benefit of the medicinal product over existing (and approved) treatments. Furthermore, the EMA offers advice for post authorization safety studies (PASSs) to improve the design of studies meant to collect further information on a product's safety once it is on the market.

With regard to timelines, once the request (including a briefing document) is submitted to the EMA, a validation of usually 3-4 weeks will take place. Once the briefing document (with possible revisions as per the request of the agency) is validated, the procedure (Day 0) will start with the first SAWP meeting. During the second SAWP meeting (one month after the first one), it will be decided whether there is a need for a meeting with the Applicant. If no meeting is required, the SAWP will finalize its recommendations and the final advice letter will be release by the CHMP at Day 40 of the procedure. If a meeting is deemed appropriate, it will take place at the third SAWP meeting (hence at Day 60) and followed by the approval of the final advice letter by the CHMP at Day 70 of the procedure.

It should be noted that the EMA charges an administrative fee for advice (reduced fees are available if the developer has a small and medium enterprise (SME) status, for ATMP, and orphan drugs). Importantly, patients (or patients' representatives) may be called upon to provide comments or attend meetings with the EMA.

Scientific advice from EU national agencies

In addition to the EMA, most if not all EU national agencies do offer scientific advice. Fees are variable depending on the agency, topics, and number of questions. The procedure is highly similar: in most agencies, a meeting request should be submitted including proposed meeting dates and questions. Some agencies do request the briefing document at the same time (as EMA does). Once the Agency validates the request, confirmation of the meeting date will be sent to the developer as well as the deadline to provide the briefing package and the presentation. Indeed, all scientific advice at the national level includes a meeting during which the questions are discussed. Following the meeting, minutes and/or final advice (written) will be sent to the developer.

Simultaneous national scientific advice

Developers can receive scientific advice nationally from EU national competent authorities (NCAs) or from the EMA. Depending on the overall clinical development, when national advice is sought, it is often requested from more than one NCA. To address this situation and aim towards more harmonization and optimization, in February 2020, the Innovation Network started a pilot program for simultaneous national scientific advice

(SNSA) to provide an avenue for regulatory interactions for developers in which scientific advice can be requested from two NCAs simultaneously, optimizing the quality and consistency of such advice.[14] The initial phase was a success and the SNSA has been extended until August 2024.[15] The participating NCAs for the pilot program are from Austria, Belgium, Czech Republic, Finland, Germany, Hungary, Italy, Norway, Poland, and Spain (participation has been kept optional for NCAs from other member states as well). SNSA meetings and topics for discussion are identical to single national scientific advice currently offered by NCAs.

Developers can apply for a SNSA by sending an informal letter of interest to one of the two selected NCAs or by using an existing application form. The applicant should propose two preferred NCAs (i.e., based on the list of NCAs volunteering for the SNSA pilot), after which the NCAs accept or deny the request for advice. In case one NCA is not able to join the SNSA, the applicant can suggest an alternative NCA, keep the procedure as a standard national scientific advice request, or withdraw the whole application. Assuming both NCAs can participate, one NCA will take over the lead of the procedure (by mutual agreement with the other NCA) as the coordinating agency and coordinate the advice procedure, acting as the main contact point with the applicant and the other NCA. The timeline of the SNSA will also be mutually agreed on by both NCAs, respecting the preferred dates of the applicant as far as possible. Briefing documents and questions need to be sent to both NCAs separately, considering special requirements with regard to submission timelines, template, scope, content, and extent of the documents of each NCA; and assistance can be provided by the coordinating NCA. The formal validation of the scope, focus of questions (and corresponding rationales) contained in the briefing documents will be reviewed independently by each NCA. In case of any queries (e.g., validation questions raised by one of the NCAs towards the applicant), the coordinating NCA will get in touch with the applicant. The applicant is not allowed to add new questions or change questions or data during the course of the SNSA procedure. Both NCAs will be represented by the respective national experts equal to the national procedures. The meeting minutes will be drafted by the applicant based on the common template provided and sent to each NCA for review and comments. The final document reflects the formal SNSA opinions from both NCAs, based on mutual agreement between them. Potential requests for clarification from the applicant (e.g., on the scientific regulatory opinions provided in the context of the formal SNSA) might be accepted and handled in agreement between both NCAs and in compliance with their respective procedures, whereas new questions from the applicant would be dealt with in a follow-up advice request. Fees will be based on the cost regulations of each NCA involved.

PRIME scheme and meeting

The PRIME (PRIority MEdicines) scheme provides early and enhanced scientific and regulatory support to medicinal products that have the potential to significantly address patients' unmet medical needs. Any sponsor engaged in the exploratory clinical trial phase of development (i.e., prior to reaching a pivotal/registration study and at a stage at which the EMA advice can be used to refine the development) can submit an eligibility request to enter the PRIME scheme. Sponsors should base their request on the availability of preliminary clinical evidence in patients indicating the promising activity of the medicinal product and its potential to significantly address an unmet medical need (proof of concept). Applicants from the academic sector and micro-, small-, and medium-sized enterprises may submit an eligibility request at an earlier stage of development if compelling nonclinical data in a relevant model provides early evidence of potentially promising activity (proof of principle) and first-in-man studies indicate adequate exposure for the desired pharmacotherapeutic effects and tolerability.

As one of the unique features of the PRIME scheme, the sponsor has an opportunity to participate in the PRIME kick-off meeting with the CHMP/CAT rapporteur and their multidisciplinary assessment team, including chairs of CAT, SAWP, COMP, PDCO, or PRAC, as appropriate. The PRIME meeting usually takes place two to three months after a product is accepted into the PRIME scheme, and its purpose is to discuss future steps in development support, including technical and scientific assistance.

In addition to the kick-off meeting, the agency will provide scientific advice at key development milestones

and involve additional stakeholders such as health-technology-assessment bodies, in order to facilitate quicker access for patients to the new medicine.

Parallel joint scientific consultation with regulators and health technology assessment bodies

In an effort to coordinate the advice from HTA and regulators, a pilot phase of joint advice was launched in the EU, now under the format of a joint consultation. There are strict eligibility criteria to apply and currently 8 meetings are granted per year. These meetings are 3 hours in duration and EMA as well as HTA may attend on a voluntary basis.

It is not clear at the date of release of this chapter what the future of such joint consultations will be.

Pre- submission interactions with the EMA and meetings with (co)rapporteurs

Pre-submission interactions (PSI) with the EMA address product-specific legal and regulatory issues, and are intended to facilitate the validation of the marketing authorization application. For PSI, applicants should submit a briefing package that includes questions raised to the EMA (e.g., overall compliance of the intended submission package with applicable regulatory requirements, possible gaps in knowledge that could be useful to discuss). If a meeting is deemed appropriate, EMA will contact the applicant.

Pre-submission meetings (PSM) with the (co)rapporteurs in charge of the evaluation of the MAA per se are meant to discuss scientific aspects and to support applicants in submitting applications for smooth evaluation. There is no specific timetable, though these meetings should take place as soon as the (co) rapporteurs are appointed by the Agency (usually around 6 months prior to the filing of the MAA).

EMA-FDA Programs

Parallel Scientific Advice

The EMA and FDA jointly offer the parallel scientific advice (PSA) program to provide a mechanism for staff from both the EMA and FDA to concurrently convey to sponsors their views on scientific issues during the development phase of new medicinal products. These interactions are meant to increase dialogue between the two agencies and sponsors from the beginning of the life cycle of a new product, provide a deeper understanding of the bases of regulatory decisions, optimize product development, and avoid unnecessary testing replication or unnecessary diverse testing methodologies. The agencies conduct PSA procedures according to the confidentiality arrangement between the European Commission, EMA, and FDA.

PSA procedures usually occur at the request of the sponsor, though, in special circumstances, the EMA or FDA may also initiate the PSA process in full cooperation with the sponsor. PSA requests should focus on specific questions or issues involving the development of a medicinal product for which the sponsor desires to have further scientific input from both the EMA and FDA. The PSA procedures should focus on sharing information and perspectives. Following PSA meetings, sponsors should have a clearer understanding of the agencies' respective requirements and perspectives regarding the development program discussed and, if divergent, the reasons for the divergence. The FDA and EMA consider the best candidates for PSA to be important medicinal products being developed for indications lacking development guidelines or for those indications for which existing EMA and FDA guidelines differ significantly.

Sponsors wishing to nominate a product for a PSA should address a single "Request for PSA" letter to both emainternational@ema.europa.eu and US-FDA-EUR@fda.hhs.gov. In this letter, the sponsor should provide the following information: the product in development; why a discussion with EMA and FDA staff would be beneficial to the product's development; specific questions requiring clarification; the desired goals for the meeting; and an explicit authorization for the agencies' comprehensive exchange of all information relevant to the product, including trade secret information. Any fees applicable for scientific advice at both agencies are unaffected by the PSA status. If both agencies grant the PSA request, the sponsor will receive an email from each agency acknowledging the agreement and indicating the primary contact person at each agency. The PSA process generally corresponds to the 70-day timeline of SAWP at the EMA and the timeline for a Type B meeting at the FDA. The designated primary contact for each agency will coordinate final meeting logistics with the sponsor, including timelines for submission of pre/meeting

background information to both agencies. The two agencies will conduct a pre-sponsor meeting teleconference or videoconference (usually around day 60 of the 70-day timeframe) to discuss the sponsor's questions prior to the meeting. The two agencies may also conduct a post-sponsor teleconference or videoconference if needed. The detailed timeline for this parallel review can be found here: https://www.ema.europa.eu/en/documents/other/timeline-european-medicines-agency-fda-parallel-scientific-advice_en.pdf

If a sponsor's request for a PSA is not granted, the sponsor is free to pursue a scientific advice procedure with each agency individually, following each agency's normal procedural rules. Both agencies may also engage in a Consultative Advice procedure, as described below.

Consultative Advice

The Consultative Advice procedure allows sponsors to request scientific advice from one regulatory agency and concurrently notify the other regulatory agency of the request.[16] At the invitation of the first agency, the second will participate in the sponsor meetings or teleconferences, as able. Unlike the parallel scientific advice process, the second agency will be expected to only engage on top level issues. The review and sponsor meeting will follow the timelines of the regulatory agency from whom the sponsor initially seeks scientific advice. Only the initially contacted regulatory agency will provide written scientific advice in accordance with standard agency meeting procedures.

MHRA (UK)

Scientific Advice

A sponsor can ask for scientific advice from MHRA at any stage of the initial development before submitting an application for a marketing authorization (MA), and during the pre-submission period for a variation to an existing MA.[17] The types of advice a sponsor seeks from MHRA can be on the topics of quality aspects (e.g., the chemical, pharmaceutical, and biological testing), nonclinical and clinical aspects, pharmacovigilance plans and post-authorization safety study protocols, an application for a variation or renewal, advice before publishing advertising for a medicinal product, and changes to labelling of packaging leaflets for medicinal products. It may be possible to request scientific advice for a broader scope that would cover a wide range of issues and would not relate to only one development product. Broader scope issues include any practical issues concerning study design, risk management plans, and complex issues of drug/device combination products.

The option to have a joint meeting with the MHRA and the National Institute for Clinical Excellence (NICE) is available. This option is useful if the sponsor would like to have a discussion regarding study design that can cater to both regulatory and NICE requirements. The MHRA does not have pre-defined meeting slots but the average lead time between submission of a meeting request and the date of the meeting is about 3-4 months.

To request a meeting, the process is similar to EU national agencies, i.e., submission of a meeting request (MHRA form) that includes proposed dates, administrative information, and proposed questions.

Innovation Office (IO)

Although the 'standard' Scientific Advice process is certainly useful for ATMP/CGT, the Innovation Office (IO) provides some more targeted options that are probably more relevant for cutting-edge technologies.[18] The MHRA Innovation Office offers regulatory information, advice, and guidance to developers of innovative medicines who seek early regulatory interactions in the UK. The IO is open to all innovative queries, particularly those that challenge the current regulatory framework. Examples of topics open for discussion with the Innovation Office include medicines, medical devices, and manufacturing processes. For queries about regenerative medicines in particular, the IO offers a Regulatory Advice Service for Regenerative Medicine in the form of consolidated information and guidance, reviewed by four independent and UK-based agencies (Health Research Authority (HRA), Human Fertilisation and Embryology Authority (HFEA), Human Tissue Authority (HTA), National Institute for Health and Care Excellence (NICE)) in addition to the MHRA specialists.

MHRA request that interested parties submit their query and they will receive a single, consolidated, considered, and confidential response within 20 working days (depending on the complexity of the query) with regulatory information, advice, and guidance to help

plan their project. In some cases, when experts consider the issues to be complex or requiring clarification, a regulatory advice meeting may be proposed to discuss the details of the query in more depth.

Innovative Licensing and Access Pathway (ILAP)

ILAP aims to accelerate the time to market, facilitating patient access to medicines. These medicines include new chemical entities, biological medicines, new indications, and repurposed medicines. The ILAP is open to both commercial and non-commercial developers of medicines (UK-based and or global). It comprises an Innovation Passport designation and a Target Development Profile (TDP) and provides applicants with access to a toolkit to support all stages of the design, development, and approvals process.[19]

The first step in the ILAP is the Innovation Passport (IP) application. The IP is the mandated entry point to the ILAP and is open to developers at the preclinical trial stage through to the mid-development program point. Eligibility criteria for the IP include:

- The condition is life-threatening or seriously debilitating, and there is a significant patient or public health need.
- The medicinal product fulfills one or more of the following specific areas:
  a.) innovative medicine such as an ATMP or new chemical or biological entity or novel drug device combination;
  b.) medicines being developed in a clinically significant new indication for an approved medicine;
  c.) medicines for rare disease and/or other special populations such as neonates and children, elderly, and pregnant women; and
  d.) development aligning with the objectives for UK public health priorities such as the Chief Medical Officer, Department of Health and Social Care (DHSC), or Life Sciences Sector Deal (including those in Devolved Administrations, where appropriate)
- The medicinal product has the potential to offer benefits to patients.

Sponsors that wish to apply for an IP should complete the submission form, after which they will be invited to meet with the MHRA to discuss how their product fulfils the three criteria (usually within 4-6 weeks following receipt of the application form). Following the meeting, the partners (AWTTC, MHRA, NICE, and SMC) will jointly consider if the criteria have been fulfilled and the outcome will be informed within 4 weeks. Once the Innovation Passport has been acquired, then onward access to the core ILAP components (Target Development Profile assistance, kick-off meeting, etc.) have additional associated timelines.

PMDA (Japan)

The PMDA provides opportunities for meetings between sponsors and the Agency to allow for feedback and guidance during clinical development.[20] In clinical trial consultations for new drugs, PMDA checks whether a proposed clinical trial complies with the requirements for regulatory submission, taking into consideration the ethical and scientific aspects of the development program, the reliability of the clinical trial, as well as the safety of trial subjects. The PMDA also gives advice to facilitate the improvement of the clinical trial.

Since 2009, PMDA started providing prior assessment consultations, which add value to the development process through feedback from reviewers on CMC data, in addition to efficacy and safety feedback on the product. This consultation process constitutes part of the review of the product once the application is submitted.

For sponsors that are located outside of Japan, it is recommended to appoint a Japanese Marketing Authorization Holder (MAH). The sponsor can request meetings with the PMDA through the MAH, who can also assist with translation and interpretation, since all communications and submission forms are in Japanese.

CMC SUBMISSION CONTENT FOR CLINICAL TRIALS

Electronic Common Technical Document

The electronic Common Technical Document (eCTD) provides the backbone for providing information regarding CMCs. Modules 2 and 3 include sections and definitions for drug substance (DS) and drug product (DP). It should be noted that these data are expected to evolve over time as the sponsor optimizes production processes and formulation of the drug. Information to be

provided about the drug substance includes the proper identification, quality, purity, and potency of the active ingredient, with an emphasis on the identification and control of raw materials and the new DS. Information to be provided about the DP is similar to that which is required for the DS section, with information about the assays and acceptable results for assessing identity, potency, quality, and purity. It is also necessary to provide information about stability (evidence on how the quality varies with time under the influence of a variety of environmental factors, such as temperature) for at least the duration of the clinical trial, with the purpose of establishing the DP shelf life and recommended storage conditions. Fitting information about cell-based therapy products into the eCTD structure can be challenging due to continuous manufacturing processes and the need for clear separation between drug substance and drug product. Currently, there is an effort to update the ICH M4Q guidance to reflect the fact that the eCTD is not fit for purpose for many modalities.

Recommendations on the CMC information to be included in an original cell-based therapy investigational new drug (IND) are provided in the *Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)* guidance[23] and *Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)*[21] guidance (particularly for genetically modified cell-based therapies). In addition to providing guidance for sponsors, this guidance also instructs FDA CMC reviewers about the information to record and assess as part of an IND review, taking into consideration the various manufacturing challenges for these products. The recommended CMC information covered in this guidance includes product manufacturing and characterization information, product testing (including release and stability testing), product container closure and labeling, as well as preclinical and clinical studies. Similarly, the EMA provides recommendations in its draft *Guideline on quality, nonclinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials*.

When filing an initial IND submission, details about the following CMC information should be included: 1) drug substance (DS); 2) drug product (DP); 3) placebo formulation, if applicable; 4) labeling information for the labeled products relevant to the investigational drug; and 5) an environmental analysis for assessment of the effects of the investigational new drug or biological product on the environment (though many products qualify for an exemption from this assessment). It should be noted that, in general, the amount of CMC information submitted should be phase-appropriate, with more information required to support later stages of development. For a Phase I clinical trial, the primary focus of the CMC information is on safety.

The contents of investigational new drug (IND) application and clinical trial application (CTA) submission directly relating to CMC is to be submitted in documentation structured according to the heading of the corresponding sections of Module 3 of the eCTD. The CMC content for the Module 3 will highly depend on the specificities of the cell-based therapy product in terms of the level of information provided. Table 2-4 and 2-5 summarize recommendations for the main Module 3 sections for Drug Substance and Drug Product, respectively, on how to provide sufficient CMC information required to assure safety, identity, quality, purity, and potency of the investigational product for IND/CTA submissions. The tables combine the recommendations from FDA and EMA.

Drug substance

Cells can be classified as being either drug substance (DS) or critical starting material, depending on whether or not the cells undergo further modification. Unlike in traditional biopharmaceuticals where the drug substance (DS) and drug product (DP) typically employ separate manufacturing facilities, staff, and schedules for manufacture, autologous cell-based therapies are typically manufactured in a continuous process, where there often are no intermediates or stopping points in the manufacturing process and the same staff and facility are used for the full process. Continuous manufacturing of an unmodified cell-based therapy product involves expansion of therapeutic cells for treatment. Harvested cells cannot maintain viability without storage in suitable cryopreservation excipient(s). Therefore, harvested cells are typically processed all the way through to formulation and filled into the final container. This is different

Table 2-3: *Cell sources used in cell-based therapies*

Autologous	Cells that are removed from a person, potentially expanded or manipulated, stored, and later given back to that same person.
Allogeneic	Cells that are obtained from a healthy donor or tissue source, potentially expanded or manipulated, stored, and administered to an unrelated person.

from processes used to produce classic chemical-based pharmaceutical products. Most biologics that are active pharmaceutical ingredients (APIs) are DSs and are stored in the process container until materials are ready for formulation and filling into final containers.

For continuously manufactured cells, the DS section of the eCTD would describe the steps from harvesting cells to just prior to formulation and filling.

For modified cells involving use of other critical components, each component can be controlled and released as a DS, with a separate DS for each critical component (e.g., cells, plasmids, vectors, or gene editing tools. In fact, the FDA considers, for example, a viral vector carrying a CAR construct, as a DS). As an example, in the case of leukapheresis-derived peripheral blood mononuclear cells (PBMCs), cells are harvested prior to formulation and filling, but due to the continuous nature of the leukapheresis process, the DS is not actually stored. Alternatively, these critical components could be described as starting materials in the appropriate sections, and their placement should be discussed with FDA at the pre-IND meeting.

Cells

Cells can be obtained from donors (allogeneic) or from the same patients for whom they are intended (autologous; Table 2-3). Cells can be simply expanded or can undergo further manufacturing by genetic modification or selective culture conditions.

The FDA regulates establishments that manufacture human cell and tissue products (HCT/Ps) to ensure compliance with recommendations and requirements for current Good Tissue Practices (CGTPs) as defined under Title 21 Code of Federal Regulations, Part 1271 (21 CFR Part 1271, also referred to as the Food, Drug, and Cosmetic [FD&C] Act), Subpart D and requirements under Part 1271, Subpart E. Core CGTP requirements (§ 1271.150(b)) directly relate to preventing the introduction, transmission, or spread of communicable disease by HCT/Ps.

Establishments may request exemptions from CGTP requirements such as Donor Eligibility from the FDA. The request must be accompanied by all relevant valid scientific data and either information justifying the requested exemption from the requirement or a description of a proposed alternative method of meeting the requirement.

Requests should be submitted to the director of the appropriate FDA center. If the HCT/P is regulated solely under § 1271.10 as a 361 HCT/P, or as a biological product or a medical device regulated by CBER, requests should be sent to the director of CBER. If the HCT/P is regulated as a medical device by the Center for Devices and Radiological Health (CDRH), requests should be sent to the director of CDRH. More information can be found at: https://www.fda.gov/vaccines-blood-biologics/tissue-tissue-products/exemptions-and-alternatives

Establishments that manufacture human cells, tissues, and cellular and tissue-based products (HCT/Ps) regulated solely under section 361 of the Public Health Service (PHS) Act are required to register their establishments with the FDA and register and list their HCT/Ps within 5 days of beginning operation. Registration must be updated annually in December. Establishments must amend their HCT/P listing if a change described in §1271.25(c)(1) through (4) has occurred.

As of August 31, 2016, electronic submission of HCT/P establishment and product listing information is required under §1271.22, unless waived in certain circumstances. HCT/P establishments that must register and list electronically under 21 CFR Part 1271 should use the electronic HCT/P establishment registration system (eHCTERS) to meet the requirement for electronic submission of establishment registration and product listing.

HLA matching

To establish histocompatibility for users of allogeneic-sourced HCT/Ps, human leukocyte antigen (HLA) testing and confirmation may be required as part of the product identity tests.

Table 2-4: *Summary of CMC information for Module 3 (Drug Substance)*

Main Sections	Cell-Based Therapy Recommended Content
3.2.S.2.1 Manufacturers	Name, address, and responsibility of each manufacturer, including contractors, and each proposed production site or facility involved in manufacturing and testing.
3.2.S.2.2 Description of Manufacturing Process and Process Controls	Description of the DS manufacturing process that represents the applicant's commitment for the manufacture of the DS. For developers of cell-based therapy, this includes the following: • Information to adequately describe the manufacturing process and process controls • An explanation of the batch numbering system, including information regarding any pooling of harvests or intermediates and batch size or scale • A flow diagram (supplemented by written descriptions of each step) that illustrates the manufacturing route from the original inoculum (e.g., cells contained in one or more vials of the Working Cell Bank up to the last harvesting operation), with relevant information for all unit operations and each stage, such as population doubling levels, cell concentration, volumes, pH, cultivation times, holding times, temperature, culture media, equipment, process controls, material transfer during process steps, and storage and shipping conditions • A flow diagram that illustrates the purification steps (i.e., unit operations) from the crude harvests up to the step preceding filling of the DS including information about all steps and intermediates and relevant information for each stage (e.g., volumes, pH, critical processing time, holding times, temperatures, elution profiles, selection of fraction, and storage of intermediate) • Information about reprocessing procedures with criteria for reprocessing of any intermediate or the DS should be described, as given in 3.2.S.2.5 • A description of the filling procedure for the DS, the container closure systems used for storage of the DS (details in 3.2.S.6.), and storage and shipping conditions for the drug.
3.2.S.2.3 Control of Materials	Quality and control of materials used in the manufacture of the DS (e.g., raw materials, starting materials, solvents, reagents, catalysts); more information can be found in ICH Guidelines Q6A and Q6B. For developers of cell-based therapy products, the following should also be provided: • Summaries of viral safety information for biologically-sourced materials, including allogeneic HCT/Ps (Details in 3.2.A.2) • Source, history, and generation of the cell substrate • Information on the source of the cell substrate and analysis of the expression construct used to genetically modify cells and incorporated in the initial cell clone used to develop the Master Cell Bank, as described in Q5B and Q5D • Information about the cell banking system, quality control activities, and cell line stability during production and storage.
3.2.S.2.4 Controls of Critical Steps and Intermediates	Quality-related tests and acceptance criteria (with justification including experimental data) performed at critical steps of the manufacturing process identified in 3.2.S.2.2, as well as stability data supporting storage conditions (more information can be found in ICH Guidelines Q6A, Q6B, and Q5C)
3.2.S.2.5 Process validation and/or evaluation	Information about process validation and evaluation studies for aseptic processing and sterilization. Developers of cell-based therapy should also provide information on validation and evaluation studies to demonstrate that the manufacturing process (including reprocessing steps) is suitable for its intended purpose and to substantiate selection of critical process controls and their limits for critical manufacturing steps (e.g., cell culture, harvesting, purification, and modification). The plan for conducting the study should be described and the results, analysis, and conclusions from the executed studies should also be provided. For manufacturing steps intended to remove or inactivate viral contaminants, the information from evaluation studies should be provided in 3.2.A.2.

continued on next page

Table 2-4: *Summary of CMC information for Module 3 (Drug Substance)*

continued from previous page

Main Sections	Cell-Based Therapy Recommended Content
3.2.S.2.6 Manufacturing Process Development	Developmental history of the manufacturing process, as described in 3.2.S.2.2. The significance of the changes made during the development process should be assessed by evaluating its potential to impact the quality of the DS. Reference should be made to the DS data provided in section 3.2.S.4.4. More information can be found in ICH Guideline Q6B.
3.2.S.3 Characterization	Details about primary, secondary, and higher-order structure, post-translational forms, biological activity, purity, and immunochemical properties (3.2.S.3.1 - Elucidation of Structure and other Characteristics), as well as impurities (3.2.S.3.2). Further guidance on 3.2.S.3 can be found in ICH Guidelines Q3A, Q3C, Q5C, Q6A, and Q6B.
3.2.S.4 Control of Drug Substance	Information about specification (3.2.S.4.1), analytical procedures (3.2.S.4.2), validation of analytical procedures (3.2.S.4.3), batch analyses (3.2.S.4.4), and justification of specification (3.2.S.4.5). Further guidance on 3.2.S.4 can be found in ICH Guidelines Q2A, Q3A, Q3C, Q6A, and Q6B.
3.2.S.5 Reference Standards or Materials	Information on the reference standards or reference materials used for testing of the drug. Further guidance on 3.2.S.5 can be found in ICH Guidelines Q6A and Q6B.
3.2.S.6 Container Closure System	Description of the container closure systems, including the identity of materials of construction of each primary packaging component, and their specifications.
3.2.S.7 Stability	Stability-related tests and protocols (3.2.S.7.1), information about post-approval stability protocol and stability commitment (3.2.S.7.2), and overall stability data (3.2.S.7.3). Further guidance on 3.2.S.7 in ICH Guidelines Q1A, Q1B, Q2A, Q2B, and Q5C.

The following need to be provided in marketing applications for cord blood drug products:[22]

- A description of the serologic and DNA-based testing performed (serologic or DNA-based methods for HLA Class I (A and B) loci, and by DNA-based methods for HLA Class II (DRB1) loci)
- Sample types
- The name and location of the CLIA certified and American Society for Histocompatibility and Immunogenetics accredited laboratory that performs the initial and the confirmatory HLA typing of the final product.

The recommended extent of HLA match between the donor and recipient and the recommended cell therapy product dose are important considerations for dosage and administration specifications and, therefore, this information should be provided in the labeling for cord blood used for reconstitution of the hematopoietic system.

Blood group (ABO) and Rh type must also be tested for and identified to meet CGTP requirements for ensuring proper identification for these cord blood cell therapy products.

For all allogeneic cells or tissues (except those that meet the exceptions in 21 CFR 1271.90(a)), in addition to the donor screening and testing described above, addressing other issues such as typing for polymorphisms and human leukocyte antigen (HLA) matching, where appropriate, should be considered.[23]

Vector/Plasmid (for genetically modified cells)

Vectors are critical raw materials for the manufacture of genetically modified cell therapies as they represent an important means of transferring and integrating transgenes of interest into cells. For *ex vivo* cell therapies such as CAR-T cell products, the vector is considered a DS. CAR-T therapies use lentiviral vectors (LVs) in the manufacturing process, where third generation LVs featuring a 4-plasmid system, comprised of 3 helper

plasmids[24] and 1 self-inactivating gene transfer plasmid are most commonly used. To that end, a description of the LV manufacturing process and process controls must be included in Module 3 of the eCTD. All components used during LV production should be sufficiently described in the manufacturing summary included in an IND application, more specifically in the control of materials section of the DS section. This includes detailed descriptions of the plasmid construct generation and the DNA sequence of the entire plasmid(s).

Characterization studies will provide a comprehensive picture and knowledge of the vector, including annotated sequence analysis for the vector in the original IND submission and any additional sequence information gathered during the course of product development in subsequent submissions. Tests should be included to show integrity and homogeneity of the recombinant viral genome or plasmid and the genetic stability of the vector and therapeutic sequence.

Detailed discussion on the manufacturing process and recommended testing for LVs are discussed in Chapter 7 of this document, and recommended testing for plasmids used in vector manufacturing is covered in A-Gene chapter 5.[1] We can expect these standards to continue evolving as products are developed focused on *in vivo* modification.

Other gene editing techniques utilized to engineer cell therapy products (e.g., CRISPR) may utilize recombinant proteins (e.g., Cas9), viral vectors (e.g., AAV or LV), mRNA, or plasmids,[25] each of which may need to be described in its own DS section. Developers should utilize the same principles outlined in A-Gene to assess manufacturing controls needed to ensure the identity, safety, purity, and stability of such gene editing agents.

Drug product

For cellular therapies, due to the nature of continuous manufacturing, it is important to align with the FDA the dividing line between DS and DP, and appropriate placement in the Module 3 sections should be agreed upon with the FDA in the pre-IND meeting.

Drug labeling

Product labels for autologous products should be made on the primary container and secondary container (if applicable). Labeling information for an autologous product should include patient ID (without compromising patient privacy in accordance with local/national laws), dose, cell density, volume, date of manufacturing, expiry, storage condition, and manufacturer. Unique Patient IDs are critical to ensure that autologous products administered to patients are traceable. Details on required product labeling for autologous products, where donor-eligibility determination or donor screening/testing is not required, are presented in 21 CFR 1271.90.

Allogeneic product labelling should include lot and product ID to ensure product traceability to the donor, and may also include information regarding HLA-matching from donor to recipient, as appropriate. For investigational new drugs intended for human use, the immediate package should be labeled "Caution: New Drug, Limited by Federal Law to Investigation Use" as stated in 21 CFR 312.6(a).

Product Characterization

Robust product characterization is needed to demonstrate safety, identity, purity/impurity, quantity/strength, and potency of the final cell products. The FDA regulates the testing requirements for General Biological Products Standards in Title 21 of the CFR Part 610, which also applies to cell therapy products. In addition, recommendations on potency assay measurements, design, and validation are presented in the FDA Guidance *Potency Tests for Cellular and Gene Therapy Products*.[26] For detailed discussion on the required product testing and analytical assay development for characterization of cell-based therapies, refer to Chapter 9. For autologous cell therapy products, the starting cells from patients are often heterogeneous. Sponsors should conduct product characterization as early as possible and throughout the development cycle in order better address variability, understand CQA and set phase appropriate specifications.

ATMPs and Medical Device Combinations

The combination of ATMPs with medical devices may give rise to different regulatory scenarios from the perspective of the EMA. When a cell-based investigational medicinal product (CBIMP) incorporates a medical device as an integral part of the active substance, the medical device will be considered a starting material. When an ATMP necessitates a medical device as part of

Table 2-5: *Summary of CMC information for Module 3 (Drug Product)*

Main Sections	Cell-Based Therapy Recommended Content
3.2.P.1 Drug Product Description and Composition	Description of the DP and its composition (3.2.P.1). Further guidance can be found in ICH Guidelines Q6A and Q6B).
3.2.P.2 Pharmaceutical Development	Information about the development studies conducted to establish that the dosage form, the formulation, manufacturing process, container closure system, microbiological attributes, and usage instructions, are appropriate for the purpose specified in the application. Further guidance on 3.2.P.2 can be found in ICH Guidelines Q6A and Q6B.
3.2.P.3 Manufacture	Information about the manufacturing of the DP. This includes the name, address, and responsibility of each manufacturer, including contractors, and each proposed production site or facility involved in manufacturing and testing (3.2.P.3.1), batch formula (3.2.P.3.2), a description of manufacturing process and process controls in both flow chart and narrative form (3.2.P.3.3), controls of critical steps identified in 3.2.P.3.3 and intermediates (3.2.P.3.4), and process validation and evaluation. Viral safety evaluation should be provided in 3.2.A.2, if necessary. Further guidance on 3.2.P.3 can be found in ICH Guidelines Q2A, Q2B, Q6A, and Q6B.
3.2.P.4 Control of Excipients	Information about the testing of excipients involved with the DP. For compendial excipients, simply referencing the appropriate compendium(a) for each excipient is sufficient. For novel excipients, additional information is expected, including specifications (3.2.P.4.1), analytical procedures (3.2.P.4.2), validation of analytical procedures (3.2.P.4.3) and justification of specifications (3.2.P.4.4). If applicable, descriptions for any excipients of human or animal origin (3.2.P.4.5) and novel excipients (3.2.P.4.6) should be provided in the relevant sections. Further guidance on 3.2.P.4 can be found in ICH Guidelines Q2A, Q2B, Q3C, Q5A, Q5C, Q5D, Q6B.
3.2.P.5 Control of Drug Product	Information about the testing of the DP during manufacturing. This information includes specification(s) (3.2.P.5.1), analytical procedures (3.2.P.5.2), validation of analytical procedures (3.2.P.5.3), batch analyses (3.2.P.5.4), characterization of impurities (3.2.P.5.5), and justification of specification(s) (3.2.P.5.6). Further guidance on 3.2.P.5 can be found in the ICH Guidelines Q2A, Q2B, Q3B, Q3C, Q5C, Q6A, and Q6B.
3.2.P.6 Reference Standards or Materials	Information on the reference standards or reference materials used for testing of the DP should be provided, if not previously provided in 3.2.S.5. Further guidance on 3.2.P.6 can be found in ICH Guidelines Q6A and Q6B.
3.2.P.7 Container Closure System	Description of the container closure systems, including the identity of materials of construction of each primary packaging component and its specification.
3.2.P.8 Stability	Stability-related tests and protocols (3.2.P.8.1), information about post-approval stability protocol and stability commitment (3.2.P.8.2), and overall stability data (3.2.P.8.3). Further guidance on 3.2.S.7 in ICH Guidelines Q1A, Q1B, Q2A, Q2B, Q3B, Q5C, and Q6A.
3.2.A.1 Facilities and Equipment*	A summary of facility information, including a diagram of the manufacturing flow, movement of raw materials, personnel, waste, and intermediates, description of product-contact equipment and its use, information on products manufactured or manipulated in the same areas as the applicant's product, and cleaning, sterilization, and storage of equipment and materials.
3.2.A.2 Adventitious Agents Safety Evaluation	Information assessing the risk of potential contamination with adventitious agents, including avoidance and control of nonviral adventitious agents, detailed information from viral safety evaluation studies, and assessment of viral clearance. Further guidance can be found in ICH Guidelines Q5A, Q5D, and Q6B.

*Section 3.2.A.1 is typically submitted for US INDs, but is not required for EU IMPDs.

the final formulation, but the medical device is not an integral part of the active substance, the medical device will be considered an excipient. When the medical device is used as the container closure system or is intended to administer an ATMP and the administration device and the ATMP are marketed as a single integral product and the device is not reusable, the combination will be regulated under the medicines framework.

IND Submission to the FDA

Sponsors who wish to conduct a clinical trial with an investigational drug in the United States must, by law, do so under an investigational new drug (IND) submission, unless exempted under 21 CFR 312.2(b), 21 CFR 312.2(c), or 320.31(d), or unless a waiver is obtained under 21 CFR 312.10.17. This requirement is intended to ensure the safety of the subjects (patients and healthy volunteers) and to ensure the adequacy of trials so that marketing/licensing applications contain well-designed clinical investigations that can support safety and efficacy. The sponsor is responsible for the nature and quality of all submissions in both INDs and marketing/licensing applications. The FDA role is carried out through reviews of submissions, responses to questions posed by a sponsor, and meetings for further discussion and communication. The FDA may also conduct inspections of sites and operations associated with an IND, including targeted inspections of select clinical manufacturing and clinical trial sites.

The contents of an IND and the scope of the FDA regulatory authority with respect to an IND are described extensively in 21 CFR 312.1 through 312.42. Because sponsors may proceed with the submitted clinical trial if they are not informed otherwise by the FDA within the specified time period, the FDA must complete the initial safety reviews and make decisions on whether the proposed trial is safe by 30 calendar days after receipt of the submission.

An IND is administratively assigned to the review division with expertise in the relevant therapeutic area. Since review of a new IND focuses primarily on safety, FDA review divisions hold an internal IND safety meeting timed so that the division may provide requests to and receive responses from the sponsor for relatively minor technical issues that, if not brought to attention, might otherwise lead to a clinical hold.

The information submitted in a new IND must ensure safety for the proposed clinical trial before the sponsor can be allowed to proceed. If a clinical trial is not considered safe to proceed, it will be placed on clinical hold, with appropriate notification to the sponsor of the reason for the hold and the information required to lift the hold. Procedures for instituting a clinical hold and for reviewing and acting on a response to a clinical hold are described in 21 CFR 312.1 through 312.42.

In certain situations, FDA review staff may recommend submission of a pre-IND meeting request to discuss the sponsor's development program. For example, sponsors submitting a new IND with clinical data obtained from extensive development outside the United States and a later phase clinical trial protocol would benefit from a pre-IND meeting. Similarly, it would be valuable to gain FDA agreement via a pre-IND meeting prior to submission of a new IND with a complex clinical trial intended to support a new indication for a marketed drug in a new patient population where the risks and benefits of therapy differ from the approved indication. Agreements made during the pre-IND meeting ensure that the sponsor and the FDA are aligned on the scope of existing data that may be used to support the application and the proposed later phase development trials.

If a sponsor submits an IND without having previously conducted a pre-IND meeting or if the IND contains complex submission materials, FDA review staff are still required to complete the safety review in 30 calendar days. Given the complexity of cell-based products in general, it is recommended to conduct a pre-IND meeting prior to submitting an IND to mitigate risks of clinical hold.

With respect to manufacturing, the review of an IND concentrates on determining if there are any reasons to believe the manufacturing or controls for the clinical trial product present unreasonable health risks to the subjects in the initial IND trials; as always, safety is the priority. Such risks could arise in cases including (but not limited to):

- A product made with unknown or impure components
- A product possessing cellular phenotypes of known or potential toxicity
- A product with an impurity profile indicative of a potential health hazard or an impurity profile insufficiently defined to assess a potential health hazard
- A poorly characterized master or working cell bank

If the FDA identifies too many unresolved CMC-related safety issues in the IND, or if the FDA identifies such issues arising during development, the FDA will issue a clinical hold on the application. The FDA also acknowledges there can be specific challenges for applications that have received (or are likely to receive) expedited designations. These challenges include possible difficulties in aligning CMC and clinical development and possible difficulties in making risk/benefit assessments (with particular regard to patient benefit) in situations in which there may be a relative paucity of CMC information.

Additional factors that can have an impact on the IND submission process include CGMP compliance, and manufacturers' licensing/registration. Upon receipt of a request by an establishment physically located in the United States that has been included as part of a marketing application submitted to a foreign regulator, the FDA will issue a letter to an identified foreign regulator conveying the current CGMP compliance status for the establishment. The FDA will issue the response letter within 30 days of receipt of the request. A CGMP declaration should only be requested if a foreign regulator does not accept a valid certificate of pharmaceutical product (CPP) and wants additional assurance of a facility's CGMP status. Foreign regulators can also find the CGMP status of an establishment by checking the inspection classifications database for the most recent inspection classification.

Master File

In an IND submission, sponsors can reference Master Files (MFs) to provide information for products used in manufacturing that the company does not own, such as ancillary materials, packaging materials, or excipients. Examples of components that can be covered by a Master File include media, media components, cell banks, viral vector platform, and manufacturing-enabling equipment. While not required by statute or regulation, MFs are submitted directly to FDA by the manufacturers of said materials (the MF holder), which provide confidential, detailed information about the facilities, processes, or articles used in the manufacturing, processing, packaging, and storing of these products.[27] If the MF holder authorizes its incorporation in writing, MFs allow sponsors to reference material without the MF holder having to disclose the contents to the sponsors.[28] MFs are neither approved nor disapproved; instead, FDA reviews the technical contents of MFs in connection with the review of the INDs that reference them. The FDA can find an MF deficient, which can have an impact on an IND that references it, including the IND going on clinical hold. In Europe, MFs cannot be used for biologic DS components but there may be NCA-specific alternatives on a case-by-case basis.

Clinical Trial Application (CTA) in the European Economic Area (EEA)

The regulatory environment for the conduct of clinical trials in the EEA is currently evolving with the entry into force, on 31 January 2022 of the new EU Clinical Trial Regulation (Regulation (EU) N° 536/2014), repealing the EU Clinical Trials Directive N°2001/20/EC.

The Clinical Trial Regulation is aimed at facilitating clinical trials across the European Union by streamlining the application procedure via a single-entry point and harmonizing the procedure for assessment of clinical trial applications. The evaluation, authorization, and supervision of clinical trials are the responsibilities of EU Member States and European Economic Area (EEA) countries.

Prior to the regulation, clinical trial sponsors had to submit clinical trial applications separately to national competent authorities and ethics committees in each country to gain regulatory approval to run a clinical trial. The Regulation now enables sponsors to submit one online application via a single online platform, known as the Clinical Trials Information System (CTIS), for approval to run a clinical trial in several European countries, making it more efficient to carry out such multinational trials.

The CTIS system is centrally administrated by the EMA and also allows transparency and access to information for any party interested in clinical trials conducted in the EEA through a searchable public website.

A transition period applies to clinical trial submission under the Regulation:

- until 30 January 2023, clinical trial sponsors may use CTIS to apply to run a clinical trial under the Clinical Trials Regulation or may choose to apply to run a trial under the Clinical Trials Directive;
- from 31 January 2023, clinical trial sponsors will need to use CTIS to apply to start a new clinical trial in the EU/EEA;
- from 31 January 2025, any trials approved under the Clinical Trials Directive that continue running will

need to comply with the Clinical Trials Regulation and their sponsors must have recorded information on them in CTIS.

The content of the CTA under the CTR is described in Annex 1 of the EU Clinical Trial Regulation (Regulation (EU) N° 536/2014) and is mainly composed of 2 parts: Part I contains scientific and medicinal product documentation; Part II contains the national and patient-level documentation.

Parts I and II will be assessed in parallel by the countries unless the sponsor has decided to submit only Part I for assessment, and later submit Part II (within two years, otherwise the Part I application in that Member State will lapse).

Timelines for approval for a CTA submitted under the CTR can vary from 60 days up to 105 days (+ 50 days for ATMPs or biologics) versus 60 days under the Clinical Trial Directive 2001/20/EC (which allows a delay of maximum 90 days for the review of clinical trial applications for ATMPs), with a possible extension by a further 90 days.

Investigational Medicinal Product Documentation (IMPD)

The central document required for a CTA is the investigational medicinal product dossier (IMPD), which is included in Part I of a CTA (submitted through the CTR) and is comprised of relevant information on quality, preclinical studies, reference clinical studies, critical analyses of the nonclinical and clinical data related to the potential risks and benefits of the proposed study, any available previously generated human data, and an assessment of the overall risk/benefit. These data are presented according to the heading structure of the eCTD, with the amount of information contained in each section depending on various factors such as product type, indication, and development phase. Advanced therapy medicinal products (ATMPs) have additional data requirements. It should be noted that CMC information in the IMPD is subject to specifications not only issued by the EMA, but also European Pharmacopoeia (Ph Eur) monographs and European Directorate of the Quality of Medicines (EDQM) standard terms database.

The data on quality aspects of Investigational Medicinal Products should be presented according to the specified structure found in common technical document (CTD) Module 3. Data requirements are known to evolve as development progresses from exploratory to confirmatory clinical trials. As such, quality data compiled in the IMPD are to reflect increasing knowledge and experience during product development. For the sake of the eventual marketing authorization, it needs to be demonstrated that the medicinal product can be produced consistently and with reproducible quality. For example, acceptance criteria for test parameters/in-process controls should be reviewed at later stages of development. Also, the addition or removal of parameters and modification of analytical methods may be necessary. In all cases, the suitability of the analytical methods used should be demonstrated. As trials progress toward marketing authorizations, confirmatory clinical trials for the product based on a mature manufacturing process and specifications are expected.

The following Guidelines provide further information on the content of the IMPDs for chemical, biological, and ATMP IMPs:

- Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials
- Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials
- Guideline on quality, nonclinical and clinical requirements for investigation of advanced therapy medicinal products in clinical trials

Cell-based investigational medicinal products (CBIMPs) often contain or consist of cell preparations of limited size and many are intended to be used in a patient-specific manner. The traceability from the recipient of the product to the donor of the cells or tissues should be ensured. The traceability system should be bidirectional (from donor to recipient and from recipient to donor). Data should be kept for 30 years after the expiry date of the product unless a longer time period is required in the clinical trial authorization.

Specific case of GMOs

The Clinical Trial Regulation does not address local GMO specific requirements, which have to be met individually for each country in addition to the CTR.

To facilitate this purpose, a Questions and Answers document has been published on the European Commission website[29] that describes the interplay of clinical trial application and GMO application in the current situation, but also with a view on the new Clinical Trial regulation No 536/2014. A prior authorization under the GMO framework can no longer be a prerequisite for the clinical trial approval, and a CTA following Regulation (EU) No 536/2014 cannot be rejected due to lack of GMO approval. However, once CTA approval has been obtained, the GMO legislation still has to be respected, so that a clinical trial cannot start until GMO approval has been issued on a country level.

Qualified persons and batch release

Each manufacturing site of ATMPs in the European Economic Area (EEA) must have at least one Qualified Person (QP). Two or more sites may have the same QP if the QP can provide their services to each of the sites in a continuous fashion. The QP's main responsibility is to verify and certify that each batch produced in the EU has been manufactured and checked in accordance with requirements for the marketing authorization/clinical trial authorization, as well as relevant regulations governing the manufacture of medicinal products, including GMP. If the product is being exported, the QP must also verify that requirements are met for relevant product specifications in the destination country.

Further information about QPs and batch release is provided in the ATMP GMP guideline released by the European Commission: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf.

GMP certification in the EU

Manufacturers must comply with EU GMP to obtain a manufacturing authorization. They can ensure that they meet all their legal obligations by following the EU GMP guidelines. In the case of developers of ATMPs, there are specific guidelines for ATMP GMP. Importers of materials into the EU are responsible for ensuring that the third-country exporter is in compliance with EU GMP.

Manufacturers of active substances intended for the manufacture of human medicines for the EU market must register with the NCA of the Member State in which the manufacturing site is located. Manufacturers of finished products must also ensure that the active substances used have been manufactured in compliance with GMP. Importers of active substances intended for the EU market are also required to register. In addition, the competent authority of the country in which the consignment is produced needs to confirm that the consignment conforms to GMP standards equivalent to those in the EU, unless a waiver applies.

In the EU, NCAs are responsible for inspecting manufacturing sites located within their own borders. Manufacturing sites outside the EU are inspected by the NCA of the MS where the EU importer is located, unless a mutual recognition agreement (MRA) is in place between the EU and the country concerned. If an MRA applies, the authorities mutually rely on each other's inspections and the requirement for batch testing of products on entry into their territories may be waived. If products are imported directly into more than one Member State from a manufacturing site outside the EU, there may be more than one NCA responsible for inspecting the site. The EMA facilitates cooperation between the involved authorities. Inspections are carried out according to a risk-based approach and are especially important if there is suspicion of non-compliance. After inspecting a manufacturing site, EU competent authorities issue a GMP certificate or a non-compliance statement, which is entered in the EudraGMDP database.

For products derived from blood or blood plasma, the EMA is responsible for coordinating inspections of the establishments in which collection, testing, processing, storage, and distribution is carried out under the plasma master file (PMF) certification procedure. More information on the PMF certification procedure can be found at: https://www.ema.europa.eu/en/human-regulatory/overview/plasma-master-file-pmf-certification.

Differences between IND and CTA

INDs and CTAs are composed of overlapping yet non-identical components. The IND consists of several forms specific to the FDA, all nonclinical study reports (including validation reports of bioanalytical methods), nonclinical summaries, CMC information, the clinical protocol, and the IB. Once an IND has been cleared by the FDA, multiple studies can be conducted under the same IND, as per the FDA legal requirements as described in 21 CFR 312.22. These studies usually must use the same investigational drug and be used in patients with the same

disease (i.e., the same indication), but there are recent Guidances that speak to multiple versions of a product and those involving umbrella or basket trials that can also be discussed with FDA. For a CTA, the main documents are the protocol, informed consent form, IB, and IMPD, as well as several EU-specific and ethics committee-related documents; the range of nonclinical data required in an IND is not submitted. However, there are specific GMP-related documents that are required for a CTA that are not required for an IND. Additionally, in the EU, each interventional clinical study requires a new CTA, in contrast to a cleared IND.

INDs have set clearance timelines of 30 days, whereas CTAs have variable timelines ranging from 60 to 106 days, depending upon whether the applicant is asked to provide additional information through Requests for Information (RFIs). If an applicant fails to respond to RFIs within the deadline, the application will lapse; therefore, it can be said that deadlines are stricter for a CTA. CTAs are not subject to clinical holds like INDs; the CTA is either approved (perhaps with mandatory changes) or rejected. However, in certain circumstances CTA approval could be revoked by the country.

An IND Sponsor can amend an IND with changes to a clinical protocol, but FDA is not obligated to respond on a PDUFA time frame. However, with CTAs each substantial protocol amendment requires CTA approval.

Cell-based therapy product CMC information for IND versus CTA

In the EU, CTAs are in the remit of NCAs even for ATMPs that will later be approved under the centralized procedure. In addition, since there is a regulatory framework that includes regulations and directives, with the latter being recommendations to be implemented by national law, CTAs are evaluated using criteria derived from a wider range of regulatory bodies and legal source documents than is the case for FDA-reviewed INDs. Consequently, there can be challenges when filling in CMC information for the respective applications. Though the content follows the same eCTD structural guidelines in both IND applications and CTAs, CMC information in an IMPD, in general, has different (and in some cases more) specifications than CMC information in an initial IND (pre-Phase I). In particular, submission contents of an IND often fail to meet or lack data with respect to requirements and recommendations that are delineated in applicable EMA guidelines and European Pharmacopoeia (Ph Eur) monographs. Thus, applicants based in the United States can be relatively prone to receiving grounds for non-acceptance upon review of the CTA, even if their initial IND applications do not result in a clinical hold (especially taking into account agency response deadlines), and can, subsequently, be forced to withdraw the CTA or receive a rejection.

For example, for 3.2.P.3.4 (Control of critical steps and intermediates), US-based sponsors may fail to take into account the acceptance criterion for acceptable bioburden prior to sterilization by filtration that is in line with EU requirements. If submitted in an IMPD, this might be questioned by EU regulatory authorities because the acceptance criterion will not match expectations.

Submission of amendments

Changes in the manufacturing of cell therapy products can be categorized as minor, moderate, or major changes. Depending on the potential impact of the change on the product quality, amendments to an IND might be required.

FDA

After an IND is opened, a number of supporting documents may be submitted to the IND during its life cycle. The types of IND submissions and amendments are separated into four categories: submissions with a specific regulatory-mandated timeline; safety-related submissions; product development submissions without regulatory-mandated timelines where communication to the sponsor is often critical and recommended; and other submissions that may overlap any of the preceding three categories and where communication with the sponsor may be needed (e.g., general correspondence, final reports, annual reports, drug quality amendments). In general, the FDA considers review of safety-related amendments to be the top priority. Other high-priority amendment submissions are those that apply to new Phase II and Phase III adaptive trial designs, new Phase III protocols, and post-marketing requirement protocols. Lastly, IND amendments for products granted breakthrough therapy designation are treated with high priority as well, with intense involvement

of senior managers and experienced review staff in a proactive collaborative, cross-disciplinary review. It should be noted that the administrative Form FDA 1571 document should accompany most IND submissions to indicate the content and purpose of the submission and that all IND submissions should include summaries that allow FDA staff to understand the regulatory and developmental context of the submissions.

CMC-related changes are classified as information amendments (as opposed to protocol amendments), which are reported by the developers, routed to the relevant FDA staff, and assigned to reviewers as needed. Typical amendments include minor changes in manufacturing process, batch size change or updated batch data, and new labeling. The most important amendments are those that could possibly affect the safety of the product. These include (but are not limited to): 1) changes in the container closure system affecting product quality; 2) changes resulting in different impurity profiles; and 3) changes from synthetic to biological sources (human or animal) of a drug substance.

Information amendments to an IND are submitted through the same electronic portal as for the original IND, the Electronic Submissions Gateway (ESG): http://www.fda.gov/ForIndustry/ElectronicSubmissionsGateway/default.htm.

CTA substantial amendments (EEA)

Under the new CTR, substantial amendments to a CTA and accompanying documentation must be submitted for approval to the concerned countries via CTIS. Modifications to a trial are regarded as 'substantial' when they are likely to have a significant impact on the safety or rights of the subjects and/or the reliability and robustness of the data generated in the clinical trial.

It is, in principle, the responsibility of the sponsor to assess whether a modification is to be regarded as 'substantial'. This assessment is to be made on a case-by-case basis in view of the above criteria. In case of doubt, sponsors are encouraged to contact the relevant countries.

The sponsor should also assess whether a substantial modification (or the combination of a number of substantial modifications) leads to changes in the clinical trial to an extent that it has to be considered as a completely new clinical trial, which would require an application for a new trial authorization. For example, unplanned introduction of a new IMP, a change of the main objective, primary endpoint of the clinical trial in all phases, or an unplanned and unjustified addition of a trial arm or placebo group are considered as resulting in a new clinical trial and would therefore require a new trial authorization.

Information about changes in the CMC or quality aspects of the IMPD, can be found in the EMA: *Guideline on quality, nonclinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials*.

The following changes to the manufacturing process of the medicinal product have a high likelihood of being substantial:

- Major changes to the product formulation
- Changes to storage conditions
- Changes to test procedures of the DS or DP
- Changes to test procedures of non-pharmacopoeial excipients
- Changes to the manufacturing process of the DS or DP
- Changes to the release or shelf-life specifications of the DS or DP (widening or deletion of acceptance criteria, addition of specification for safety/quality concerns)
- Changes to the immediate packaging material

Once the application is validated and assessed, a written notice is sent to the applicant within 49 to 95 days (99 to 145 days for ATMPs) after receipt.

Requests for Out of Specification Exemptions

In some cases, a cell therapy product does not meet the quality requirements as specified in the regulatory submission (whether IND or CTA). In such a case, the product is considered to be out of specification (OOS) and is ordinarily not eligible for delivery to patients in the clinical trial. For example, a given instance of a cell therapy product may contain fewer cells than is specified in the CMC section of the regulatory submission and is, therefore, OOS. However, OOS products can, in select cases, still be delivered if a positive benefit/risk assessment is made with respect to the patient's status. The process for requesting OOS exemptions differs between regulatory agencies and is described below for the FDA and the EMA.

FDA

The process for requesting OOS exemptions under FDA regulatory jurisdiction is to email the RPM/CMC reviewer with a description of the OOS and the status of the patient and request a teleconference for discussion. The purpose of the teleconference is to arrive at a conclusive risk/benefit assessment with respect to safety for the patient. The PM/CMC reviewer will follow-up with an OOS-granted or OOS-rejected email. All correspondence regarding the OOS is then submitted as an IND CMC information amendment.

EEA

In EEA, there is no common rule for OOS exemption requests and this aspect is not regulated under the CTR. Instead, the sponsor, physician, and individual countries are invited to communicate when delivery of an OOS product is required. After a request from the treating physician is received, the manufacturer should provide the treating physician with its evaluation of the risks and notify the physician that the OOS product is being supplied to the physician at their request. Subsequently, the sponsor should inform the relevant national competent authority.

As an example, in the Netherlands, the process for reporting to the relevant competent authority is to send a notification of an OOS ATMP to the Central Committee for Research Involving Human Subjects (CCMO), an office within the Medicines Evaluations Board (MEB), by e-mail to tc@ccmo.nl within 48 hours after delivery of the product, with the subject stating 'Notification of OOS ATMP' and the relevant file number (NL number). The notification to the CCMO must consist of the following: a description of the OOS with a risk analysis that takes into account impact on product quality, safety, and efficacy; a statement from the investigating physician; and a statement from the sponsor as to whether or not the product has been administered.

Further details can be found in:
https://www.ccmo.nl/onderzoekers/standaardonderzoeksdossier/d-productinformatie

Life Cycle Management

Both the FDA and the EMA adhere to life cycle management principles defined by ICH. The ICH Quality Guidelines ICH Q8, Q9, Q10, and Q11 define and discuss concepts important for the assessment of CMC changes across the product life cycle. These documents detail a harmonized approach to technical and regulatory considerations for life cycle management with the aim of benefiting patients, industry, and regulatory authorities by promoting innovation and continual improvement in the biopharmaceutical sector, strengthening quality assurance, and improving supply of medicinal products. Additional goals include the facilitation of the management of post-approval CMC changes in a more predictable and efficient manner and the demonstration of how increased product and process knowledge can contribute to a reduction in the number of regulatory submissions. The latest ICH Quality Guideline, ICH Q12, addresses the commercial phase of the product life cycle (as described in ICH Q10). In addition, in line with prior ICH Quality Guidelines, ICH Q12 provides a framework to facilitate the management of post-approval CMC changes in a more predictable and efficient manner and is intended to demonstrate how increased product and process knowledge can contribute to a reduction in the number of regulatory submissions. In certain ICH regions, the current ICH Q12 guideline is not fully compatible with the established legal framework with regard to the use of explicit established conditions (ECs) and with the product life cycle management (PLCM) concept. These concepts will, however, be considered when the legal frameworks are reviewed and, in the interim, to the extent possible under the existing regulation in these ICH regions.

The concept of ECs provides a clear understanding between the MAH and regulatory authorities regarding the necessary elements to assure product quality and identify the elements that require a regulatory submission, if changed.

The Post-Approval Change Management Protocol (PACMP) is a regulatory tool that provides predictability regarding the information required to support a CMC change and the type of regulatory submission based on prior agreement between the MAH and regulatory authority. Such a mechanism enables planning and implementation of future changes to ECs in an efficient and predictable manner.

The PLCM document serves as a central repository for the ECs and the associated reporting category for changes made to ECs. The document also captures how a product

will be managed during the commercial phase of the life cycle including relevant post-approval CMC commitments and PACMPs.

An effective Pharmaceutical Quality System (PQS) as described in ICH Q10 and compliance with regional GMPs are necessary for implementation of ICH Q12. In particular, management of manufacturing changes across the supply chain is an essential part of an effective change management system. ICH Q12 provides recommendations for robust change management across multiple entities involved in the manufacture of a pharmaceutical product. It outlines the complementary roles of regulatory assessment and inspection, and how communication between assessors and inspectors facilitates the use of the tools included herein.

ICH Q12 outlines approaches to facilitate changes to marketed products and provides detailed guidance to enable changes to analytical methods to be made with immediate or other post-implementation notification. ICH Q12 also describes science- and risk-based approaches for stability studies in support of the evaluation of CMC changes.

In summary, the tools and concepts covered by ICH Q12 are complementary and are intended to link together different phases of the product life cycle. Pharmaceutical development activities result in an appropriate control strategy, the elements of which are considered to be ECs. All changes to an approved product are managed through a firm's PQS; changes to ECs must also be reported to the regulatory authority. Where the regulatory system provides for categorization of post-approval CMC changes for reporting according to risk, the MAH may propose reporting categories for changes to ECs based on risk and knowledge gained through enhanced pharmaceutical development. A system with risk-based reporting categories also facilitates the use of post-approval change management protocols, which provide predictability regarding planning for future changes to ECs. The PLM document is a summary that transparently conveys to the regulatory authority how the MAH plans to manage post-approval CMC changes.

DESIGNATIONS TO EXPEDITE DEVELOPMENT

The United States, European Union, and Japan have established expedited pathways to support accelerated development and regulatory approval for medicinal products that have the potential to address unmet medical needs. These pathways provide opportunities for developers to engage with regulators during the development process and participate in accelerated review programs within each agency.

US

Regenerative medicine therapies to treat, modify, reverse, or cure serious conditions may be eligible for several FDA expedited programs.[30] The FDA has developed five expedited pathways that are relevant for cell-based therapies: Fast Track (FT) designation, Breakthrough Therapy designation (BTD), Regenerative Medicine Advanced Therapy (RMAT) designation, priority review designation, and accelerated approval. Cell-based therapy products, including those that received FT, BTD, or RMAT designation, may also be eligible for priority review designation and accelerated approval. Sponsors may apply for and receive more than one designation for a given product, but sponsors should apply for each designation separately. Information that supports more than one designation may be submitted in each separate designation request.

Manufacturing and product quality are important factors to consider when filing requests for expedited programs. The sponsor of a product that receives an expedited drug development designation should be prepared to pursue a more rapid manufacturing development program to accommodate the accelerated pace of the clinical program. The Type B multidisciplinary meeting (e.g., after an IND sponsor is granted a BTD or RMAT) is an opportunity to discuss this with the FDA. If sponsors receive an expedited designation, they should be prepared to propose a commercial manufacturing program that will ensure availability of quality product at the time of approval. The proposal should consider estimated market demand, the commercial manufacturing development plan, manufacturing facilities, and a life cycle approach to process validation. Additionally, the proposal should include a timeline for development of the manufacturing capabilities with goals aligned with the clinical development program. Sponsors of such products should allow for an earlier submission of the CMC section (including product quality information) for timely review, and, critically, for inspection activities. Coordination with the sponsor and contract manufacturers may be necessary to ensure that manufacturing facilities and equipment are ready for inspection during review of the clinical section of the application.

Requests for expedited programs may be indicated in the relevant submission. The Office of Tissues and Advanced Therapies (OTAT) will notify the sponsor no later than 60 calendar days after receipt of the designation request as to whether the requested designation has been granted. If OTAT determines that the designation request was incomplete or that the drug development program does not meet the criteria designation, OTAT will include a written description of the rationale for such determination. If a designation has been granted but, later in development, the product no longer meets the qualifying criteria, then CBER may rescind the designation.

The criteria and key features of each expedited pathway is summarized in Table 2-6 below. Further guidance on accelerated pathways, including the specifications for requests for each designation, can be found in the *Expedited Programs for Regenerative Medicine Therapies for Serious Conditions* guidance document.[31]

Fast track designation

As specified and described in Section 506(b) of the FD&C Act (as added by section 112 of the Food and Drug Administration Modernization Act of 1997 [FDAMA] and amended by section 901 of the Food and Drug Administration Safety and Innovation Act of 2012 [FDASIA]), an IND can receive fast track designation if it is intended to treat a serious condition, and if nonclinical or clinical data demonstrate its potential to address an unmet medical need in patients with such a condition. The fast track designation provides advantages for facilitating development and expediting review of the product. The request for a fast track designation must be made either concurrently with submission of an IND application or as an amendment to an existing IND and should ideally be made no later than the pre-BLA meeting. The fast track designation will not be granted if an IND is on hold or is placed on hold during the designation review.

Breakthrough therapy designation

As specified and described in section 506(a) of the FD&C Act (as added by section 902 of FDASIA), an IND may qualify for breakthrough therapy designation (BTD) if it is intended to treat a serious condition, and if preliminary clinical data indicate that the product may demonstrate substantial improvement over available therapies with regard to one or more clinically significant endpoints. This designation is available for all treatment modalities, including cell-based therapies, and incorporates all the benefits of fast track designation and more, including intensive FDA guidance on efficient drug development, and an organizational commitment to involve senior management in facilitating the product's development program.

The request for a breakthrough therapy designation can be made either concurrently with submission of an IND application or as an amendment to an existing IND. A request should ideally be made no later than the EOP2 meeting. Because preliminary clinical data are required for granting the breakthrough therapy designation, the FDA expects that, in the majority of cases, a breakthrough therapy designation request will be submitted as an amendment to an ongoing IND. The breakthrough therapy designation will not be granted if an IND is on hold or is placed on hold during the designation review.

RMAT designation

The RMAT designation is the only expedited pathway that is specifically relevant to regenerative medicine therapies, such as cell therapies, gene therapies, therapeutic tissue engineering products, and human cell and tissue product. The designation was introduced in December 2016 as part of the 21st Century Cures Act.[32] A combination product can also be eligible for RMAT designation when the biological product constituent part is a regenerative medicine therapy and provides the greatest contribution to the overall intended therapeutic effects of the combination product (i.e., the primary mode of action of the combination product is conveyed by the biological product constituent part).

When determining whether preliminary clinical evidence is sufficient to support the RMAT designation, CBER considers factors, including but not limited to, the rigor of data collection, the consistency and persuasiveness of the outcomes, the number of patients or subjects and the number of sites contributing to the data, and the severity, rarity, or prevalence of the condition. In addition, CBER considers the potential that bias (e.g., bias in the study design, treatment assignment, or outcome assessment) may be a factor in the evidence provided in support of the RMAT designation.

The request for an RMAT designation must be made

Table 2-6: *Summary of Expedited Pathways in US*

	Fast Track (FT)	Breakthrough Therapy (BTD)	Regenerative Medicine Advanced Therapy (RMAT)	Priority Review	Accelerated Approval
Year established	**1997**	**2012**	**2017**	**1992**	**1992**
Qualifying criteria	Must treat serious condition Clinical or nonclinical data demonstrates that the therapy has the potential to address unmet medical needs for such disease or condition. **OR** Must be designated as a qualified infectious disease product	Chemical, Biological and Regenerative Medicines Must treat serious condition Preliminary clinical evidence indicates that the therapy may demonstrate substantial improvement on a clinically significant endpoint(s) over available therapies.	Meets the definition of regenerative medicine therapy Must treat, modify, reverse, or cure a serious or life-threatening disease/ condition Preliminary clinical evidence indicates that the therapy has the potential to address unmet medical needs for such disease or condition.	Must treat a serious condition Must provide a significant improvement in safety or effectiveness **OR** Any supplement that proposes a labeling change pursuant to a report on a pediatric study under 505A **OR** An application for a drug that has been designated as a qualified infectious disease product **OR** Any application or supplement for a drug submitted with a priority review voucher	Must treat a serious condition Provides a meaningful advantage over available therapies Demonstrates an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality (IMM) that is reasonably likely to predict an effect on IMM or other clinical benefit (i.e., an intermediate clinical endpoint).
Key program features	Frequent written communication Actions to expedite development and review Rolling review	Same as FT, plus: Early and more frequent communications with FDA during development. Rolling submission and review Priority Review	Same as BTD, plus: Early discussion of potential surrogate or intermediate clinical endpoint Post-approval advantages for those with accelerated approval	Shorter review of marketing application (6 months compared with the 10-month standard review)	Approval based on the effect on a surrogate endpoint or an intermediate clinical endpoint

either concurrently with submission of an IND application or as an amendment to an existing IND. The request should contain a concise summary of information that supports the RMAT designation, as described in the Expedited Programs for Regenerative Medicine Therapies guidance.[34] As opposed to breakthrough therapy designation, the RMAT designation does not require evidence to indicate that the drug may offer a substantial improvement over available therapies. An RMAT designation is not the same as an approval and does not change the statutory standards for demonstration of safety and effectiveness needed for marketing approval. The application success rate for RMAT designations from 2017-2022 is 38%.[33]

Both BTD and RMAT can be rescinded based on substantial changes to product/CMC without showing comparability during clinical development.

Table 2-7: *Comparison of benefits and requirements for Breakthrough Therapy and RMAT designation*[34]

	Breakthrough Therapy Designation (BTD)	Regenerative Medicine Advanced Therapy (RMAT)
Use of preliminary clinical evidence to support designation	✓	✓
Use of preliminary nonclinical evidence to support filing	✗	✗
Frequent interactions with FDA for discussions e.g. study design, extent of safety data required to support approval, dose-response concerns, and use of biomarkers	✓	✓
Eligible for priority review	✓	✓
Eligible for accelerated approval	✓	✓
Restricted to regenerative medicines	✗	✓
Show substantial improvement on clinically significant endpoint(s) over available therapy	✓	✗
Early interactions with FDA to discuss and determine potential surrogate or intermediate endpoints in support of accelerated approval	✗	✓
Regulatory interactions with FDA should be more focused around manufacturing issues	✗	✓

Priority review

As specified and described in the Prescription Drug User Fee Act of 1992, products (including those that received the fast track, breakthrough therapy, or RMAT designation) may be eligible for priority review if they meet the criteria for priority review at the time the marketing application is submitted. Sponsors of regenerative medicine therapies should consider discussing eligibility for priority review at the time of a pre-biologics license application (pre-BLA) meeting with CBER.

A regenerative medicine therapy may receive priority review if it treats a serious condition, and, if approved, would provide a significant improvement in the safety or effectiveness of the treatment for the condition. A decision about granting priority review is made within 60 calendar days of receipt of the marketing application or efficacy supplement. If the priority review designation is granted, CBER has a 6-month goal (reduced from 10 months for standard review) for reviewing the BLA or efficacy supplement.

The request for a priority review designation must be made with the original BLA or efficacy supplement.

Accelerated approval

Under the accelerated approval program, the FDA may consider accelerated approval for products for serious conditions that fill an unmet medical need (to the extent that the product provides a meaningful therapeutic benefit over existing treatments) based on whether the product has an effect on a surrogate or an intermediate clinical endpoint. A surrogate endpoint used for accelerated approval is a marker (including laboratory measurements, radiographic images, physical signs, or other measures) that is thought to predict clinical benefit, but is not itself a measure of clinical benefit. For the purposes of accelerated approval, an intermediate clinical endpoint is a measurement of a therapeutic effect that can be measured earlier than an effect on irreversible morbidity and mortality (IMM) and is considered reasonably likely to predict the drug's effect on IMM or other clinical benefit. The FDA bases its decision on whether to accept the proposed surrogate or intermediate clinical endpoint on the scientific support for that endpoint. Studies that demonstrate a drug's effect on a surrogate or intermediate clinical endpoint must be "adequate and well controlled" as required by the FD&C Act. The FDA has published examples of surrogate endpoints

that have been used in approved drug development programs under the basis of accelerated approval.[35]

In general, the accelerated approval pathway has been used primarily in settings in which the disease course is long and an extended period of time would be required to measure the intended clinical benefit of a drug. Accelerated approval is also potentially useful in acute disease settings where the intended clinical benefit can be demonstrated only in a very large study because the clinical event that would need to be evaluated to demonstrate clinical benefit occurs rarely. The FDA encourages sponsors to communicate with the agency early in development concerning the potential eligibility of a drug for accelerated approval, proposed surrogate endpoints or intermediate clinical endpoints, clinical trial designs, and planning and conduct of confirmatory trials. It should be noted that a sponsor seeking accelerated approval may also need to prepare for a more rapid pace for other aspects of the drug development, such as manufacturing. Drugs approved under the accelerated approval program still need to be tested in clinical trials using endpoints that demonstrate clinical benefit, in trials known as Phase IV confirmatory trials. If the drug later proves unable to demonstrate clinical benefit to patients, the FDA may withdraw approval.

If the FDA determines that there are grounds for withdrawal, the agency may ask the applicant to request withdrawal of approval under § 314.150(d) or notify the applicant of an FDA proposal to withdraw approval in a notice of opportunity for hearing (NOOH). The NOOH generally will state the proposed grounds for withdrawal of approval. Upon receipt of a NOOH, an applicant has 15 days to file a written request for a hearing. If an applicant does not request a hearing within 15 days, the applicant waives its opportunity for a hearing. An applicant may also, of its own volition, request the agency to withdraw approval of an application approved under accelerated approval.

Europe

Similar to the U.S., Europe (more specifically the EEA) has established expedited pathways to support accelerated development and regulatory approval for medicinal products that have the potential to address unmet medical needs. These pathways (Table 2-8) provide opportunities for developers to engage with regulators during the development process and participate in accelerated review programs.

PRIME scheme

The PRIority MEdicines (PRIME) scheme supports accelerated development of clinical programs to facilitate earlier patient access for unmet, serious medical needs. The PRIME scheme leverages on existing procedures and tools provided by the EMA with a commitment to engage more closely. This scheme broadly corresponds to the FDA breakthrough therapy designation. Between May 2016 and 2022, the success rate for PRIME applications for ATMP was 24%.[36]

Accelerated assessment

Sponsors may apply to the EMA for accelerated assessment for submissions. The accelerated assessment procedure is provided for by recital 33 and Article 14(9) of Regulation (EC) No 726/2004. Accelerated assessment reduces the timeframe for CHMP to review a marketing authorization application under the centralized procedure from 210 days to 150 days. Applications may be eligible for accelerated assessment if the CHMP decides the product is of major interest for public health and therapeutic innovation. Requests for accelerated assessment should be made at least two to three months before submitting the marketing authorization application. Applicants must also prepare justifications for why the product is expected to be of major public health interest, particularly from the point of view of therapeutic innovation.

The EMA also recommends that applicants request a pre-submission meeting six to seven months before submission to prepare for evaluation under accelerated assessment. In this meeting, applicants can discuss their proposal for accelerated assessment with EMA staff from CHMP, PRAC, and CAT, as needed. Requests for pre-submission meetings should be sent electronically to the EMA together with supporting documentation via the EMA Service Desk; required forms can be found at https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/accelerated-assessment. The CHMP decisions will be communicated to the applicant and the reasons for accepting or rejecting the request will be summarized in the CHMP assessment report.

Conditional Marketing Authorization (CMA)

Introduced in 2006 through Regulation (EC) No. 507/2006 according to Regulation N°726/2004, CMA is a mechanism by which an innovative medicine

Table 2-8: *Summary of Expedited Pathways in Europe*

	PRIME	Accelerated Assessment	Conditional Marketing Authorization	Authorization under Exceptional Circumstances
Year established	**2016**	**2004**	**2004**	**2004**
Qualifying criteria	Address unmet medical need Provide a major therapeutic advantage over existing treatments Based on early clinical data	Request should be made at least two to three months before submitting a marketing-authorization application Important in terms of public health and innovation Fulfills an unmet need Strong evidence	Filling an unmet medical need Pertaining to life-threatening, serious, or emergency disease, or orphan products Company must be able to provide clinical data comprehensively Positive benefit/risk balance	Applicants are not able to provide clinical data comprehensively because of rarity of the disease, for example Applicable to life-threatening or serious disease
Key program features	Enhanced interaction and early communication with sponsors Accelerated assessment and scientific advice	Reduce the timeframe for marketing authorization to 150 days	Active for one year only with an annual renewal of the approval until the EMA converts the approval to standard authorization Enables early approval while confirmatory	Applicants do not need to submit comprehensive data

addressing an unmet medical need can be made available for market supply as early as a positive benefit-risk balance supported by a sufficient body of clinical data can be demonstrated. The sponsor must then renew the conditional approval on an annual basis to ensure that the benefit-risk balance is monitored while further clinical trials are performed such that data are obtained to enable the conditional approval to be converted to a standard approval later. CMA is, therefore, a strategic way of providing therapies to patients who may lack access to treatment options that can be administered in a timely manner.

Distinction from authorization under exceptional circumstances

EMA may also grant a marketing authorization in absence of comprehensive clinical data under exceptional circumstances. Unlike CMA, where marketing approval is granted in the likelihood that the sponsor will provide such data within an agreed timeframe, EMA can grant authorization under exceptional circumstances when comprehensive data cannot be obtained even after authorization. This route normally does not lead to a standard marketing authorization. For more information and comparison between these authorization routes, see: https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/pre-authorisation-guidance

Japan

In Japan, expedited pathways that are relevant for gene therapies are: Priority Review, Conditional and Term-Limited Approval, Conditional Approval, and Sakigake (Table 2-9). Priority Review lessens the target review date to nine months and is available for medications that fulfill an unmet need. The Conditional and Term-Limited Approval pathway is for regenerative medicines

that show promising early phase results. The Conditional Approval pathway is targeted for highly useful and effective drugs treating serious diseases. The Sakigake early access scheme was introduced in 2014 to expedite innovative assets and was implemented in 2015.

Conditional approval

Under the PMD Act, an RMP can obtain conditional approval on the basis of safety and predicted probable efficacy demonstrated in early clinical trials. Conditional approval for an RMP under the PMD Act lasts for a maximum of 7 years, during which time the applicant is required to perform the later-stage trials that will be required for subsequent full approval. It should be noted that this time roughly corresponds to Phase II and Phase III clinical trials under most circumstances. If these trials are not performed or if the data obtained from them are considered inadequate to support full approval, the product must be withdrawn from the market at the end of the 7-year conditional approval period. Additionally, PMDA recommends that the following factors should be kept in mind with respect to conditional approval: 1) evaluation frequently needs to be done based on a small number of study participants due to patient scarcity; 2) controlled clinical trials are usually difficult to perform; 3) cellular heterogeneity makes it difficult to evaluate based on a fixed/limited number of study participants; and 4) there are frequently cases when

Table 2-9: *Summary of Expedited Pathways in Japan*

	Priority Review	Conditional and Term-Limited Approval	Conditional Approval	Sakigake
Year established	**2004**	**2004**	**2004**	**2016**
Qualifying criteria	No standard existing therapy or superior clinical usefulness as compared with the existing products in terms of quality of life of patients, efficacy, or safety Applicable to serious disease	Promising results of early-Phase I/II registration trials in terms of efficacy and safety Sponsors must conduct post-marketing clinical studies and so on to confirm the efficacy and safety and resubmit applications for regular approval within a predetermined period Only for regenerative medicines	No standard therapy exists or superior clinical usefulness is demonstrated as compared with the existing products in terms of quality of life of patients, efficacy, or safety Applicable to serious disease It is difficult or would take too long to conduct a confirmatory study Exploratory clinical studies show efficacy and safety Surveillance or clinical studies must be conducted as a post-marketing requirement.	Products for diseases in dire need of innovative therapy Applied for approval firstly or simultaneously (defined as submissions within 30 days of each other) in Japan Prominent effectiveness can be expected based on nonclinical and early phase trials
Key program features	Target total review time is nine months	Valid for no more than seven years	Conditional approval for drugs Priority Review	Prioritized consultation Prioritized review Review partner Substantial post-marketing safety measures Rolling submission and review

it is difficult to conduct rigorous statistical analysis. While items 1, 2, and 4 in the preceding list are considerations that are important for medicinal products in general, item 3 is unique to cell therapy products.

Sakigake designation

The sakigake (the meaning of which is "pioneer") designation was introduced in 2015 as the PMDA priority review system for innovative therapies targeting an unmet medical need. This designation roughly corresponds to the EMA PRIME scheme and the FDA breakthrough therapy designation. To qualify for the sakigake designation, a medicinal product must meet the following conditions: 1) be initially developed and submitted for authorization in Japan; 2) must be an innovative therapy for a disease or condition with an urgent unmet need; and 3) must demonstrate high efficacy in early stage clinical trials. To be considered an innovative therapy for an urgent unmet need, the product should possess a new and different mechanism of action compared to currently authorized products and treat either a serious life-threatening disease or a chronic disease that leads to the deterioration of patient quality of life.

The benefits of the sakigake designation include the following:

- Prioritized PMDA consultation (scientific advice), with the meeting taking place 1 month, rather than 2 months, after submission of the briefing documents
- Scope for extensive consultation prior to submission of the marketing application
- Accelerated review of the eventual marketing application, targeting review within 6 months rather than 12 months
- Enabling submission of Phase III study data after submission of the marketing application
- Assignment of a PMDA concierge to facilitate an efficient development program and marketing application process
- Implementation of specific post-authorization safety measures, including extended follow-up (over 10 years)

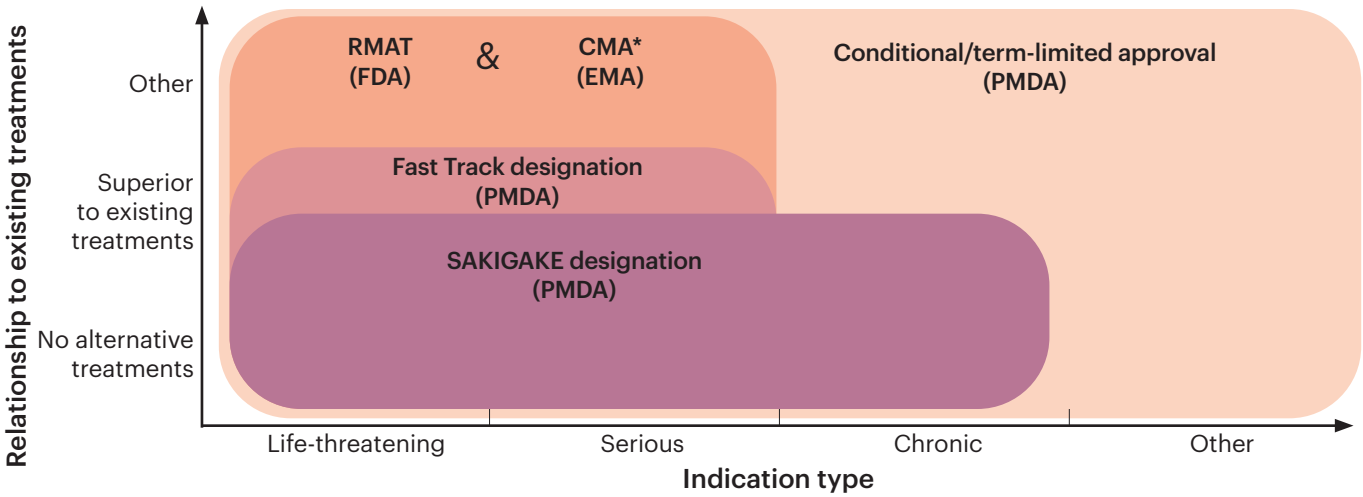
Overall comparison of accelerated pathways to other agencies

Figure 2-2 shows a comparison of Japanese accelerated regulatory pathways to those of agencies in the United States and Europe.

Comparability

This section focuses on providing supplementary guidelines about the best practices to establish product comparability for cell-based products. These include unmodified cellular products (i.e., *ex vivo* expanded NK, T cells, or MSCs) manufactured from autologous

Figure 2-2: *Comparison of PMDA, FDA, and EMA accelerated regulatory pathways*



Source: FDA, EMA, PMDA web sites
*Can also be used in emergency situations of if designated as an orphan medicine

and allogeneic source materials, gene modified cellular products including CAR-T, gene edited cellular products, and iPSC-derived cell-based products. Most elements of comparability as presented in Chapter 8 of A-Gene[1] are applicable to cell-based therapies (Table 2-10). To better highlight major differences in comparability approaches in this chapter, we will focus on some key differences and two relevant case studies.

COMPARABILITY STUDIES FOR CELL-BASED PRODUCTS

For cell-based products, establishing the comparability study design could be impacted by the nature of product, i.e., autologous versus allogeneic product. Although comparability of the DP is essential in both cases, for allogeneic-derived products there could be a need for establishing comparability of the DS (not shown), and if manufactured, the MCB and WCB as well (Figure 2-3).

For cell-based products, donor-to-donor variability is considered to be the primary source of complexity associated with establishing product comparability, whether one is comparing two different manufacturing processes or two different facilities. This variability is commonly addressed using either healthy donor material or preferably patient materials that are split for establishing product comparability that are manufactured by different processes (Figure 2-4A), or the same product manufactured by the same process at two different facilities (Figure 2-4B).

Another consideration is related to the choice of healthy donor material versus material obtained from patients in the autologously sourced setting. Although it is recommended that for establishing comparability (when process change is introduced, or additional facility is added) the most optimal starting biological material is patient-derived, sometimes it is not feasible to obtain patient materials. For this reason, FDA has shown flexibility historically with regards to the use of healthy donor materials as a substitute for patient-derived materials. However, manufacturers are expected to demonstrate that the product can be reliably manufactured from patient-sourced material post-comparability studies by

Table 2-10: *Applicable sections and page number from* A-Gene *Comparability chapter*

Section	Page number in A-Gene
Regulatory Requirements for Managing Manufacturing Changes	194-197
Introduction and Background	201
Description of Change and Rationale for Introducing Change	201
Categorization of Changes	201
Comparison of CQAs: potency, purity, strength, identity, and safety	202-203
Predefined Approach to Establishing Product Comparability	203
Well-Defined Acceptance Criteria to Establish Analytical Comparability	203
Detailed Analytical Procedure	203
Sampling Plan and Statistical Analysis	204
Statistical Strategy for Comparability Assessment	204-205
Overview of Commonly Used Statistical Approaches and Applicability to Process Comparability Assessment	205-209
Applying SPI as the Predetermined Acceptance Criteria	209
Process Validation for New Processes	211
Stability of Products Manufactured Using a New Process	212
Comparability Study Conclusion	213

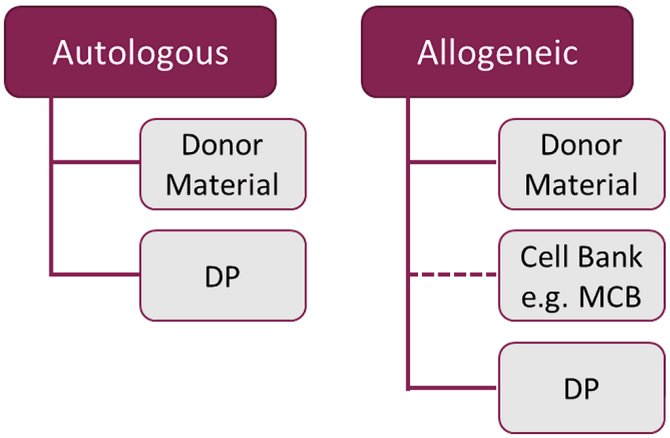
performing in the future, for example, during PPQ runs.

There are additional considerations that could impact the strategy for establishing comparability for autologous versus allogeneic off-the-shelf products. For allogeneic products, it is prudent to test more than one, preferably three donor sources, in order to establish comparability in a setting where donors are continuously introduced into the manufacturing process. For changes in facility, split starting materials can be useful.

Regardless of autologous or allogeneic, examples of changes include change of facility, change of process, change of ancillary materials, change in manufacturing-enabling equipment, and/or change of starting materials. In some cases, however, it is not feasible to perform a side-by-side comparison., for example when the starting materials are changed from MSC-derived to iPSC-derived (or even two different iPSC induction processes), when moving away from a facility that is no longer operational where the legacy product was manufactured, and/or the reagents or material for the old process are no longer available. In such cases, the use of historical lot data could be valuable for a comparative basis. In the event that side by side comparison is not feasible, it is also possible to compare the quality attributes of the product after certain changes are introduced using post-change materials as well as pre-change materials retained and stored appropriately. The availability of such samples for clinical batches may allow for limited comparative studies for cell-based products, as long as they are supported with stability data.

In all cases, where comparison of CQA for the DS/DP is performed, it is highly recommended that the materials are tested using assays that are adequately qualified.

Figure 2-3: *Potential differences in product intermediates for autologous vs allogeneic products*



In cases where the assays have changed, the validity of historical data could be further complemented with the use of reference materials generated from product manufactured previously. In the situation where the assay is performed at two different sites of manufacturing, FDA's expectation is to demonstrate that assays are properly transferred and deemed to be equivalent in terms of sensitivity, accuracy, linearity, and precision.

Another critical aspect of comparability study is availability of highly qualified potency assay for the drug product. Potency assay development is described in Chapter 9, but it is important to emphasize that the more reflective the potency assay is of bioactivity (or surrogate therein) of the product *in vivo* and in patients, the better. With this, the more readily available analytical data can be used to support product comparability. The use of surrogate markers that are not shown to reflect the

Figure 2-4: *Example starting material split strategy for establishing CAR-T product comparability*

(A) Process 1 versus Process 2 in same facility, and (B) Process 1 in one facility versus Process 1 in another facility

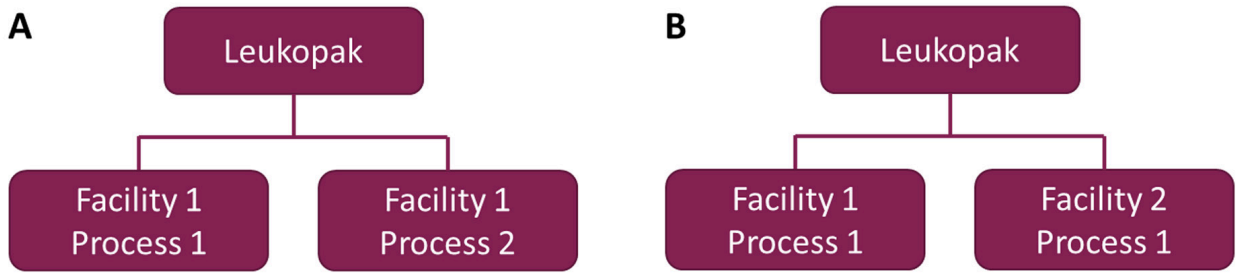


Figure 2-5: *Phase-dependent, risk-based approach to establishing product comparability*

	Risk Level			
Development Stage	**Low**	**Moderate**	**High**	**Highest**
Phase I	●			
Phase II	●	●		
Before Phase III or Pivotal Study		●	●	
During Phase III or Pivotal Study			●	●
During clinical study when combining clinical data before and after change is necessary			●	●
After Phase III or Pivotal Study and before Licensure				●
After Licensure				●

biological activity of the product is not recommended as a basis for establishing product comparability in the late phase of the product development cycle.

Although donor eligibility for off-the-shelf products is not necessarily considered one of the parameters to be used for establishing product comparability, it is important that donor eligibility and testing for relevant adventitious agents should be part of mandatory safety testing performed by IND Holders, as appropriate for the donor source. If the biological starting material, e.g., Leukopaks, are sourced from different vendors, and/or they are stored differently (i.e., fresh vs. cryopreserved), this can impact comparability and should be included as part of the comparability exercise.

Another important consideration is the phase-appropriateness of the comparability exercise. Regardless of the phase of development, it is recommended for sponsors to have early interaction with the FDA on their comparability plans, and provide a draft comparability protocol to the FDA in advance of execution, in order to take into account any advice the Agency may have on the protocol itself. Figure 2-5 shows a phase-dependent, risk-based approach to establishing product comparability during development. In general, the earlier major CMC changes can be implemented, the better (such as between the first-in-human study and pivotal trial), with comparability addressed (with FDA concurrence) prior to initiation of the pivotal trial.

Table 2-11 provides some examples of these changes and their categorization as minor (not requiring comparability) or major (requiring comparability). In some cases, a change in the type of donor material from one tissue to another may represent a change that requires a separate IND submission, in which case the FDA should be consulted for confirmation on impact.

Below we provide case studies of cell-based therapy products where manufacturing changes have been introduced: an autologous CAR-T that has been gene-modified via lentiviral vector in order to express the CAR transgene (Table 2-12), and an allogeneic iPSC-derived MSC (Table 2-13). First, the most relevant attributes to the study should be determined, as well as the methods and procedures that should be used to measure these attributes. A risk- and science-based approach allows prioritization of the relevant attributes, which may include biological activity, potency, identity, and purity (refer to Chapter 4 for discussion on risk assessment tools). These attributes are key elements of comparability studies along with standard safety tests such as sterility and endotoxin.

Based on the risk assessment and assay considerations, it is possible to select a subset of release tests to evaluate comparability with the highest relevance to product quality and effectiveness. These tests must be performed on the final DS/DP, but it is also important to monitor the process

Table 2-11: *Examples of changes and Associated Risk Categories*

Type of Change	Change Category	Note
Process changes re: cell washing	Minor	
Process changes re: multiple changes	Minor to Major depending on type of changes	Cumulative minor changes could potentially require establishment of comparability
Process changes re: starting material from cord blood to bone marrow	Major	Potentially may require a new IND; consult the appropriate review division
Process change: change of manufacturing from adherent to suspension cultures	Moderate to Major	
Change of facility 1 to facility 2 of the same process	Major	
Change of MCB for manufacturing of iPSC or MSC products	Major	

by conducting additional in-process testing (which could be a subset of the tests described here). Statistical considerations are covered in A-Gene as stated in Table 2-10. Table 2-12 summarizes an example of a list of attributes to be measured and the test methods for a CAR-T cell product. These attributes need to be prioritized, some of which are considered critical, such as purity and potency. Not all attributes are listed; process-related impurities are one example of other attributes that should be assessed and prioritized.

For MSC products derived from iPSCs, perhaps the most challenging exercise in establishing product comparability is when the MCB for MSC or iPSC is changed for a variety of reasons, which is outside of scope of this case study. To illustrate unique challenges in establishing comparability we will focus on a hypothetical product, MSC derived from differentiated iPSCs. The change involves major changes in manufacturing and using a different donor material. Table 2-13 summarizes a list of attributes to be measured in the comparability study and the test

Table 2-12: *Relevant list of product attributes and test methods for an autologous CAR-T cell product*

Potential changes include process/CMC changes within the same facility (Figure 2-4A), or change in facilities (include splitting source materials between two sites; performing same analytics at two sites) (Figure 2-4B).

Attributes	Test	Method Procedure
Cell counts	Measure total number of cells	Dye-based automated cell counter
Cell viability	Measure live cells/total cells ratio	Dye-based automated cell counter
Safety - Sterility	Measure of microbial contamination	USP <71>
Safety - Endotoxin	Measure of endotoxin	LAL (USP <85>)
Safety - Mycoplasma	Measure of mycoplasma	USP <63>
Safety - RCL	Replication Competent Lentivirus	qPCR / ddPCR
Safety - VCN	Measure vector integration into host cell genome	qPCR
Identity	CD3; CAR expression	Flow cytometry
Potency	Target cell killing	Cell based assay; ELISA (for surrogate marker if data supports)
Safety-*in vitro* proliferation	Measure IL-2 independent expansion	Cell-based assay
Purity - Contaminating cell types	Measure other cell types (B cells, NK cells)	Flow cytometry

Table 2-13: *Relevant list of product attributes and test methods for an iPSC-derived MSC product*

Attributes	Test	Method Procedure
Cell counts	Measure total number of cells	Dye-based automated cell counter
Cell viability	Measure live cells/total cells ratio	Dye-based automated cell counter
Safety - Sterility	Measure of microbial contamination	USP <71>
Safety - Endotoxin	Measure of endotoxin	LAL (USP <85>)
Safety - Mycoplasma	Measure of mycoplasma	USP <63>
Safety - Tumorigenicity	Aberrant cell growth	Cell-based
Identity MSC cell surface Marker	Relevant cell surface CD markers	Flow cytometry
Potency	Coculture immunosuppression assay	Cell based assay
Purity - Cell types and composition	Relevant cell surface CD markers; Measure cell types	Flow cytometry
Safety - Undifferentiated iPSC	Residual iPSCs	Flow Cytometry
Genome stability	Measure changes in genome structural or point mutation and / or deletions	STTR (qPCR), optical imaging method, FISH, karyotype (G-banding)

methods for this hypothetical MSC product. These attributes need to be prioritized, some of which are considered critical, such as purity and potency. Not all attributes are listed; process-related impurities are one example of other attributes that should be assessed and prioritized.

Standards

Cell and gene therapy is a rapidly developing field of medicine, with many promising products in development that could help manage and potentially cure a wide range of diseases that are intractable, chronic, and even terminal. Given that the cell therapy field is currently at a tipping point, with disruptive innovation pushing the boundaries of science and a number of products poised for commercialization (with a few already on the market), robust standards that can support developers in ensuring the safety, efficacy, and quality of products must be established. This section will provide an overview of the current landscape of standards development in cell therapy.

BENEFITS OF VOLUNTARY CONSENSUS STANDARDS IN CELL THERAPY

Standards are considered to be voluntary rules, conditions, characteristics, or physical materials that an organization can adopt to make a process safer, more efficient, or better aligned with the practices of other organizations in their industry. In general, standards can be considered to be either documentary or non-documentary.

Cell-based medicinal products are one of the key modalities of newly emerging regenerative therapeutics. The number and diversity of the product platforms in current use has exploded over the last ten years. However, given that relatively little experience has accumulated in the years that cell therapy has been a part of the therapeutic landscape, there are relatively few ways for developers across the research and industrial sectors to achieve consistency in areas such as protocol development, process infrastructure, and product quality testing and assurance. This lack of consistency and experience has made it challenging for stakeholders involved in cell therapy development and manufacturing to operate with a sense of certainty and provide patients in need of these essential and groundbreaking therapies with the confidence that products are of the maximum possible quality. The ongoing absence of standards to support safe and efficient practices that reduce the burden on companies seeking regulatory approval for their products may result in promising therapies being unable to successfully transition from the clinical development phase to being commercially available for the benefit of patients. The appropriately

targeted, field-wide development of standards will help to address the previously tolerated shortcomings.

Specifically, standardizing equipment, methodologies, and testing methods will result in a number of benefits for industry stakeholders. Standards establish a base of legitimacy on which patients, regulators, and investors can rely—standards development will instill the public with confidence that development of cell therapy is adequately informed by thoroughly researched best practices and is, therefore, a safe and effective option for treating a wide range of diseases. In addition, from an operational standpoint, standardization can help industry stakeholders streamline business practices by allowing for more efficient coordination of efforts throughout the entire supply chain, as well as improving the predictability of costs and resource management. In effect, barriers to entry into the clinical development space may be lowered for smaller companies or academic researchers, thus facilitating the delivery of therapies to patients. Standards can assure regulators that the fundamental processes underlying the development of a new therapy are sound. This assurance, in turn, allows regulators to review a product more rapidly. A smoother, less uncertain regulatory review process increases industry stability, lowers perceived risk to investors, and accelerates market availability of products, all of which serve to increase access and options for patients. Lastly, standards can greatly aid the regenerative medicine therapy community as a whole by enhancing the ability of developers to collaborate and share knowledge with others. This can reduce the potential for redundant efforts to be undertaken and serve to patch up the relatively fragmented state of knowledge that characterizes the emerging state of this field.

CURRENT LANDSCAPE OF STANDARDS IN CELL THERAPY

Currently, additional research is needed to assess the safety and efficacy of cell therapies for commercial use, which is becoming more widespread with the regulatory approval of several products in recent years. Furthermore, factors such as variations in manufacturing, measurement, and analytical techniques across developers of experimental cell therapy products make it difficult to evaluate product quality and safety, and to assess the impact of manufacturing changes or innovations intended to improve product safety and efficacy. A common set of standards in cell therapy will advance development of treatments for a number of diseases.

Standards Developing Organizations (SDOs) play a critical role in the publishing of consensus standards that are universally recognized. There is a big overlap between SDOs involved in cell therapy and gene therapy. A list of these organizations and the scope of their standards development efforts are presented in the Standards chapter of the A-Gene document.[1] In addition, organizations like the Standards Coordinating Body (SCB) helps accelerate such efforts by facilitating the use and development of standards in response to the diverse needs of stakeholder groups, including government and regulatory agencies, product developers, raw materials providers, and clinicians and healthcare professionals. An interactive database of the current regenerative medicine standards landscape is available at https://portal.standardscoordinatingbody.org/.

RECENT AND ONGOING EFFORTS IN CELL THERAPY STANDARDS DEVELOPMENT

Various organizations are developing and publishing technical standards using a consensus-based development process to benefit the broad regenerative medicine community. The examples below show a few standards that were recently published and are currently in development in the cell-based therapy field.

1. Testing and characterization standards for cellular therapeutic products (ISO 23033)

This effort (ISO 23033:2021 Biotechnology—Analytical methods—General requirements and considerations for the testing and characterization of cellular therapeutic products), led by The International Organization for Standardization (ISO), aims to define terms related to characterization of human cells for therapeutic applications, as well as provides general requirements for the testing of cellular therapeutic products intended for human use. It covers considerations for the characterization and testing of cellular therapeutic products, including approaches to select and design analytical methods that are fit-for-purpose and considerations for setting specifications for the analytical methods. Such considerations are critical to establishing CQAs for cell therapy products. Aspects of this document

are applicable to starting materials (including those for tissue-engineered products) and intermediates of cellular therapeutic products. This effort has been published and information relating to the effort can be found at: https://www.iso.org/standard/74367.html.

2. Ancillary materials standard for production of cell and gene therapy products (ISO/CD 20399)

The quality of ancillary materials used in the production of cell-based medicinal products will determine the safety and efficacy of the final cell therapy products. This document (ISO/CD 20399 Biotechnology—Ancillary materials present during the production of cellular therapeutic products and gene therapy products) provides guidance to suppliers and users of ancillary materials to improve the consistency and quality of ancillary materials of biological (human and animal) and chemical origin through an appropriate level of documented lot-to-lot consistency in the aspects of identity, purity, storage and stability, biosafety, and performance.

This standard will combine ISO/TS 20399-1, ISO/TS 20399-2, and ISO/TS 20399-3 into an international standard while adding further information on the relationship between ancillary material suppliers and users and quality considerations.

3. Cell collection and apheresis standards (BSR/PDA 08-202x)

The Parenteral Drug Association (PDA) is leading the development of a cell collection and apheresis standard, which is intended to minimize the variation during the apheresis collection process. This effort will result in creation of a standard leukapheresis manual/standard operating procedure (SOP) template to help align the format of cell collection requirements for product manufacturers and sponsors. This will aid both manufactures and apheresis sites in minimizing mistakes and increasing quality of starting materials for cell therapy. This standard is intended to clarify and minimize the variation in basic steps of the apheresis process while allowing for flexibility for manufacturing processes when needed.

4. Requirements for human and mouse pluripotent stem cells (ISO/DIS 24603)

Pluripotent stem cell (PSC) lines hold unique characteristics and behavior due to their capability for both self-renewal and differentiation into multiple cell types. However, the stem cell phenotype can be changed by suboptimal cell culture technique, prolonged passage, or changing the culture conditions. Accordingly, mouse PSCs have been used to establish our fundamental understanding of PSC biology, and these discoveries have been translated into human PSC research to drive the development of new human cell-based *in vitro* assays and potential regenerative medicines.

Mouse PSCs and human PSCs have become widely studied in this field, and many significant scientific advances have been made by using these PSCs, giving clues to the development of cell therapies. It is therefore critical to ensure that cell lines used in this field have been prepared and documented appropriately and have the correct identity and characteristics to help assure reproducibility in PSC-based studies.

This standard (ISO/DIS 24603 Biotechnology—Biobanking—Requirements for human and mouse pluripotent stem cells) specifies establishment, expansion, preservation, maintenance, characterization, quality control, storage, and distribution requirements for the biobanking of mouse and human PSCs. This document is applicable to all organizations performing biobanking with mouse and human PSCs used for research and development, excluding cell lines used for *in vivo* application in humans, clinical applications, or therapeutic use. This document aims to meet the current demand for the standardized PSC procedures of biobanks and builds on international consensus agreed by PSC resource centers (International Stem Cell Banking Initiative [ISCBI], 2009).

This effort is currently underway and information relating to the effort can be found at: https://www.iso.org/standard/79046.html.

5. Cryopreservation Standards for Cell Therapies, Gene Therapies, and Regenerative Medicine Manufacturing (ANSI/PDA 02-2021)

Cryopreservation is a key step in the manufacturing of cell therapies as many process parameters could affect cell recovery, viability, and function. This standard aims to present a best practices approach on how to prepare, cryopreserve, and recover cells, cell lines, and cell-based tissue products. It provides general, non-cell type specific guidance and considerations when developing a robust

protocol for freezing, storage, and recovery of primary cells or cell lines in research, development, and manufacturing of cell and gene therapy products, as well as potential sources of variability and mitigation strategies. The best practices and guidance in this document provide general procedural support for cryopreservation of cell-based products during both early and late phases of product development. This document provides guidance on how to ensure that your fit-for-purpose cryopreservation process is effective and validated appropriately based on the needs of your manufacturing process. This document has been published and more information can be found at https://www.pda.org/bookstore/product-detail/6498-pda-standard-02-2021.

6. Determining and interpreting cell viability

Being able to measure cell health and response to different stimuli is critical to understanding a cell therapy's quality, safety, and efficacy. With various available methods to measure cell viability, it is often challenging to identify the most appropriate method or assay for a given therapy or cell type. In addition, test methods can be difficult to interpret due to lack of understanding of what the assays measure and how measured parameters correlate with cell viability.

The cell therapy field has identified the need for this analytics and testing standard, with the goal being to develop a flexible, non-protocol-based standard around guidelines and considerations in designing and using cost-effective assays that yield accurate and precise cell viability results. These efforts will guide the fit-for-purpose decision-making process for developing a cell viability assay, with potential to develop a reference standard for cell viability assays. Possible areas of standardization include data type (e.g., number of living/dead cells), assay parameters (e.g., incubation time), stock cultures and testing environments, criteria for methods selection, cell sampling time during culture/manufacturing process, data recording, documentation, and interpretation, as well as assessing impact of patient variability.

A new effort in ISO to address this topic has begun development. This is being led by NIST and other U.S. experts. It will address how to assess cell viability for cellular therapeutic manufacturing.

NEEDS AND GAPS FOR STANDARDS IN CELL THERAPY BY FUNCTIONAL AREA

Variations in manufacturing, measurement, and analytical techniques across developers of cell therapy products often cause difficulties for evaluating product quality and safety and addressing the impact of manufacturing changes or innovations. A common set of standards in cell therapy will advance development of treatments beyond the realm of clinical trials, to safe approved treatments for genetic diseases and syndromes. The following list shows a few examples of needs and gaps for standards development in the cell therapy sector.

Chain of Identity

Chain of custody (COC) and chain of identity (COI) are records used in the regenerative medicine therapy manufacturing process to ensure product safety and quality. COI is a record associated with a single patient, including health records both before and after treatment. COC records all points of transfer and control for a product, including product starting material and all in-process manipulations through the point of delivery.

COC and COI currently lack standardized definitions and strategies. Because of this, stakeholders throughout the regenerative medicine supply chain often use different tracking systems or formats, which can potentially result in inaccurate or incompatible records. Such errors create a risk of administering the wrong product to a patient or the inability to administer a product due to delays. Standardization of this area would ease communication between the manufacturers and apheresis sites while also minimizing burden on the apheresis sites.

Data acquisition and management

The data generated throughout the research, development, and application of regenerative medicine therapies can inform efficient innovation of therapeutic products and help demonstrate the efficacy, safety, or regulatory compliance of a product. This data needs to be organized and formatted.

The quality, accuracy, and completeness of the existing body of data varies. Forms to be organized exist for acquiring and recording data, but there is no standard to encourage their use, which makes it difficult to compare similar data. Additionally, companies do not have a streamlined method to share their data while retaining

their intellectual property (IP) and maintaining patient/donor privacy.

Standardization of guidelines and protocols to increase data quality, make data easier to share, and establish mechanisms to safeguard IP and patient privacy would help advance cell therapy and ease life cycle management and communication. There are many potential areas to standardize that could address this, such as data management and storage plans, data registry/database characteristics, data elements (form fields), application programming interfaces (APIs), methods to study long-term effects of therapies in patients, and management of metadata.

IMPLEMENTING STANDARDS IN CELL-BASED THERAPY MANUFACTURING

The SCB has teamed up with the American National Standards Institute (ANSI) to create standards packages tailored to specific regenerative medicine application areas. The SCB has so far curated three standards packages: 1) Tissue Engineering Standards Addressing Product Quality and Characterization Package, 2) Cell Therapy Standards Addressing Product Quality and Characterization Package, and 3) Tissue Engineering Standards Addressing Analytical and Testing Methodologies Package, with more planned for other application areas.

The packages aim to make it faster and easier for regenerative medicine product developers to identify standards that can help stakeholders. These packages save time and money by offering insight into efficient methodologies and practices, and improve the safety, consistency, and comparability of regenerative medicine products. These packages can also facilitate a smoother regulatory approval process for new therapies.

The SCB plans to continue developing more standards packages in collaboration with ANSI. In addition to the large, broad packages that cover several methods with varying applications, the SCB also plans to create smaller, more targeted packages, focused on specific applications to help guide users through an entire process.

FDA-supported courses for standards implementation

There is a need for guidance in implementing current regenerative medicine standards. Currently the SCB is working on developing courses for standards implementation. These would be focused on helping stakeholders interpret and understand how standards can be put into place in their manufacturing processes. These courses will each focus on individual critical standards for the regenerative medicine field. The first course will focus on standards for cell counting (ISO 20391-1:2018 Biotechnology—Cell counting—Part 1: General guidance on cell counting methods and ISO 20391-2:2019 Biotechnology—Cell counting—Part 2: Experimental design and statistical analysis to quantify counting method performance). Currently the SCB is working to develop courses for nucleic acid synthesis (ISO 20395) and ancillary materials (ISO 20399). Further courses for additional standards are also planned.

STANDARDS IN CELL THERAPY AND REGULATORY APPROVAL

The FDA evaluates consensus standards for appropriateness for the review of medical device safety and performance by formally recognizing, all or partially, or not recognizing consensus standards. Manufacturers may submit declarations of conformity to FDA-recognized consensus standards to demonstrate they have met relevant requirements in the Federal Food, Drug, and Cosmetic Act (FD&C Act). When used appropriately, this may reduce the amount of supporting testing documentation typically needed in a premarket submission. The informal and formal recognition processes are discussed below.

FDA Informal Recognition of Voluntary Consensus Standards

Voluntary consensus standards can be defined as standards developed by voluntary consensus standards bodies. The Food and Drug Administration Modernization Act of 1997 (FDAMA) (Pub. L. No. 105-115) and the 21st Century Cures Act of 2016 (Pub. L. No. 114-255) amended section 514(c) of the Federal Food, Drug, and Cosmetic Act (FDC Act) to require FDA recognition of voluntary consensus standards. The FDA has used such standards to develop and evaluate performance characteristics of dosage forms, testing methodologies, manufacturing practices, product standards, scientific protocols, compliance criteria, ingredient specifications, labeling of drug products, and other technical or policy criteria.

In addition, the FDA Center for Drug Evaluation and Research (CDER) has drafted a document titled *CDER's Program for the Recognition of Voluntary Consensus Standards Related to Pharmaceutical Quality Guidance for Industry*. This guidance describes a proposed program at CDER to make public a comprehensive listing of informally recognized voluntary consensus standards related to pharmaceutical quality. The program, once established, will facilitate submissions by external stakeholders and CDER staff proposing voluntary consensus standards related to pharmaceutical quality for informal recognition. CDER believes that this informal program, which is different than the formal recognition standards program in the FDA Center for Devices and Radiological Health, will help promote innovation in pharmaceutical development and manufacturing and streamline the compilation and assessment of marketing applications for products regulated by CDER. It should be noted, however, that even if an applicant decides to use one of the CDER informally recognized voluntary standards, CDER may request that the applicant provide additional information to support an IND application or a marketing application. In addition, the applicant's use of an informally recognized consensus standard will be strictly voluntary. CDER has issued this draft guidance to obtain public comments on the proposed program. After CDER considers submitted comments, CDER will establish this program and describe it by publishing a final guidance. Thus, though not yet formalized, this draft guidance, when finalized, will comprehensively represent the current thinking of the FDA.

FDA Formal Recognition of Standards

In addition to informal recognition, the FDA also issues formal recognition of standards. The FDA recognizes consensus standards are standards that the FDA has vetted and determined are appropriate to support clearance or approval of a device.[37] The purpose of the FDA formal recognition of consensus standards is to streamline the premarket review process. This formal recognition allows companies to submit a declaration of conformity with a recognized standard in a premarket application, rather than submit complete data and test reports demonstrating conformity with a standard.

The FDA maintains a formal database of recognized consensus standards. This database consists of national and international standards recognized by the FDA to which manufacturers can declare conformity and is a component of the information that regulators can use to make an appropriate decision regarding the clearance or approval of a submission.

In the January 2020, FDA Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)—Guidance for Industry, the FDA recognizes three types of reference standards: 1) certified reference standards (e.g., USP compendial standards); 2) commercially supplied reference standards obtained from a reputable commercial source; and 3) other materials of documented purity, custom-synthesized by an analytical laboratory or other noncommercial establishment.

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CHAPTER 3

Generation of Quality Target Product Profile

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Chapter 3 | Contents

Concept of QTTP and its role in the development process

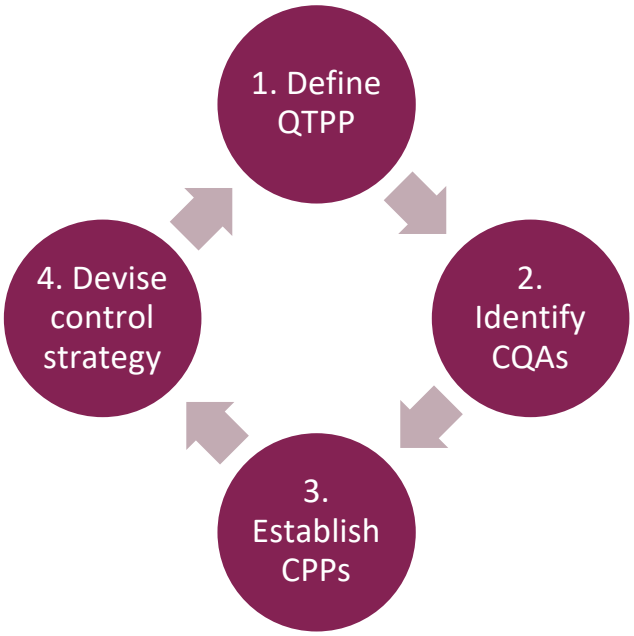
The Quality Target Product Profile (QTPP) provides developers of cell-based therapy products with a powerful tool for considering and assessing all elements of the production process that could have an impact on the quality, safety, and efficacy of the final product, and can help developers to focus resources and efforts on the most critical aspects of production. As first defined in ICH guideline Q8 (R2), the QTPP is a "prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product."[1] The concept of the QTPP follows directly from approaches taken for the target product profile (TPP), in which developers establish the goals for the drug development program (e.g., the target indication, target vein-to-vein time) and define safety- and efficacy-related information for labeled use. The QTPP focuses the concepts established in the TPP to guide chemistry, manufacturing, and controls (CMC) development, and is meant to identify, define, and justify quality characteristics to ensure safety and efficacy expectations established by the TPP.

The QTPP is an integral element of a Quality by Design (QbD) approach, in which quality of the product is considered at the earliest possible stage of development prior to clinical manufacturing. As part of this framework, the information contained in the QTPP should contain the Critical Quality Attributes (CQAs), defined to be "physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality",[1] which are identified through risk assessment (Chapter 4). Upon CQA identification, a series of development studies should be performed to quantify the relationship between process parameters and CQAs, resulting in the establishment of the critical process parameters (CPPs), defined as process parameters that have been proven to have a direct impact on the CQAs of the manufactured product. Additionally, the CQAs captured in the QTPP should provide the information required to define a design space and control strategy. Cumulatively, the QTPP serves as a link between the TPP and process validation steps necessary for ensuring the required quality standard.

The QTPP should be established at the research phase and be updated regularly throughout all phases of the product life cycle, up to the Biologics License Application (BLA) phase. A successful QTTP provides an understanding of the factors that will ensure the quality, safety, and efficacy of a specific product, as well as a comprehensive overview of the entire CMC module of the BLA. Once the product is approved, the QTPP serves as the product standard-bearer that grounds continuous process improvement and, therefore, is essential to the entire product life cycle.

Figure 3-1 depicts the path of QTPP maturation through the development process, as well as the relationship of the QTTP to the major stages of product development.

Figure 3-1: *QTPP maturation through product development*



Use of the quality target product profile

When initiating development of a pharmaceutical product, developers should begin drafting a list of desired characteristics targeted for the new product. This target product profile (TPP) is an instrument intended to facilitate a common vision across all disciplines to guide the development, conduct, and analysis of clinical trials by focusing on strategic product label claims.

The TPP encompasses a broad range of attributes, some of which are likely to evolve as development progresses and product knowledge becomes refined. Throughout a product's life cycle, the TPP provides the focus among all areas involved, forming the foundation through all stages of development and all disciplines that contribute to the development program of a medicinal product, including research, nonclinical, clinical, manufacturing, quality, and regulatory teams. It allows all medical, technical, and scientific information to be consolidated within one development tool or document.

Because it links safety and efficacy requirements to the final product, the TPP is considered the starting point for the development of a control strategy (Chapter 10). The TPP serves as the cornerstone to defining the QTPP and derived CQAs that should be monitored in order to assess the consistency of the manufacturing process. For a Cell and Gene therapy (CGT) product, as for any product, the TPP is a dynamic summary of the CGT product development that changes as knowledge increases, steering toward the goal of creating a commercialized product.

Once the initial TPP is agreed upon, a QTPP can be developed that focuses on the desired product characteristics and sets development goals. The QTPP is a tactical implementation of the strategic vision outlined in the TPP. As with the TPP, the QTPP may also define targets for each stage of development. It establishes the characteristics that a product must possess to safely and reproducibly deliver the therapeutic benefit promised on the final product label.

The QTPP should capture the entire body of knowledge about the product. This may include research experiences, available literature, small-scale development activities, risk assessment and mitigation plans, regulatory requirements, and regulatory guidance. The QTPP embodies the refined list of CQAs that enable product quality and consistency, and as the body of knowledge expands, the QTPP should evolve, as clinical experience and additional process understanding progresses and the TPP matures. QTPP updates typically consider any preclinical studies, first in-human (FIH) studies, Phase I/IIa trials, and subsequent clinical trial data, as well as product and process design changes, manufacturability, route of administration, dosage form, key market-specific requirements, and data from similar platforms.

Typical contents of the QTPP can be categorized into:

- **Drug Product Design Attributes:** broadly defined as those attributes that illustrate the physical description of the drug product
- **Drug Product Quality Attributes:** broadly defined as those attributes that relate specifically to drug product safety, identity, strength, purity, potency, or quality (SISPQ)

Table 3-1 lists the examples to consider for inclusion in the QTPP.

Table 3-1: *Attributes to consider for a cell-based therapy product QTPP.*

	Category of Attributes	Examples
General Property	Therapeutic Indication	Relapsed or Refractory Diffuse Large B-Cell Lymphoma, r/r Acute Lymphoblastic Leukemia
	Shelf life	years
	Storage Conditions	2-8°C, cryopreserved in vapor phase LN_2 <-130°C
	Container Closure System	Bag, vial, sterile-sealed
	Dosage Form	Liquid suspension, tissue equivalent, cryopreserved, fresh
	Dose Regimen	Daily, monthly, single infusion
	Delivery volume per dose	mL, mL/kg
	Method of administration	IV administration
Drug product Quality Attributes	General attributes	pH, osmolality
	Appearance	Color, opalescence, visible particulates
	Safety	Microbial testing that, depending on the nature of the product, is likely to be based on a multidimensional approach encompassing in-process and final-product testing
	Identity	Tests to distinguish the specified cells through physical or chemical characteristics of the cell line (i.e., phenotype, genotype, or other markers; qPCR of transgene; tissue-specific gene expression)
	Content	Total cell number, cell concentration (cells/ml), active (transduced) cells/ml
	Purity and impurities	Tests to assess product purity, considering the product (e.g., live cells, dead cells, cellular impurities, residual vector, process-related impurities such as residual media components, DMSO, anticoagulant)
	Potency	Measure of the relevant product biological functions. Methods to assess product biological activity are based on the different elements involved with the mechanism of action (MoA), often multiple tests evolving from specific markers in early stage to more functional assays at later stage.

QTPP for Cell-based Therapy

The starting materials and manufacturing processes of a drug development program can have fundamental effects on the quality characteristics of the final product. Therefore, one of the purposes of the QTPP is to provide information that readers can use to identify places and variables in the production process where quality can be compromised or drift from the required quality standard. Considering this, developers should aim to identify the potential CQAs of the product early in the development lifecycle. CQAs and, consequently, CPPs for the manufacturing process are product-specific and should be defined on a case-by-case basis; any available published data on similar products should also be considered. Acceptance criteria for CQAs and CPPs are defined through process characterization. As the process is validated and more clinical experience is gained, refinements in these specifications can be made. Most production processes for cell-based therapy products are still in early days of development, but several key areas should be captured or addressed by the QTPP.

Of note, cell-based therapy products are subject to considerable inherent variability of the cellular starting material, which may have a further impact on the consistency of the manufacturing process and final product. As a result, these potential inconsistencies could cause a misalignment between quality standards and clinical results (safety and efficacy). It is important to clearly establish a link between quality and the clinical performance of the product. Drug product quality attributes for cell-based therapy products should consider safety-,

potency-, purity-, identity-, and functionality-related parameters, which will be discussed in depth below. Note that the details of the associated testing for these attributes are discussed in Chapter 9 of this document.

SAFETY

Cell-based therapy products, being composed in part or in whole of cells, present several safety concerns based on factors unique to cells, such as differentiation and proliferative potential, as well as the potential for local and systemic toxicities that could arise thereof. Additionally, cells are subject to infection by adventitious agents, immune response, variability based on formulation, and improper quality assessment due to potential deficiencies in donor testing, particularly if used in an allogeneic application. To address these risks, a safety profile that captures safety parameters through categories such as microbial assurance, cellular impurities, non-viral and viral integration sites, genomic instability, as well as manufacturing residuals, is an important component of the QTTP.

It should be noted that safety assessment for cell-based therapy products can be challenging because of factors such as inconsistent approaches to product characterization, inadequately defined product parameters that anticipate adverse events, and a lack of standardized approaches in evaluating *in vivo* host responses. These could result from a poorly defined TPP or QTPP, which impacts CQA and overall analytical strategy (control, characterization). In particular, sterility of the final product is difficult, if not impossible, to fully guarantee. This is because cell-based therapy products cannot be terminally sterilized due to their biologically active state; therefore, aseptic controls and processing at the relevant stages of the manufacturing pipeline are needed to maintain sterility. As a result, regulatory authorities such as FDA and EMA recommend the use of closed manufacturing systems whenever possible. One potential sterility assurance strategy is to perform in-process sterility testing (of both media and drug substance/product intermediates) and only release products for administration to the patient if pre-harvest testing results are all negative. If in-process testing is difficult or not possible, surrogate testing may also be performed (for example, testing the last liquid with which the cells came into contact). Another solution is to use rapid assay methods, such as gram stains, which, while not highly sensitive, are quick and can detect gross contamination. Lastly, since batch sizes can vary and, in some cases, yield small amounts of materials for analysis, sterility testing that matches regulatory guidelines in terms of sample size volume is often not possible; in these cases, in-process or surrogate testing should be performed.

Furthermore, the specific type of cell-based therapy products can pose their own unique safety-related challenges. To illustrate some of the specific complications that can arise when determining the safety profile, it can be useful to consider the cases of human pluripotent stem cells (hPSCs) and chimeric antigen receptor (CAR)-T products. Both of these cases are briefly described below.

hPSCs

It is known that the contamination of a differentiated cell therapy product with undifferentiated stem cells, incompletely differentiated progenitor/precursor cells, or functionally mature cells, all pose potential safety concerns for recipients of the final product. In the case of hPSCs, expression characteristics of both stem cell-derived and functionally differentiated cell-derived products are present. Therefore, it can be seen that a single cell therapy substance can pose the full range of safety risks that exist for a given safety parameter, a characteristic that does not apply to many traditional pharmaceutical or biologic products.

CAR-T

Given that CAR-T cell manufacturing hinges on the intentional incubation of patient-derived T cells (purified from apheresis material) with viral vectors to deliver, integrate, and express the transgene of interest, there are several safety-related concerns during the manufacturing process. Among these are the potential for recombination events leading to off target integration, insertional mutagenesis, and the presence or creation of replication-competent vectors.

With regard to the choice of viral vector, both gamma retroviral vectors (γRVV) and lentiviral vectors (LVV) deliver RNA that is reverse-transcribed into DNA. However, their safety profiles are different, primarily due to the ability of LVV to transduce non-proliferating cells. Nonetheless, third-generation platforms offer the best safety profile and commercial viability by ensuring

efficient gene transfer and absence of replication-competent virus. For both viral vectors, the main safety concern is in regard to potential insertional mutagenesis. Because viral vectors mediate the integration of the genetic payload into the host cell genome, insertional mutagenesis is a possible occurrence in all cases involving integrating vectors. Insertional mutagenesis can, in turn, cause oncogenicity, depending on the site of insertion. It has been shown that lentiviral integration patterns favor sites away from risk-prone cellular promoters, while gammaretroviral integration more frequently occurs near transcriptional start sites. Therefore, the use of lentiviral vectors can mitigate the potential for insertional mutagenesis. However, it should be noted that the potential for insertional mutagenesis also appears to depend on the transgene, the promoter, and the target cell type. Lastly, oncogenic potential may be increased if replication-competent vectors are present in the final vector process materials (all vectors to be used should be replication-defective). Assays for the presence of replication-competent vectors (RCR or RCL) among product cells and media should be performed to ensure that the final CAR-T product is safe for infusion into patients.

Additionally, it should be noted that, due to the mechanism of attack on cancer cells implemented via CAR-T cells, "bystander" cells may become targeted by CAR-T cell therapy products as an unintended consequence of treatment. In particular, CAR-T cells may attack healthy B-cells, thereby exposing some patients to increased risk for infections.

POTENCY

According to 21 CFR 600.3, potency is defined as the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result. Tests for potency consist of either *in vitro* or *in vivo* tests (or both) that have been specifically designed for each product to indicate its functional activity (potency). Potency assays include tests to indicate and confirm the biological activities of the product and tests to measure activity of all ingredients considered necessary for activity of the product. These tests can be direct measurements of biological activity (*in vitro* or *in vivo* depending on product-specific attributes), surrogate measures (immunochemical, molecular, biochemical) that are correlated to but not direct measurements of biological activity, and assay matrix approaches (combination of assays based on product-specific attributes).

The matrixed approaches to potency that are recommended for protein-based therapeutics are particularly important for cell-based therapies. While there are functional assays in place for CAR-T products, the complex nature of the mechanism of action (MOA) for these therapies presents a limitation to potency assay development. Current cytokine release and cytotoxicity assays are often not predictive of the product's clinical outcome. It is therefore necessary to generate and monitor data throughout the clinical life cycle and use statistical methods and correlative analysis to determine relationships between the product CQAs and the clinical outcomes. This exercise allows for a better understanding of the manufacturing variables that impact product quality and clinical outcomes, which enables the appropriate control strategies to be implemented in the commercial setting.

PURITY

Provisions to remove possible contaminants or impurities should be made early on in CMC development. As CMC development progresses, an emphasis should be placed on validating manufacturing processes that either remove or introduce contaminants. Contaminants should be removed or prevented in accordance with limits set based on safety data from relevant preclinical and/or clinical studies. Common contaminants in final cell-based therapy products include product-related impurities (which originate from the heterogeneous nature of the starting cellular material for cell-based therapies) such as of non-T cells, residual tumor cells, non-viable and non-transduced T cells, and extracellular vesicles; as well as process-related impurities such as cell culture media (especially in cases in which animal-derived supplements such as fetal bovine serum are used), matrix components, residual beads, residual reagents (e.g. DMSO), cellular DNA, antibodies, product isoforms, helper viruses, viral vectors or associated components (including empty capsids), and leachables and extractables from manufacturing components. Additionally, because of contingencies such as batch mix-up or the accidental transduction of

other cells, unintended cell populations also pose major contamination risks for both allogeneic and autologous products. There are also risks posed by process substances such as contaminated reagents, cesium chloride (CsCl), and chromatography media.

IDENTITY

Protocols to test final product identity should be established early in the CMC development process. Tests for identity should be able to identify the product as designated on the final container and package label. Tests should also be able to distinguish the product from any other product being produced and/or processed in the same facility. Some methods for identity-testing cell-based therapy products include (but are not limited to): flow cytometry, PCR, and genome sequencing-based assays (such as those to identify short tandem repeats (STRs)). The appropriate application of these methods will vary according to the particularities of the manufacturing context at hand.

STABILITY

Tests for stability should demonstrate that the product is within acceptable quality limits for the duration of the planned clinical study. Stability testing should be done in the context of planned storage conditions, planned formulation, manufacturing holding steps, stressed conditions, shipping/transport, and clinical in-use hold steps, and thus should prioritize testing the attributes that could potentially change over time in cryostorage. Acceptable limits and ranges for stability should be established incrementally through the stages of CMC development, with data from preclinical lots in Phase I informing initial limits, and further refinement during Phase II and Phase III (especially with ongoing data collection for setting the expiry date of the product and required/recommended storage conditions).

Evolution of the QTTP during development

Over the course of product development, changes to manufacturing or processing stages are likely to occur. While it is impossible to know the full range of changes that could occur, the QTPP should aim to highlight at least some of the most likely areas at which changes could occur and establish possible steps that could be taken to maintain product quality. Elements of the cell-based therapy product development process subject to possible change include: ancillary materials (cytokines, antibodies, media, etc.), vector supplier, manufacturing site, formulation/concentration, analytical procedures, and prospects of scale-up. When assessing changes in manufacturing, establishing knowledge/understanding of the impact of specific process steps or unit operations on a particular CQA is critical because even small changes could result in unpredictable effects on safety, efficacy, stability, and other aspects of cell-based therapy product quality.

Cells often vary greatly in different parameters that could impact target product quality. These parameters include (but are not limited to): growth potential, cell sample composition, genetic stability, and phenotype. It is particularly important for developers of cell-based therapy products to be able to define CQAs early in the development process so that the relevant factors can be identified and addressed when/if they appear during the course of production. To this end, developers of cell-based therapy products should consider factors such as morphology, phenotypic markers, potency, and genetic stability. It is also important to define the maximum population doublings (PDs), both for autologous and allogeneic cell-based therapy products, because any degree of cell-count variability in the final product may result in variability in the nonclinical and clinical results. A major aim with regard to developing an effective QTPP should be to monitor these factors as the development program evolves. Diligent tracking of these factors will, in turn, allow the developer to update the QTPP according to new findings if necessary.

Some other factors that warrant specific commentary with respect to the in-process evolution of cell-based therapy product development programs are process- and product-related impurities in autologous cell-based therapy products and changes in target and non-target cells during expansion. Impurities can present special challenges for developers of autologous cell-based therapy products when defining CQAs that can be used as standards for updating the QTPP because, though it is commonly assumed that there are no cellular impurities in the autologous cell-based therapy product (because the source donors and patients are identical), the administration of the cells does not always follow the physiological

place and magnitude; higher cell doses may be given to unconventional places where the presence of certain cell types may be harmful in terms of safety or may even lower efficacy. Therefore, clinical experience must be used to inform and update the CQAs that define purity-related quality standards. With respect to changes during expansion, cells are especially subject to change during expansion when growth factors and cytokines are used to drive high proliferation. In order to address this tendency, developers should establish cell selection and isolation steps in the production process (particularly if the starting material is heterogeneous and the final cell composition is critical).

More generally, optimization and process validation studies should always be used to provide a clear rationale behind all changes made to processing and manufacturing steps. Differences in processing and manipulation conditions should be evaluated and validated with commercial production in mind. Additionally, when the manufacturing process is changed, the risk assessment must be repeated in order to identify whether the given change is a minor or major one and to consider the possible impact on the quality, safety, and efficacy of the final product. In many cases, it may be sufficient to demonstrate that the product has not changed on a quality level as defined in the QTPP. For the required comparability studies, one should utilize not only release test methods, but also extended characterization and comparison of in-process controls. It is also important that the changes do not impact the stability of the product. If the quality of the product does, in fact, change, additional clinical studies may be required to demonstrate unchanged safety and efficacy. As new data accumulate throughout the development process and new CQAs are identified, the QTPP should be updated in order to assess the need for comparability studies and to evaluate possible gaps that may need to be filled before further clinical studies or submission of the BLA. The QTPP enables a proactive approach to product design as any process changes would need to result in targets defined within the design space documented in the QTPP, ensuring process modifications are implemented based on no changes to product quality.

An example of an early, pre-IND QTPP for an autologous CAR-T product is presented in Table 3-2. Note that the values are demonstrated by units of measurements, but no targets are prescribed as these are heavily dependent on each product's individual modality and target indication.

During later stages of development when sufficient clinical data has been gathered, correlation between CQAs and clinical efficacy and safety can be made. This correlative analysis exercise can be the basis of justification for widening the product specification acceptance criteria, which can then be used to update the QTPP throughout the product life cycle. As discussed earlier, product impurities (e.g., non-viable cells, presence of other cell types) can present challenges for cell-based therapy products since their presence may impact the product safety or lower efficacy. Using clinical experience to inform and update the CQAs that define purity-related quality standards is therefore critical. For example, in tisagenlecleucel, cell viability that is below the specification (80%) has been shown to not compromise its clinical safety or efficacy in patients with acute lymphoblastic leukemia, non-Hodgkin lymphoma,[3] or diffuse large B-cell lymphoma.[4] By expanding this specification to the broader range observed in the clinical data, delay of product release caused by unnecessarily stringent specifications, and consequently delivery to patients, can be prevented. The QTPP should then be updated to reflect this new design space of the product based on no changes to product quality.

QTPP for allogeneic cell-based therapy products

The QTPP for an allogeneic product can differ substantially from that of an autologous product as allogeneic cell therapy products possess a few unique additional attributes. Such additional attributes can be safety-related as allogeneic products in general pose more safety concerns than autologous products, particularly with regard to presence of adventitious agents that may be present in the donor material. To that end, the QTPP of an allogeneic product may include attributes reflecting the presence of Relevant Communicable Diseases and Disease Agents (RCDAD), such as HIV 1 and 2, Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human transmissible spongiform encephalopathy (TSE), among others. Consequently, in addition to robust control and testing strategy, the allogeneic approach requires additional factors to ensure safe administration to patients, such as donor screening and testing. Additionally, genomic stability is important to monitor in allogeneic

Table 3-2: *Example QTPP for autologous CAR-T (pre-IND).*

General Properties			
Property	**Target**		
Indication	Hematologic malignancies		
Geographic scope	Global: test to USP, EP, JP compendia where possible		
Shelf life	12 months at intended storage conditions		
Storage conditions	≤-120°C (vapor phase liquid nitrogen)		
Container closure	Selected to support product compatibility and stability and to ensure sterility (cryobag)		
Delivery volume	Variable based on patient weight		
Method of administration	IV administration		
Product Attributes			
Attribute Category	**Attribute**	**Target**	**Method / Justification**
General attributes	pH	Appropriate for the formulation to support product stability and compatible with the route of administration	
	Osmolality	Appropriate for the formulation to support product stability and compatible with the route of administration	
Appearance	Color / opalescence	Colorless to yellow	
	Visible foreign particles	Negative	
Content / strength	Dose range	XX CD3+ transduced cells per weight - or - XX ml/kg body weight	Cell growth profile and transduction efficiency ranges to be determined from dose needs
	Cell concentration	XX cells/ml	Automated cell counter
	Product / container volume	XX ml	Volume to be determined to support the dose
	In-use stability	< XX minutes	Stable at room temperature before infusion
Safety	Sterility	Negative	USP <71> / Eur Ph 2.6.27
	Mycoplasma	< XX	USP <63> / Eur Ph 2.6.7
	Endotoxin	< XX	USP <85> / Eur Ph 2.6.14
	Bacterial contamination	Negative	Gram staining
	Replication competent lentivirus (RCL)	Negative	qPCR / ddPCR
	Vector copy number (VCN)	< XX	qPCR
Identity	% CAR+ CD 3+	> XX	Flow cytometry

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Table 3-2: *Example QTPP for autologous CAR-T (pre-IND)*

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Purity	Residual beads	< XX beads/mL	Microscope imaging
	Residual BSA / serum	< XX pg/ml	ELISA
	Cell viability	> 70% [2]	Automated cell counter
	% B cells	< XX	Flow cytometry
	Product related impurities (% NK cells, % monocytes, etc.)	< XX	Flow cytometry
Potency	Cytotoxicity	> XX% CD19+ cell apoptosis	Cell killing
	IFN-γ cytokine release	Report result	ELISA

cell-based therapies as long-term culture of cells can alter the genomic composition and lead to chromosomal abnormalities, which in turn can affect protein expression, cell function, safety, and purity. The QTPP for an allogeneic product can therefore include characterization of changes in genome structural or point mutation and/or deletions. Overall, a risk-based approach should be taken for selection of attributes to characterize for a particular product to best inform the product development strategy. For discussion on qualification and testing of starting material and components, donor eligibility determination, as well as appropriate product testing and characterization for allogeneic cell-based therapy products, refer to Chapter 5. Additionally, to establish histocompatibility for users of allogeneic products, human leukocyte antigen (HLA) testing may be performed as part of the product identity tests. Discussion on HLA matching is presented in Chapter 2. The methods and timepoints for genomic characterization in allogeneic product manufacturing are discussed in Chapter 9.

Conclusion

In closing, as many cell-based therapy products proceed toward late-stage development and BLA-enabling activities, it is going to be increasingly important to take lessons from biologics and vaccines in applying a Quality by Design approach in product development. One of the most important things when taking and implementing QbD principles in cell-based therapy is to establish the QTPP early during development. The importance of a QTPP cannot be overemphasized as it, as well as the next step—CQA identification, dictates the rest of the product development activities. Being able to define the QTPP and CQA early enables product developers to manage comparability across products and process changes.

Various risk-based approaches are used throughout the product development to establish manufacturing processing operations and define the control of critical quality attributes, the control of raw materials, and the areas for further investigation. Such assessments are performed by a cross-functional team of subject matter experts to ensure that the process delivers a product that satisfies the QTPP in a reproducible manner.

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CHAPTER 4

Risk Assessment and Critical Quality Attribute Identification

CHAPTER LEAD

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Chapter 4 | Contents

Due to similarities in risk and criticality assessment approaches across various types of cell-based therapy products, some of the overall content and flow of this chapter was based on, but adapted from when applicable, Technical Report No. 81: Cell-Based Therapy Control Strategy, a document published by the Parenteral Drug Association (PDA).

The authors of this A-Cell chapter acknowledge the work of the authors and editors in constructing the PDA document. For further details on the PDA document, please refer to: Parenteral Drug Association. Technical Report No. 81: Cell-Based Therapy Control Strategy. https://www.pda.org/bookstore/product-detail/4638-tr-81-control-strategy-for-cell-therapy. February 2019.

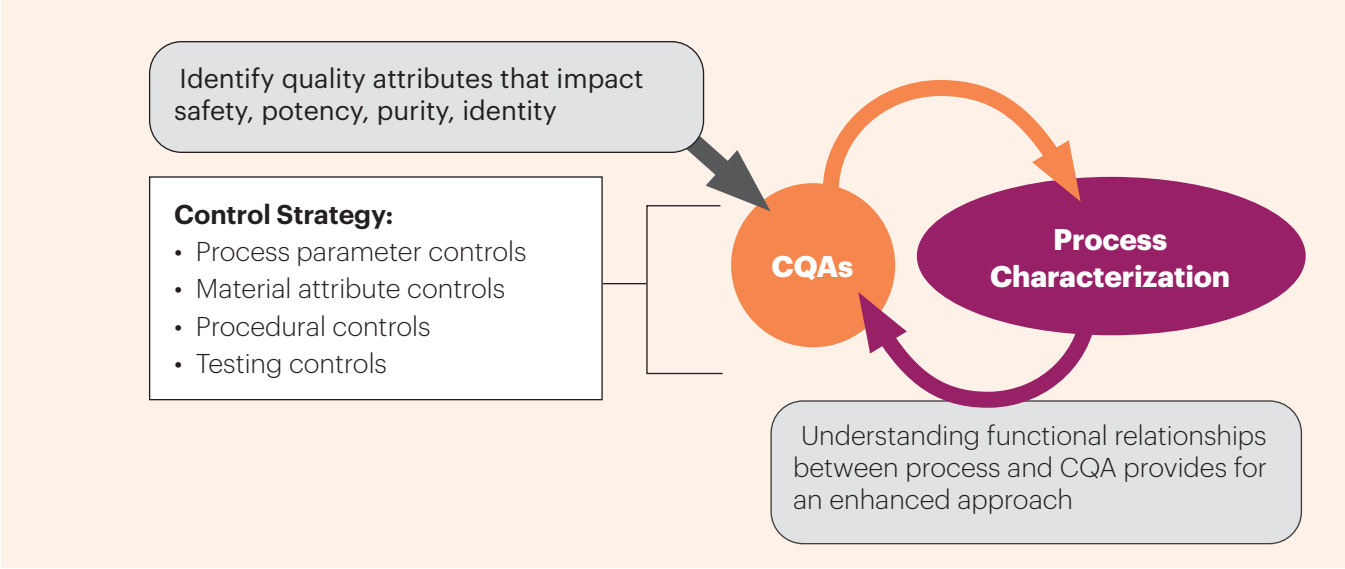
Definition of a Critical Quality Attribute

Cell therapy product manufacturers must ensure that the quality of their products are acceptable with respect to patient safety and efficacy (depending on stage of development) and are consistent with regulatory requirements for the drug product (DP) and drug substance (DS). Critical quality attributes (CQAs) define the desired product quality of the manufactured product and are defined in International Conference on Harmonization (ICH) Guideline Q8(R2) as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.[1]

CQAs are related to resulting patient safety and product efficacy and are therefore used by manufacturers to assure suitability of each product batch for use. CQAs are important to generate clinical data, demonstrate lot-to-lot consistency and in-process attributes, determine relationships between product quality attributes and safety and efficacy, support meaningful specifications for release, and show product comparability after manufacturing process changes. Developing a good understanding of the inter-relationships between CQAs and clinical outcomes and between CQAs and critical process parameters (CPPs) is fundamental to the implementation of an effective control strategy. Ideally this will result in predictable process performance, lot to lot consistency, and knowledge of the potential impact of any planned changes.

As discussed in Chapter 3, CQAs are defined during the drug development process. Initially, a quality target product profile (QTPP) is drafted that describes the design criteria for the product, and from this an initial

Figure 4-1: *CQA considerations during development*



list of quality attributes is defined. Quality attributes are identified and scored in relation to their impact on efficacy and/or patient safety. This is typically performed by a cross-functional team through a risk assessment to define and rank the quality attributes as critical (CQA), potential (pCQA), or non-critical (non-CQA). Risk assessments are also used to link material attributes and process parameters to the drug product CQA. Along with process characterization studies, the accumulated knowledge can be used to identify critical process parameters (CPPs), define an initial control strategy, and confirm its suitability through Process Performance Qualification (PPQ).

Understanding product CQAs is perhaps the most critical aspect for establishing a suitable manufacturing process as well as establishing process controls for assuring product quality and consistency. CQAs serve as benchmarks to enable the selection of operational ranges. Most importantly, control of CQAs is used to ensure that every batch of final product is as safe and effective as possible.

Figure 4-1 summarizes the key considerations related to defining CQAs with respect to the overall development process. To define CQAs, first all quality attributes that could affect safety, potency, purity, and identity are identified. It is important to establish these early in development as this is critical to managing changes during development. For example, it is very common to start a manufacturing process with a research-based process up to Phase I, and upon realizing the need for scaling, transition to a different manufacturing platform. As CQAs relate to patient safety and efficacy, detrimental impacts to CQAs can predict a detrimental impact to clinical outcomes. Changes to CQAs could also result in the conclusion that batches of product post-change are not comparable to batches pre-change and therefore the inability to group clinical data. Therefore, it is highly advisable to create the QTPP and initially identify CQAs prior to first-in-human studies, and to test these CQAs from an early stage of clinical development.

Various risk-based approaches are used throughout the manufacturing process to establish processing operations and define the control of CQAs, the control of raw materials (including critical material attributes), and the areas for further investigation. Risk assessments are performed using scientific understanding and experience and product knowledge to identify areas that pose a risk of failure to deliver a quality product that satisfies the QTPP. Risk assessment will inform a prospective process characterization plan, i.e., the high-risk parameters and materials that should be investigated in process characterization. Process parameters are defined as critical or non-critical based on study data and additional operational information. These will then be followed by an initial control strategy derived from current product and process understanding, which includes process parameter controls, material attribute controls, procedural controls, and testing controls, as discussed in Chapter 10.

DEFINITION OF A QUALITY ATTRIBUTE

A quality attribute is a distinctive characteristic that relates to the safety, identity, purity, potency, or stability of a product. Refer to Chapter 3 for a discussion about these product attributes.

Cell-based therapy products are generally more complex than biopharmaceutical products, and therefore, potentially have more quality attributes that must be controlled to minimize their potential impact on safety and assure efficacy. It is not feasible to perform a comprehensive assessment of all possible attributes; therefore, selecting the right attributes to control during manufacturing is of paramount importance to developing a viable and effective control strategy.[2] To define which attributes are critical and necessary to the control strategy, all relevant quality attributes should be considered and ranked during risk assessment. The identification of a CQA does not indicate that a test will be performed to assess that CQA. Some CQAs may either be highly challenging to test analytically and therefore only feasible to study using non-clinical and/or clinical studies (e.g., stem cell engraftment), or it may be unnecessary to test the CQA since the manufacturing process design will always control the CQA. Manufacturers must grade their product CQAs by ranking the attributes that have the most influence on the safety and efficacy of the product first.

TYPES OF QUALITY ATTRIBUTES

The QTPP serves as the starting point for establishing CQAs. Manufacturers start by grouping product attributes into categories based on classification (e.g., appearance, identity, safety, strength, purity, potency) to generate a comprehensive list of quality attributes. CQAs are distinct from tests; there may be tests that are capable of assessing multiple CQAs, and conversely, multiple tests may be required for a single CQA. However, the name for the CQA and test may be interchangeable, e.g., sterility, bacterial endotoxins.

With the variety of DPs, each with their unique characteristics, it is not easy to provide a simple solution for defining and measuring quality attribute that will fit all cases. Refer to Chapter 9 for more information on some of the tools available to measure quality attributes for cell-based therapy products.

DEFINING MEASURABLE QUALITY ATTRIBUTES

Identification of CQAs is a multidisciplinary effort utilizing all available sources of relevant information, including scientific publications and pre-clinical and clinical data, in conjunction with formal risk assessments.[2]

Identifying potential CQAs is an integral part of manufacturing process development. With progressive clinical development and correlational analysis of quality attributes, measures of product safety and efficacy ultimately provide evidence as to whether a product attribute affects the clinical outcome.[2] Due to the complexity of cell and gene therapy and the low patient numbers for some rare diseases, this might only be achieved during commercial use of a product; therefore, use of a risk-based analysis is recommended to identify CQAs.[2] Analyzing the criticality of the quality attributes early in the development cycle will enable the development of controlled manufacturing processes, defining the control strategy for clinical manufacturing, and testing for process development and comparability studies.[2]

It is recommended to have the QTPP defined so its content can serve as the basis for establishing potential CQAs.[2] As a starting point for a systematic approach, product attributes are grouped into categories based on classification. A comprehensive list of quality attributes should be generated.[2] Examples for these categories for cell therapies and genetically modified cell-based therapy products are presented in Table 4-1.

After grouping, each quality attribute (e.g., percent cell viability, percentage of cells with the desired phenotype, residual cell culture media components such as bovine serum albumin [BSA]) should be assessed for its risk (including severity and uncertainty) to product quality, patient safety, and intended mode of action (efficacy). This assessment should be supported by reliable scientific data. If experimental data is unavailable or unreliable, coherent scientific reasoning that addresses the potential impact of a given attribute (i.e., examining worst-case outcomes) must be provided.[2]

Table 4-1: *Example attribute categories for cell-based therapy products*

Category	Attributes for cell therapy product	Additional attributes for genetically modified cell-based product
Safety	Bacterial endotoxins, sterility, mycoplasma, adventitious viruses	Replication competent lentivirus (RCL), vector copy number (VCN), off-target edits
Identity	Expression of surface markers specific to the intended cell phenotype (e.g., T-cell marker CD3+)	Confirmation of genetic modification (e.g., CAR expression)
Purity and impurities	Cellular impurities, dead cells/cell viability, process-related impurities (e.g., residual media components, DMSO, anticoagulant)	% untransduced cells, residual vector
Potency	Functional response, biological activity	
General attributes	pH, osmolality	
Appearance	Color, opalescence, visible foreign particles	
Content (Quantity, strength)	Total cell number, cell concentration, viability	Quantity of genetically modified cells

Risk Assessment as Basis of Criticality Determination

Risk assessments serve as the basis for defining controls and developing a strategy for managing risk. Assessing risk follows a systematic approach to support risk decisions using accurate, analyzable, and well-organized information. Whether part of a formal risk management strategy or not, risk assessments are also integral to an organization, because they help increase the scope and accuracy of its institutional knowledge of the risks and hazards within its own processes, systems, and operational business models. Organizations should maintain formal documentation describing their quality risk management processes at the level determined through their risk assessment.

Managing risk as it relates to quality is generally based on a structured set of processes that allow manufacturers to assess and control risks throughout the life cycle of a product.

The first step to assessing risk involves identifying all possible points of failure that exist in the current manufacturing process. Manufacturers should use all factors available to them, such as historical data, theoretical analysis, and the informed opinions of experts.

After identifying possible sources of risk, manufacturers must perform a risk analysis using qualitative and quantitative methods to estimate the amount of risk due to each source. Associations can be established for the probability that a harmful event occurs, the severity of said event, and the ease of detecting the harmful event. If data are scarce or if the project is in early or preclinical stage, efficacy and safety risk can be identified based mainly on severity/detectability only. In these cases, it is recommended to add an uncertainty rating as these risks may be theoretical.

The final step to assessing risk is to perform a risk evaluation where the results of the identification and analysis steps are compared, and proper context is added to each risk with respect to the complete manufacturing process.

Risk assessment is an ever-evolving process that starts with bench research, develops through the clinical stages, continues through product approval, and finishes with commercial manufacturing. With a wide array of entities involved throughout the risk assessment process, ongoing communication related to the existence, probability, severity, acceptability, and detectability of risks with others in the industry and regulators is vital.

DEFINITION OF RISK

In cellular therapy, as in other biopharmaceutical manufacturing contexts, patient safety is a primary concern. Risk is the metric used to assess the potential amount of danger a situation, variable, or product may pose to a patient, with a focus on safety as a chief concern. The goal of risk assessment is to minimize the potential for harm to patients while maximizing the therapeutic benefits. It should be noted that some safety attributes of the cell-based therapy such as sterility, endotoxin, and mycoplasma can be considered obligatory and therefore do not need to be risk assessed. Efficacy risks must also be considered, although their severity is often lower than that of safety risks.

Attributing risk to any given situation may not be straightforward when factoring in business- and operations-related matters. One such factor is the multitude of stakeholders involved in decisions that shape the manufacturing program development, as each stakeholder bringing their own subjectivity can lead to varying degrees of uncertainty and severity. These diverse influences may result in inaccurate or disproportionate risk estimates, potentially exposing patients to side effects or ineffective therapies.

TOOLS FOR RISK ASSESSMENT

Risk assessment for identification of CQAs follows the principles outlined in ICH Q9, which defines risk analysis as "the qualitative or quantitative process of linking the likelihood of occurrence and severity of harms." It is often unclear whether an attribute listed in the early/pre-IND QTPP is critical; risk assessment can inform whether data generation is needed to determine that criticality. Several scoring methods can be used to assess the criticality of a particular attribute.

Risk ranking

Criticality assessments can utilize qualitative or semi-quantitative measurements. A quantitative risk assessment requires describing and ranking the risk. Several scoring scales exist to assess the criticality of a particular attribute. For products where no previous knowledge or understanding is available, a simple system can be implemented.

Severity x Uncertainty scoring

Severity refers to how significantly the attribute would impact product safety or efficacy. Severity scoring is particularly helpful in situations where the range of risks and the potential consequences to be managed are diverse and difficult to compare using other methods. Severity scoring has the advantage of allowing for both quantitative and qualitative assessment of risks within the same organizational framework. If severity scoring is properly applied at key points throughout the product life cycle, starting at the pre-IND phase and through to licensure and post-approval, developers will be able to identify the attributes that pose the highest levels of risk, and therefore will be able to implement effective precautionary measures and

Table 4-2: *Example criteria for impact (severity) scoring of product attributes*[2]

Severity	Value	Impact
Low	1	Variability in attribute has minor or negligible potential for decreased safety/efficacy. Marginal patient impact or minor transient adverse effects are expected based on historical experience.
Medium	3	Variability in attribute may have moderate potential for decreased safety or efficacy within the clinical history of the product. Attribute may result in manageable adverse effect, but significant patient impact is improbable.
High	10	Variability in attribute may have potential for severe effect on patient. Potential significant change in safety/efficacy or risk/benefit profiles. May result in a serious (reversible or irreversible) adverse effect.

Table 4-3: *Example criteria for uncertainty scoring of product attributes*

Uncertainty	Value	Prior Knowledge	Pre-Clinical Studies	Clinical Studies
Low	1	Well characterized effect based on extensive in-house data (*in vitro*, *in vivo*, or clinical). Large body of knowledge in the literature for a similar class of products.	Demonstrated relevance of animal model results. Extensive *in vitro* and *in vivo* studies for this product.	Significant clinical experience with this product.
Medium	2	External published literature available on similar class of products. Well characterized effect known. Some available internal data (*in vitro*, *in vivo*, or clinical) from this or similar class products.	Only limited *in vitro* or *in vivo* data available for this product.	Only limited clinical experience with this product.
High	3	Limited scientific understanding, no published external scientific literature and no internal data from this or similar class products.	No data available for this product.	No data available for this product.

mitigation strategies. Descriptions used to define severity are shown in Table 4-2. Severity may be scored as low (1), medium (3), or high (10).

Uncertainty refers to the level of confidence in assessing the criticality of an attribute. As cell and gene therapy products (CGTPs) are a novel class of therapy, and the literature and in-house experience of the sponsors are consequently small when compared to the large body of knowledge available for pharmaceutical proteins, uncertainty levels are expected to trend higher.[2] Uncertainty may be scored as low, medium, or high. Those attributes for which there is limited knowledge (i.e., high uncertainty) should be the subject of characterization studies or a relevant clinical study.[2] Descriptions used to define uncertainty as it relates to the level of confidence in understanding an attribute are shown in Table 4-3.

After assigning severity and uncertainty scores, the two are multiplied together to assign an overall attribute criticality, or risk score. Table 4-4 shows an example CQA classification matrix. Note that there are multiple ways to assign an attribute as critical versus potentially critical based on this example matrix. Risk must be determined by the organization, depending on the level of risk it is willing to accept.[2]

The resulting list of attributes classified as CQAs and pCQAs (potential CQAs) is used during early development and reviewed and revised periodically during accumulation of the product knowledge based on the availability of clinical and product characterization studies across the life cycle of the product.[2]

Preliminary hazards assessment (PHA)

As defined by ICH Q9, the preliminary hazards assessment tool is a risk assessment approach based on applying prior experience or knowledge of a hazard or failure to identify future hazards, hazardous situations, and events that might

Table 4-4: *Example product attribute criticality assessment outcome*

		Uncertainty		
		1 (Low)	**2 (Medium)**	**3 (High)**
Severity	**1 (Low)**	1 (non CQA)	2 (non CQA)	3 (pCQA)
	3 (Medium)	3 (pCQA)	6 (pCQA)	9 (pCQA)
	10 (High)	10 (CQA)	20 (CQA)	30 (CQA)

Table adapted from PDA Technical Report No. 81; Cell-Based Therapy Control Strategy, with permission of PDA, Inc.

cause harm, as well as to estimate their probability of occurrence for a given activity, facility, product, or system.[3] The PHA consists of: 1) identifying the possibility that the risk event will happen, 2) the qualitative evaluation of the extent of possible injury or damage to health, 3) a relative ranking of the hazard using a combination of severity and likelihood of occurrence, and 4) the identification of possible remedial measures.

Similar to risk ranking, PHA is based in part on severity, but unlike risk ranking, PHA uses likelihood as the other parameter instead of uncertainty. Likelihood refers to the probability that, should a quality attribute stray outside of accepted ranges based on the most recent understanding and knowledge about the attribute (drawn from literature, clinical, and non-clinical studies relevant to the product in question or similar products), the occurrence will affect the safety or efficacy of the product. When limited clinical data are available for a particular quality attribute, likelihood is to be assessed conservatively. In a similar way to how risk ranking scores are determined, the risk priority number (RPN) of PHA is calculated as Severity x Likelihood. The direness of the risk posed by the attribute in question can then be determined based on its relative placement along the priority scale compared to the other attributes being assessed. Because the assessment of likelihood depends on prior knowledge, PHA is particularly useful when performing risk assessment in existing systems.

PHA is most commonly used early in the development of a project, at a time when there is little information on design details or operating procedures. Thus, results of PHA can inform process and facility design, as well as serve as a pointer for further study of quality attributes using other risk management tools.

Other risk assessment tools are available, such as the Failure Mode and Effects Analysis (FMEA) as referred to in ICH Q9. FMEA provides a method to evaluate potential failure modes for processes and their likely effect on outcomes or product performance and is calculated as Severity × Occurrence × Detection. Since it requires scoring for occurrence and detectability, FMEA relies on product and process understanding and is typically performed in later stages of development (e.g., during the control strategy stage) where data is available to predict occurrence and detectability. FMEA can be applied to equipment and facilities and might be used to analyze a manufacturing unit operation and its effect on product or process. It identifies elements/operations within the system that render it vulnerable. The output/results of FMEA can be used as a basis for design or further analysis or to guide resource deployment. PDA Technical Reports Nos. 44, 54, 54-4, and 60, which discuss quality risk management and product life cycle management, also offer examples of suitable risk assessment tools and explain their use.[2]

EXAMPLES OF UNCERTAINTY AND SEVERITY DETERMINATION BASED ON PRIOR KNOWLEDGE

Cell extrinsic expansion rate

For autologous CAR-T cells, each batch corresponds to a different donor, and is therefore characterized with cell growth rate that is patient-dependent and may greatly vary between batches. Cell expansion rates will ultimately influence the cell yield and viability of the final product, both being CQAs. The quantity (dose) of active substance (CAR-T cells) needed for a positive clinical outcome and to minimize risks to patient safety will require an understanding of multiple factors including the mechanisms of action and intended patient population.

To achieve a target cell number and meet the dose size requirements, different cell seeding densities may be implemented during the CAR-T therapy manufacturing process. However, there may be an associated high level of uncertainty due to limited prior knowledge of the correlation between various cell culture parameters that may influence cell expansion rate and the actual cell growth. Given this uncertainty, setting too tight of a product specification on final cell yield and viability poses a risk of the product not meeting the specification.[4]

The decision on seeding density may rely on prior knowledge on cell size, cell composition (% of each cell type), and patient health and prior therapy.[5] Developing prior knowledge linking culture conditions to cell type behavior in culture is key to developing process controls and predictability for the CQAs.[6]

Residual beads process impurities

During the manufacture of some cell-based therapies, such as CAR-T cells, reagent-coated beads are commonly used (refer to Chapter 8 for details on the use of beads in the activation step of CAR-T cell manufacturing). These

beads are considered ancillary materials used in the cell production process. Thus, sufficient bead removal or reduction has to be demonstrated prior to administration of the final product to patients. Residual beads could elicit a pharmacological or toxicological effect in the patient, e.g., due to unwanted T-cell activation *in vivo,*[7] thus posing a risk to patient safety. Therefore, limits are often set for residual bead counts as part of the control strategy.[8]

While there are no standard acceptance criteria for residual bead impurity in the final DP due to the various types of beads (and reagents), broad range of indications, and method of delivery, studies for CAR-T production typically report >5-log depletion of beads throughout the manufacturing process to establish proof of safety.[9,10] To determine the criticality of residual bead impurity to the product CQA, an uncertainty score of 2 can be assigned due to the presence of some data in the literature. Due to reported acute toxicity of residual beads in intravenous safety studies in animal models,[11] a severity scoring of 10 can be assigned.

Media supplement impurities

Effective cell therapies are dependent on optimal cell culture conditions, including the culture media selection. Choosing an appropriate medium that is conducive to the activation and expansion of T cells, as well as transduction with the CAR construct, is key to maintaining cell health and producing efficacious CAR-T products. Traditionally, media supplemented with serum (from animal or human origin) are widely used in the manufacturing process, but these supplements come with a range of challenges. Serum can vary in quality (critical material attributes) between batches due to its complex composition of a large number of constituents, and possibly result in inconsistency of the final CAR-T product efficacy.[12] In some cases, high concentrations of serum have been shown to inhibit cell growth.[13] Additionally, components of serum may also introduce adventitious agents into the production process, inhibit genetic modification such as transduction, resulting in adverse effects in the patient such as hypersensitivity reactions.

In CAR-T therapies, it has been shown that infusion of CAR T cells with less differentiated phenotypes (e.g., naive-like or central memory T-cells) is associated with prolonged *in vivo* persistence and superior anti-tumor effects, at least for liquid tumors.[14,15,16,17,18] Several studies have indicated that such anti-tumor function is dependent on the serum composition in the media. In one study where three media supplements were evaluated, i.e., fetal bovine serum (FBS), human AB serum (ABS), and human platelet lysate (hPL), CAR-T cells cultured in hPL-supplemented media exhibited the least differentiated T cell phenotype and displayed superior proliferative and anti-tumor effects *in vitro* and in a mouse xenograft model.[19] Another study compared human serum to a xeno-free concentrated growth factor from whole blood fractions, and reported that cells expanded in the latter showed enhancement of transduction in activated T cells, as well as superior engraftment in a neuroblastoma model and potency *in vivo.*[11]

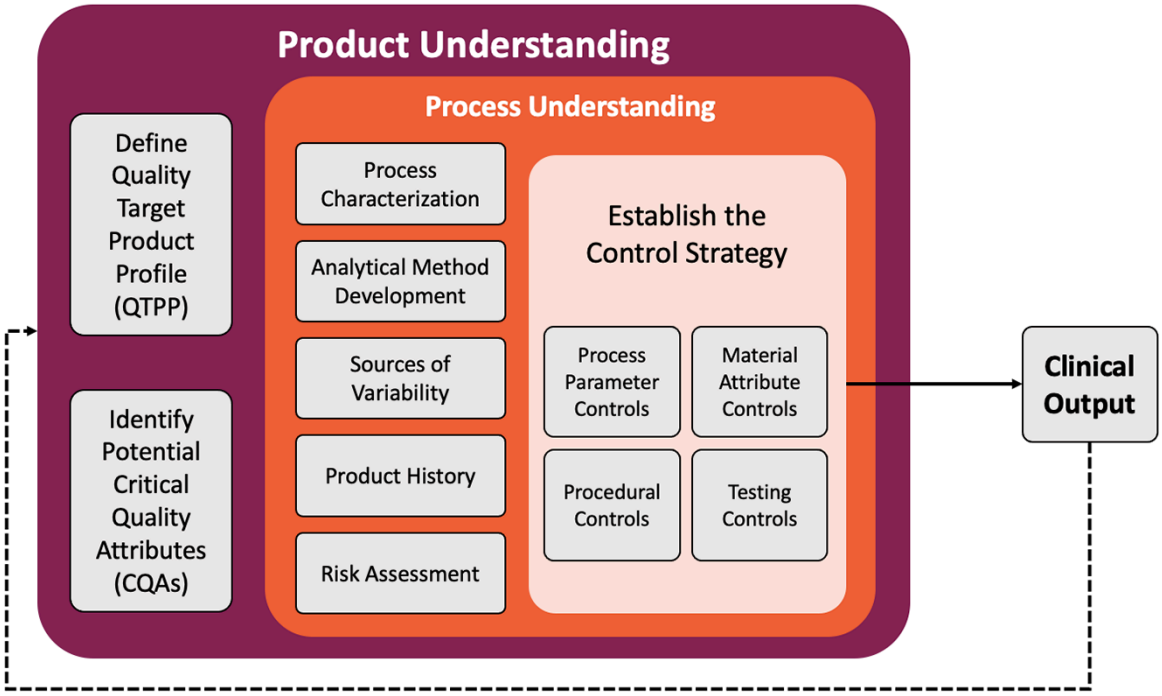
These studies highlight the potential criticality of serum concentration in culture media on overall potency of CAR-T therapies. In this example, due to the presence of some data in the literature, an uncertainly score of 2 can be assigned, and due to the potential risk to patient safety from serum impurities, a severity scoring of 10 can be assigned. If the potency assay and metric used in these published studies are not relevant to the product in development, additional data can be generated to properly evaluate the effect of serum concentration on the intended potency using a Design of Experiment (DOE) approach.

CQA Life Cycle Management

It is critical to establish the product CQAs as early as possible since this is the foundation for a rational, risk-based approach to product development and product life cycle management. Early determination of CQAs allows early data collection. This approach should provide knowledge and experience for product understanding and the ability to adopt a risk-based approach to planned and unplanned changes (comparability assessments) essential for efficient life cycle management. The expectations of regulatory agencies for the content of clinical trial applications, marketing applications, and post-submission changes are consistent with this understanding of risk.

CQAs are dynamic and should be continuously reevaluated and revised throughout development,[20] as driven

Figure 4-2: *Systematic and iterative approach to identifying clinically relevant CQAs*



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by increased product knowledge. The cycle of knowledge starts with identifying potential critical quality attributes (pCQAs) and subsequently defining these as either critical or non-critical as experience is gained. A pCQA that was initially not proven to be critical can later be reevaluated based on further information or drug product experience. If found to not have any direct impact on the quality of the final drug product, these can be dropped out during development. This is also pertinent to the re-assignment of criticality to process parameters.

PHASE APPROPRIATE CQA DETERMINATION

Throughout development, knowledge of what is a CQA will evolve based on development history and increased product understanding as more robust characterization is implemented. The FDA recognizes that identifying product-specific and clinically relevant CQAs can be challenging. Accordingly, the agency encourages a systematic approach involving several steps starting with the identification of several candidate CQAs for each product and the development of qualified assays to measure such candidate CQAs.

Achieving confidence in CQA measurement is critical, as CQAs are affected by the robustness of the analytical method that measures them.[21,22] It is important to note that not every CQA will have a qualified method; some CQAs can be assessed within early phase with minimally developed methods or feasibility testing. The knowledge gathered during the product development cycle forms a scientific basis for establishing meaningful specifications.

In addition, there should be a systematic approach to correlate potential CQAs (pCQAs) with product quality. Clinical outcomes form the basis for establishing which pCQAs are in fact CQAs.

Figure 4-2 shows a holistic integrated strategy to identifying CQAs through product understanding, process understanding, and control strategy. This highly systematic and iterative process allows the identification of pCQAs by addressing all essential parts of the manufacturing process. Development phases will refine pCQAs based on increased product and process characterization.[23] For attributes identified during the pre-clinical phase (prior to first-in-human studies and platform knowledge), *in vitro* metrics are often found to be not predictive of *in vivo* activity. Further pCQA

characterization will be guided and influenced by clinical data as they become available, by long-term safety profiles as they become established, and by increased understanding of patient heterogeneity and variability. This further characterization will confirm the true criticality of various quality attributes and allow refinement of pCQAs. Attributes that are found to correlate with clinical outcomes will become confirmed CQAs.[24]

CORRELATION OF CQAS TO CLINICAL OUTCOMES

To explore potential associations between quality attributes (e.g., purity, potency) and clinical outcomes (e.g., adverse reactions, efficacy), correlative analysis is often used. By using statistical methods, subject-matter reviews, and advanced analytics infrastructures, these analyses can inform the true criticality of quality attributes as well as defining targets and ranges for manufacture and batch release. Correlative analyses also facilitate a better understanding of how variation within quality attributes impact both product performance and clinical outcome. The result may enable streamlining the manufacturing process by pointing out where flexibility lies in the process or the product.

Examples of clinical factors and CQAs examined in correlative analysis for CAR-T cell products are presented in Chapter 10, where numerous genotypic, phenotypic, and functional CQAs and patient-specific characteristics can be evaluated for their relationship to clinical outcomes of interest. This chapter also covers the use of correlative relationships to set or widen acceptance criteria for certain CQAs based on the impact of variability within the particular CQA, e.g., a suitable acceptance criteria for cell viability, since it has been shown that cell viability below the lower limit originally defined did not compromise the clinical safety or efficacy of tisagenlecleucel in patients with acute lymphoblastic leukemia, or non-Hodgkin lymphoma.[25] This approach uses knowledge from clinical outcome to revise the control strategy to focus on preventing the use of batches that would be predicted to result in suboptimal efficacy and/or a detrimental impact to patient safety. Additionally, this practice can minimize delays in drug product release and subsequently, delivery to patients due to unnecessarily stringent specifications.

RISK ASSESSMENT THROUGHOUT THE PRODUCT LIFE CYCLE

The progression from the initial QTPP to the commercial product includes an iterative learning process, where the initial envisioned cell-based therapy product often undergoes substantial revision and evolution as it translates from the scientific bench to the patient. Ideally, product and process changes will be deliberate and planned. Changes made to the manufacturing process may potentially impact the product's critical characteristics (CQAs and pCQAs) and therefore clinical outcomes. Failure to detect the potential impact of these changes during late-phase clinical trials or post-approval could potentially affect product quality, effectiveness, and ultimately, commercial success of the product. It is therefore important to reevaluate risks associated with the process changes throughout the product life cycle.

While manufacturers are encouraged to introduce major manufacturing changes early in the process when possible, product developers often introduce major manufacturing changes late in the product development life cycle. A common example is a change in manufacturing platform as manufacturers scale up their process from small-scale flask systems to scalable suspension bioreactors when the yield of the initial manufacturing process is not sufficient to support later phase trials and predicted commercial demand. It is therefore important for manufacturers to develop a comparability plan as early as possible in product development, preferably before a Phase I trial. A comparability exercise is used to assess whether pre- and post-change drug products are comparable. In this context, comparable implies that the CQAs are highly (or sufficiently) similar such that patient safety (and efficacy if relevant) is not detrimentally impacted, and importantly, clinical data from pre- and post-change products can be considered to come from the "same product". If risk assessment indicates that one or more CQAs are likely to be adversely impacted by planned changes, then it is highly advisable to re-think these changes, and there may be limited value in a comparability study.

Risk factors that affect product comparability are dependent on the complexity of the manufacturing changes, the impact on product quality attributes, and the timing during the product development life cycle. Multiple simultaneous process changes, limited product

knowledge, and later phases of clinical trials are associated with increased risk that pre- and post-change product are either not comparable or cannot be concluded as comparable without further non-clinical or clinical data.

More details on product comparability throughout its life cycle are provided in Chapter 2, which includes discussion on potential risks, reporting requirements for changes during the IND and post-approval phases, tools for establishing comparability, and essential elements of a comparability study to support a streamlined pathway for translation of research into later-stage product development and commercialization. Comparability case studies for hypothetical CAR-T products are also presented to illustrate common scenarios and problems related to manufacturing control and comparability of pre- and post-changes in products.

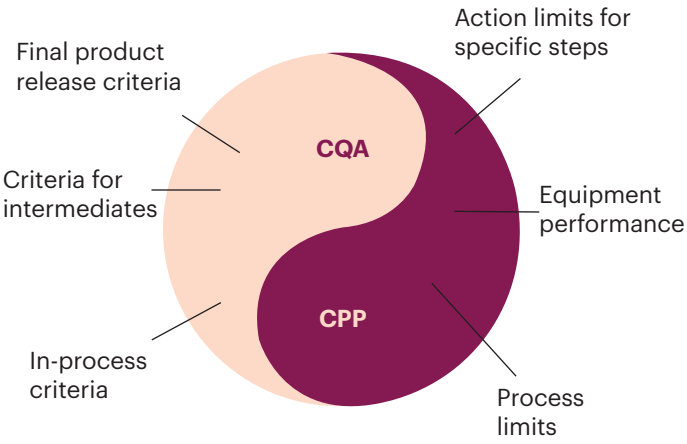
Mapping CQAs to CPPs

During product development, CQAs and CPPs are used hand-in-hand and are essential aspects of the control strategy to ensure product quality and manufacturing consistency (Figure 4-3). To identify the process parameters that must be controlled to assure the control of CQAs, product developers can work backwards from identifying: 1) what critical properties of the product need to be controlled to achieve the desired safety and potential efficacy (CQAs), 2) what process step is linked to said attribute, and 3) what parameters in that process impact or may impact the attribute (CPPs or potential CPPs). This identification exercise is followed by process characterization, identification/confirmation of CPPs and implementation of a control strategy.

Process characterization begins with process risk assessments, which can be divided into Process Attribute Matrix and Process Capability assessments. A process attribute matrix is a map containing the attributes and the unit operation the attribute is associated with. Once this is determined, process capability assessment entails evaluation of how well process parameters are controlled by comparing the performance of a process against its specifications. For example, in a CAR-T cell manufacturing process, final cell yield and viability have been determined to be CQAs since these relate to the quantity of active substance and therefore, efficacy. In the process attribute matrix, both attributes are affected by multiple steps in various unit operations, from cell collection to infusion. Example parameters within these process steps that have been found to influence the final cell number and viability include temperature during transport of the apheresis product, hold time between cell collection and start of manufacturing, volume / concentration of DMSO in the final product, and time between DMSO addition and start of cryopreservation. Quantification of the effect of these process parameters on established CQAs will drive the identification of the CPPs.

In developing a control strategy, targets and/or ranges for CPPs are defined, including: 1) normal acceptable ranges (NORs), which are the targets and intended ranges used for routine manufacture, and 2) proven acceptable ranges (PARs), which are wider than NORs and for which operation within this range should result in producing a material that meets the relevant quality criteria based on accumulated knowledge and experience. More detailed discussion on product control strategy, including further discussion on how to define NORs and PARs for a process, is provided in Chapter 10.

Figure 4-3: *Linking CPPs To CQAs are critical to ensuring product quality and manufacturing process consistency*



Adapted from FDA presentation "Early-Stage Manufacturing Considerations for Cell Therapy Products."[26]

IDENTIFYING AND SETTING ACCEPTABLE RANGES FOR CPPS

Identification of CPPs in a manufacturing process is an important step for any process development program. By performing development studies to quantify the effects of each process parameter on the CQAs, CPPs can be defined as those that strongly impact the CQAs. An example of the DOE study approach to identify CPPs for a T-cell expansion process is presented in Chapter 10. Prioritization of these characterization studies is important as it is impossible to evaluate the effect of the hundreds of interconnected parameters within a cell and gene therapy (CGT) manufacturing process. Prioritization could be based on prior knowledge from previous experience in other systems, existing product/process development experimental data, published literature, and quantifiable impacts *via* an FMEA.

Several guidelines and examples on identification of CPPs in cell-based therapy manufacturing have been published.[27,28] The next step is to set a target (NOR) and acceptable range (PAR) for each parameter based on experimental data. Process parameters have boundaries within which a given process yields an expected result that is defined in terms of CQAs. In most process steps, the process parameter acts as an input that directly affects the CQA; for example, centrifuge speed and/or duration (input) has been shown to affect cell recovery (yield) in different cell types.[29,30] Therefore, a range of speeds and time needs to be established to concentrate cells during downstream processing prior to fill-finish that will not impact the cell recovery and viability. A target value can be picked in the middle of the identified range. For example, if the study suggests that 300-500 × g centrifuge speed for 10-15 minutes can recover >85% of cells with >80% viability (i.e., the established CQA metric), a target of 400 × g for 12 minutes can be selected, with an acceptable range of 300-500 × g and 10-15 minutes. As better process understanding is gained, this range can be broadened until a failure point is hit, i.e., a centrifuge speed that results in drop in recovery or viability below the specification. Broadening the range allows higher process tolerance without changing the manufacturing process.[26]

Case Study: Risk Assessment and CQA Identification for Autologous CAR-T Cell Product

In this case study, a risk assessment is performed to determine which quality attributes are critical to guide the process characterization plan and design process validation studies. The purpose of this risk assessment is to identify and summarize the CQAs for a generic autologous CAR-T cell product introduced in Chapter 3. The ICH guideline Q8(R2) requires the identification of potential CQAs, including those related to DS, DP, and excipients, so that any characteristics that may have an impact on the desired product quality can be studied and controlled.

THE USE OF RISK ASSESSMENT TOOLS TO ESTABLISH CQAS

The risk ranking approach outlined in earlier sections is adopted for identification and assignment of CQAs. Each quality attribute is evaluated for criticality by assessing its potential impact and uncertainty as it relates to the efficacy and safety of the product. For examples of rationale for determining the uncertainty and severity scoring, refer to the earlier section titled *Examples of uncertainty and severity determination based on prior knowledge.* During stage I process validation activities, CQAs will be used to identify the CPPs for the proposed commercial manufacturing process via a risk assessment, observations from historical experience, and findings from process characterization experiments.

The identification and justification of CQAs are being performed in accordance with the Quality by Design (QbD) principles and recommendations made in ICH Q8(R2), Pharmaceutical Development and ICH Q9, Quality Risk Management, as well as the generally accepted approach described in guidance documents related to process validation. The criticality determination example presented in Table 4-5 is based on the attributes identified in the QTPP of a generic autologous CAR-T cell product introduced in Chapter 3.

Table 4-5: *Example of criticality determination using risk ranking for an autologous CAR-T product*

Quality Attribute Category	Quality Attribute	Severity Score	Uncertainty Score	Overall Ranking	Criticality
Safety	Sterility	10	1	10	CQA
	Mycoplasma	10	1	10	CQA
	Endotoxin	10	1	10	CQA
	Bacterial contamination (Gram stain)	10	1	10	CQA
	Replication competent lentivirus (RCL)	10	2	20	CQA
	Vector copy number (VCN)	10	2	20	CQA
Content / strength	Dose range (# CD3+ transduced cells per weight or XX ml / kg body weight)	10	1	10	CQA
	Cell concentration (cells/ml)	10	1	10	CQA
Identity	% CAR+ CD 3+	10	1	10	CQA
	% CD 4+ / CD 8+ ratio	3	1	3	pCQA
Purity	Residual plasmid DNA	3	1	3	pCQA
	Residual beads	10	2	20	CQA
	Residual BSA / serum	10	2	20	CQA
	Viability	3	1	3	pCQA
	% B cells	3	2	6	pCQA
	% NK cells	3	2	6	pCQA
Potency	Cytotoxicity / cell killing	10	3	30	CQA
	IFN-γ cytokine release	10	3	30	CQA

Conclusion

A prospectively planned, systematic, risk-based approach to product development is key to facilitating efficient clinical development and marketing authorization. Lessons can be learned from the development of other, more established biologics such as vaccines. The risk and criticality assessment tools presented in this chapter can be used to identify and confirm CQAs, to understand criticality of materials and process parameters, and inform a robust control strategy to provide consistent process performance and batch consistency. Risk assessment approaches allow a large pool of attributes and/or parameters to be narrowed down to a smaller number deemed important for further characterization, therefore allowing greater focus on aspects of the process that present the highest amount of risk to the overall quality of the product. An integrated and iterative strategy that incorporates product and process understanding, control strategy, and correlative analyses with clinical outcome should provide increasing CQA knowledge and experience throughout the product life cycle, leading to greater assurance of product quality.

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CHAPTER 5

Cell Collection and Starting Materials Consideration

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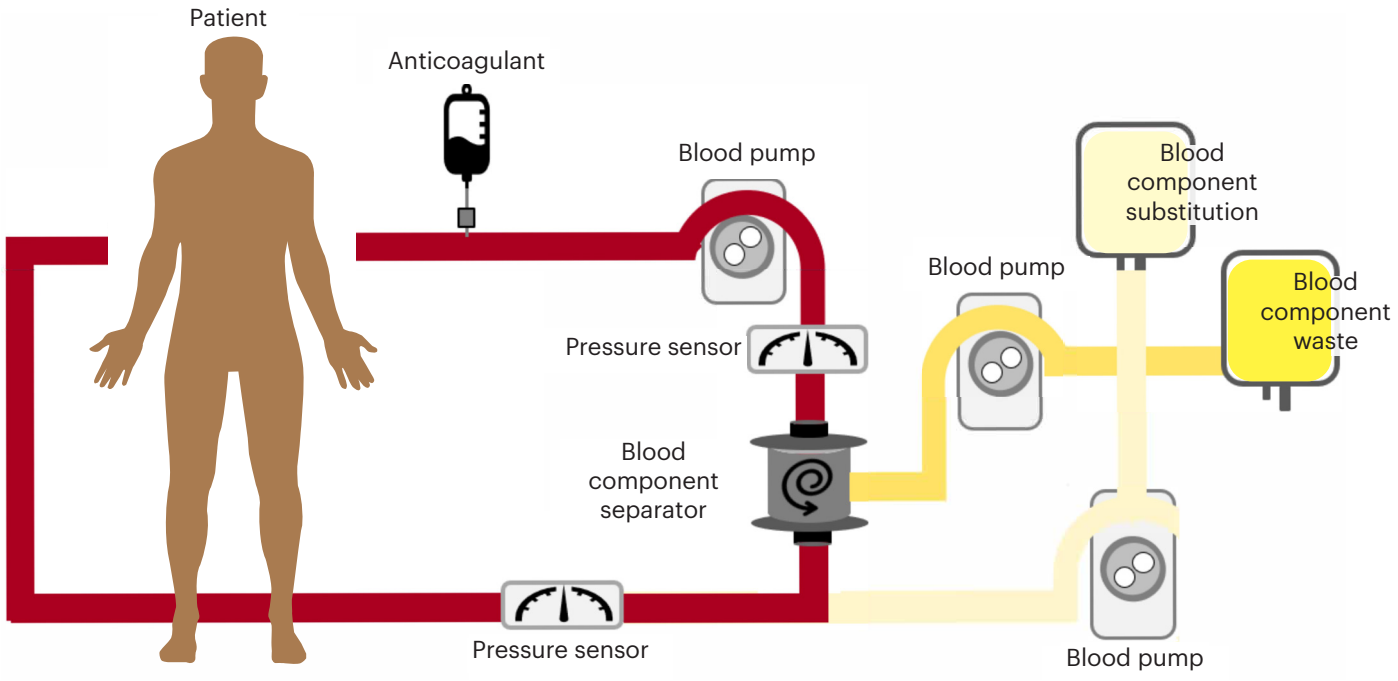
Chapter 5 | Contents

Introduction

Quality cell-based therapeutic products start with quality raw materials, whether those materials are a set of chemical or biologic reagents, or the living cells that form the basis of a cell-based therapy. The use of cell-based therapies, including CAR-T therapies, in the treatment landscape for some forms of cancer has shined a spotlight on the relatively unknown sub-specialty of apheresis, a cell collection process that lays the foundation for these therapies, whether the cellular starting material originates from the patients themselves (autologous) or healthy donors (allogeneic). Apheresis, Greek for "a taking away," is the process of separating a donor's or a patient's whole blood by centrifugation into its respective components (plasma, platelets, white cells, and red cells), and "taking away" the desired component. In the case of CAR-T therapies, the cell type of interest, i.e., white blood cells (commonly referred to as the "buffy coat" or leukopak during the apheresis process), is collected into a bag and retained, while the other blood components are returned to the patient. For autologous cell therapies, the manufacturing process begins with apheresis collection from a patient and ends with release of the therapeutic product for imminent use in that same patient. For allogeneic therapies, apheresis is performed on a donor, and the final therapeutic product is intended for off-the-shelf use in many patients.

Apheresis device selection and collection protocols can vary by application and clinical need. The process involves many variables that require detailed consideration to ensure the best possible outcome of the cell collection while minimizing negative effects on the donor, who frequently is also the intended recipient of the manufactured therapeutic. Implementation of donor eligibility criteria as well as technical patient considerations (e.g., blood volume and count, recent therapies that may depress lymphocyte count or function) ensure safe administration to patients and successful collection

Figure 5-1: *Overview of therapeutic apheresis process*



Adapted from: Asahi Kasei Medical; www.asahi-kasei.co.jp/medical/en/apheresis/product/plasma/about/cure.html

procedure, mitigati ng the risks in the therapeutic product manufacturing. In addition, the industry overall is taking steps to ensure consistent, robust apheresis processes in the clinical manufacturing context through standardization of apheresis procedures and collection targets[1] to help align cell collection requirements for product manufacturers and sponsors.

This chapter aims to cover various aspects of the apheresis process, including instrument technical operation, optimization techniques, patient preparation and monitoring, donor screening and testing, and clinic operation considerations. In many sections, the authors present considerations that are specific to one of the more commonly used apheresis systems, the Spectra Optia®.[2]

Apheresis Instrumentation Technical Considerations

The use of apheresis instrumentation for cell collections is a well-established methodology for collecting mononuclear cells (MNC) destined for curative cancer therapies. Key topics related to apheresis instrumentation include the concept of separation of blood components, extracorporeal volume, anticoagulation management, barriers to prevent contamination of the collected product, and instrument optimization techniques. A graphical overview of the apheresis process is presented in Figure 5-1.

SEPARATION OF BLOOD COMPONENTS

Most apheresis instruments used for cell collection procedures utilize continuous centrifugation methods to separate whole blood into its components based on density or specific gravity. Taking advantage of the density characteristics of whole blood cells allows the instrument to separate cells from whole blood, collect the MNC layer, and return remaining components (RBC, plasma, platelets) to the person undergoing apheresis, whether it is the patient or a donor.

When whole blood enters the apheresis instrument centrifuge it is exposed to gravitational force through centrifugal rotation that separates the whole blood into its components based on specific gravity. Red cells, being the densest cells of whole blood, collect at the bottom of the disposable kit, with the MNC layer stacking on top of the RBCs, followed by the platelets on top of the MNCs, and then the plasma floating to the top (Figure 5-2).

Separation of the cellular components from whole blood is dependent on two main variables: centrifuge speed (measured in RPMs) and the duration of the applied centrifugal force in the separation chamber. When applied to whole blood, centrifuge speed affects how tightly the cells are packed in the chamber. The faster the centrifuge speed, the more compressed the cellular portion of whole blood is (high packing factor). Conversely, the slower the centrifuge speed, the less compressed the cellular portion of whole blood is (low packing factor), as illustrated in Figure 5-3. Different packing factors are utilized based on the target blood component. Aside from these variables, the overall separation ultimately depends on the patient/donor status and vascular access options, which will be discussed in subsequent sections of this chapter.

Understanding what influences where MNCs will reside in the collection chamber allows the designers of the disposable tubing sets to engineer the placement of the collection port within the tubing set collection chamber to actively collect the target cells into a collection bag.

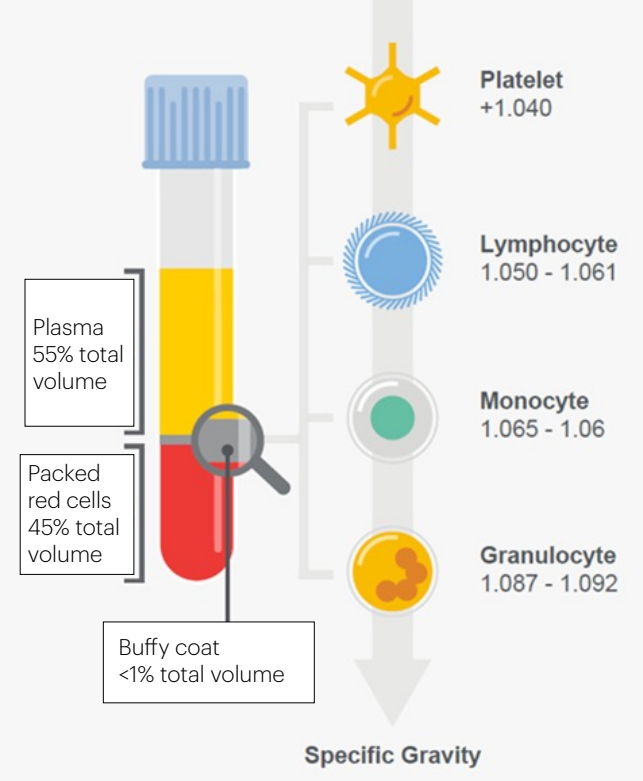
Another variable that affects the quality of cell separation, which dictates the purity and recovery of target cells in the final isolated fraction, is the rate at which the whole blood enters the centrifugal field, and thus, the rate at which the separated components exit the centrifugal field. This is referred to as dwell time. Operators of these devices have some control over how fast cells are introduced into the centrifugal field but rather limited capacity to impact large changes to the speed of the centrifuge. Whole blood requires sufficient exposure to the gravitational force in the centrifugal field to allow for cell separation. If the whole blood does not have enough dwell time to allow for the separation to occur, the result may be a less than optimal cell collection procedure.

Another variable is vascular access, which often limits overall whole blood flow through the device. Poor vascular access can contribute to inadequate cell separation in the centrifugal field resulting in instrument malfunction up to and including loss of the collection.

EXTRACORPOREAL VOLUME

Extracorporeal volume (ECV) refers to the volume of blood that is flowing through the disposable tubing set at any given time during the apheresis procedure. ECV can have a profound effect on the person undergoing apheresis.

Figure 5-2: *Separation of blood components by centrifugation*

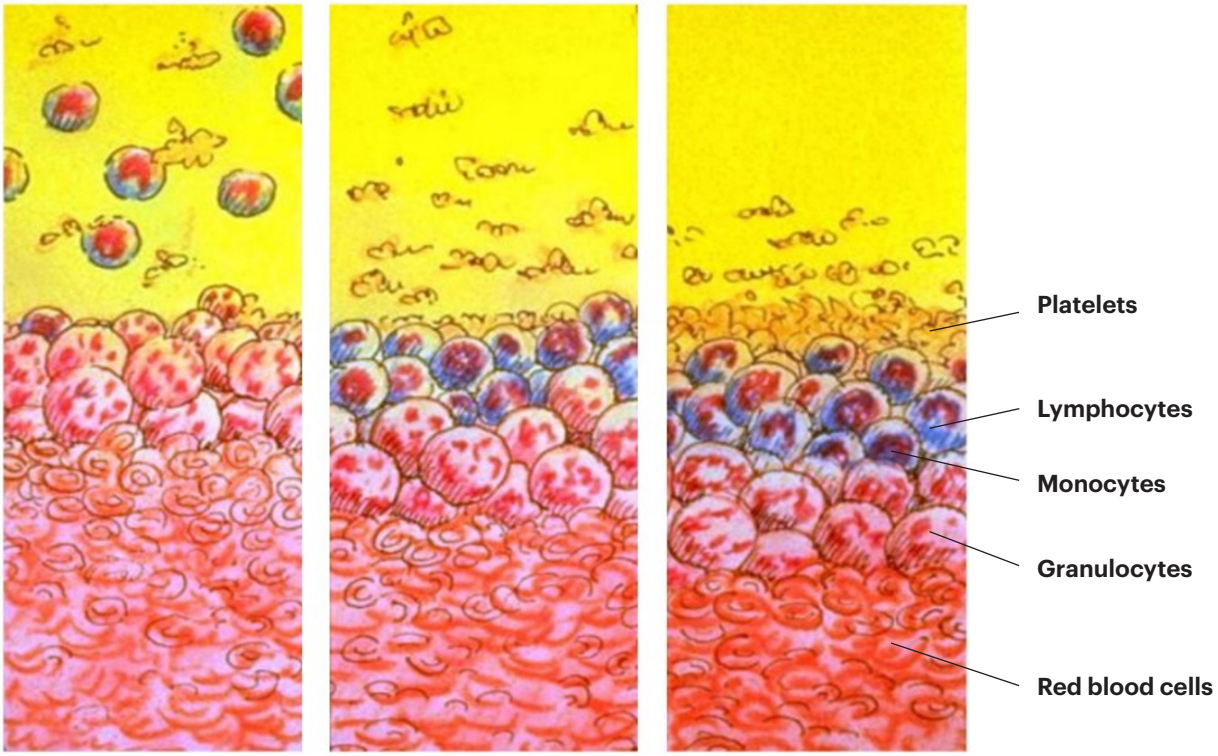


Source: Terumo Blood and Cell Technologies

A person's vasculature has a finite amount of circulating whole blood. Apheresis instruments allow for blood separation by routing whole blood through the centrifugal field via a sterile disposable kit loaded onto the instrument. During the cell collection process, a small portion of red cells are inherently retained in the disposable tubing kit. The retention of red cells in the disposable tubing set can affect the ability of the donor/patient to maintain hemodynamic stability. Prior to collection, the apheresis clinical team will need to assess the patient's total blood volume/clinical status within the defined parameters of the device's extracorporeal volume.

To ensure hemodynamic stability during the collection, it is important to know the required volume of blood in the disposable kit compared to the donor's/patient's blood volume. The ECV of the disposable kit is generally included in the manufacturer's operating manual. For most adult sized (~70kg) donors/patients with a healthy presentation, hemodynamic stability should be

Figure 5-3: *Schematic of low (left), medium (middle), and high (right) packing factor*



Adapted from "Principles of Blood Separation and Apheresis Instrumentation"[3]

maintained if the ECV is less than or equal to 15% of total blood volume (TBV). Of course, this is dependent on their individual clinical presentation. For smaller adults or pediatric subjects with low TBV, or patients with a compromised clinical condition including a low hematocrit, interventions may be required to ensure their safety during the collection. Ultimately, the clinical and apheresis teams need to evaluate how blood volume shifts, including how concomitant red cell loss will affect patients/donors based on clinical disposition, and plan for potential mitigation strategies.

Mitigation strategies

A blood prime procedure is a solution offered by the instrument manufacturers to overcome the ECV requirements of the disposable kit when clinically appropriate. Most instruments will display a warning screen to the operator if the ECV of the disposable tubing kit exceeds 10-15% of the patient's TBV and offer a recommendation to perform a blood prime of the disposable kit. A blood prime may also be necessary for anemic patients who would not be able to tolerate the volume of red cell retention in the disposable kit.

The blood prime procedure is used to offset the ECV requirement and promote patient tolerance. This procedure displaces the saline used to prime the disposable kit dead space with packed red blood cells. When the patient is attached to the instrument tubing, the packed red blood cells are delivered to the patient while their whole blood is removed into the disposable kit, thus ensuring proper volume replacement while maintaining oxygenation to promote hemodynamic stability.

ANTICOAGULATION

Effective anticoagulation of the extracorporeal circuit is a critical consideration of the apheresis procedure. Free flowing whole blood into the extracorporeal circuit vastly affects the instrument's ability to separate and collect target cells. Acid citrate dextrose formula A (ACD-A) is the most widely used anticoagulant (AC) for cell collection procedures.

The acid component of ACD-A lowers the pH of whole blood to prevent platelets from aggregating and

keeps them in suspension. The citrate component of ACD-A binds ionized calcium in the extracorporeal circuit, which prevents platelet aggregation and the activation of calcium-dependent plasma coagulation factors.[4] As a consequence of citrate delivery to the extracorporeal circuit, the patient will receive citrate at a prescribed rate with the components of blood that are returned to the patient. Citrate reactions constitute one of the top adverse events patients or donors experience from an apheresis procedure[4] and need to be monitored carefully by the apheresis team.

Citrate is actively metabolized by the liver and does not serve as an anticoagulant of the blood circulating in the patient or donor, but the patient can have a range of side effects from the citrate they receive, which can be as minor as circumoral tingling, or hand and feet tingling, to tetany reactions.[5] However, hypocalcemia can also manifest more severely as tetany, laryngospasm, cardiac conductional abnormalities, or even seizures.[4] Close monitoring of the patient or donor for symptoms of citrate reactions is critical, and the patient or donor (or family member) needs to be made aware of the signs and symptoms and given instructions about triggers for seeking medical intervention. Patients with immature organ function (e.g. liver) or at increased risk might even need cardiac monitoring.[4] As citrate reactions can progress quickly, early intervention is key.

Clinicians should consider serially monitoring electrolyte levels of the patient and could consider providing replacement infusions of calcium or magnesium to offset the binding of these electrolytes when applicable. Special considerations should also be made for patients with immature liver function or liver dysfunction to prevent citrate lock.[6] Many institutions will, at minimum, monitor electrolyte panels of the patient/donor at the beginning and end of the procedure, and these times can be adjusted if a strong reaction to citrate is suspected. Pediatric institutions may adapt a more aggressive monitoring regimen given the age and communication ability of the patients.

In the Spectra Optia system, the inlet:AC ratio is the control that ensures adequate anticoagulation of the extracorporeal circuit. The inlet:AC ratio is the amount of AC present in the extracorporeal circuit compared to the amount of whole blood. This mixture determines the concentration of AC in the extracorporeal circuit and the degree to which blood in the system is anticoagulated. It is expressed as parts of whole blood to one part of anticoagulant. For example, an inlet:AC ratio of 12:1 means there are eleven parts whole blood to one part anticoagulant. Think of this parameter as how anticoagulated the disposable circuit is. The higher the AC ratio, the more whole blood the one part of anticoagulant must prevent from coagulating, and thus the greater potential for clumping to occur. The lower the AC ratio, the less whole blood to one part of anticoagulant, and thus the less whole blood that one part of anticoagulant must contend with.

The other anticoagulant control on the Spectra Optia system is the AC infusion rate. AC infusion rate should not be confused with inlet:AC ratio. The two controls have a distinct difference for which aspect of AC management they control. Simply put, the inlet:AC ratio controls the degree that the extracorporeal circuit is anticoagulated. The AC infusion is the dose of anticoagulant the patient receives. The AC infusion rate is defined as the dose the patient receives in mL/min/LTBV (liters of total blood volume) processed.

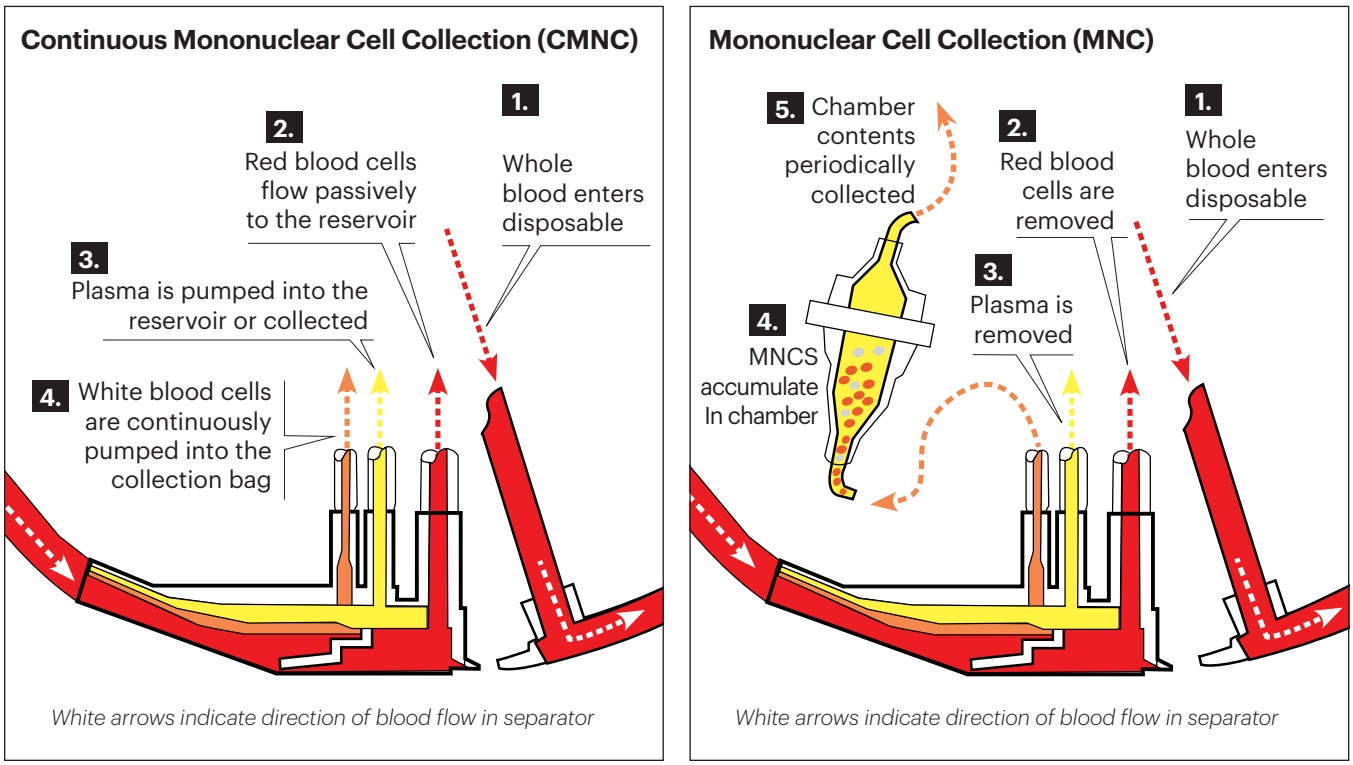
We will discuss AC infusion management in more detail in the Donor/Patient Preparation/Management section as AC infusion rate determines the delivery rate of citrate to the donor/patient, so the concept is more relevant to understand in the context of how it affects the donor/patient.

CONTAMINATION REDUCTION

Disposable tubing systems on most apheresis devices are considered functionally closed systems, which aid in maintaining the integrity and sterility of the collected cells. Manufacturers of the tubing systems have developed engineering controls to prevent the contamination of collected cell products. Some of the contamination mitigation features are described below.

- **Sterile barrier filters.** Tubing that control the flow of fluid used to prime and anticoagulate the disposable kits are equipped with filters to filter out any particulates. Sterile filters are on the final product bag for adding anticoagulant to the collected product if needed.

Figure 5-4: *Schematic of cell collection procedures*



Source: Terumo Blood and Cell Technologies

- **Sampling port.** A sample bulb system is on the final product bag to allow for aseptic samples to be obtained for product analysis.
- **Pre-attached venous access.** Some disposable kits come with a pre-attached venous access device, or sample diversion pouch, on the inlet line of the disposable kit to collect the first 15-30 mL of blood and the skin plug artifact created by the needlestick. Skin plugs are a common contamination source. The donor arm prep procedure is known to be effective, however issues may arise in frequent donors where repeated needlesticks have occurred and where the needlestick site may be difficult to clean effectively. Diverting the initial collection volume and potential skin contaminants can limit microbial access to the disposable tubing and the collection.
- **Heat sealers.** Heat sealers come with most devices to allow for aseptic sealing of the product bag once the product is ready to be transported to the cell processing lab.

Methods

Spectra Optia has two cell collection procedures available for customer use. The first, Continuous Mononuclear Cell Collection (CMNC), is a single staged cell separation procedure. Whole blood is separated by specific gravity using centrifugation and the resulting white blood cell layer is continuously pumped in mL/min to the collection bag.

The second, Mononuclear Cell Collection (MNC) is a dual staged cell collection procedure. The whole blood is separated by specific gravity first in the disposable connector, removing RBCs and plasma, then the MNC layer is routed to a collection chamber where the MNC are exposed to elutriation and separated by size. This refined MNC layer is then pumped to the product bag in batches when the system has determined the collection chamber is full of refined MNCs. The resulting cell collection efforts are not seen in the product bag until the system triggers a collection cycle. A schematic of the two collection procedures is presented in Figure 5-4.

Another important difference between the two collection protocols is the ECV required by the respective disposable tubing systems: 253-297 mL for CMNC and 147-191 mL for an MNC collection.[7] This means that the volume of blood required to be outside of the patient's body for the duration of the procedure is a key consideration and should be used to help determine a patient's tolerance to the procedure and, when appropriate, plan mitigation strategies.

There are minimal head-to-head studies comparing the CMNC and MNC collection methods for the non-mobilized donor. Of the three studies, eight data points were gathered: procedure run time, collection volume, product mononuclear collection efficiency, percentage of lymphocytes, monocytes, granulocytes and hematocrit in the product bag, and platelet loss to the patient. The only consistently replicated difference between the three studies was the significant difference in overall run time between the two procedures, with CNMC runtime being shorter.[8,9,10]

Overall, both procedures have similar outcomes of the cell types collected in the product bag. The target cell layer collection percentage was reasonably consistent between the two cell collection procedures in these three studies with CMNC showing superiority in one study regarding the percentage of lymphocytes in the product bag. Choice of protocol may be more influenced by patient tolerance (e.g., MNC collection might be preferable for well mobilized pediatric patients due to the lower ECV), operator protocol competency, and the legacy cell collection methods of the individual collection site.

OPTIMIZATION TECHNIQUES

Apheresis instruments have been developed with the operator in mind. Human factors testing is quite extensive and considered by the FDA to be an important aspect of instrument development. While these instruments monitor for conditions and situations that require operator intervention, it is always best practice that operators understand how they might tailor instrument configurations to patient conditions that may impact the ability of the instrument to collect cells efficiently.

Processing volume

Ensuring that the instrument processes adequate blood volume to obtain target cell yield is one of the most important aspects of optimizing collection procedures. The use of processing prediction algorithms adopted from site specific data is one way to take the guess work out of how much blood should be processed to obtain target cell yield.

Processing prediction algorithms can utilize pre-collection and mid-collection patient cell counts obtained from flow cytometry, whole blood processed, instrument collection efficiency, and final product cell yield to calculate the volume of blood in liters to process for future procedures. To adequately predict the processing volume and the likelihood of obtaining the cell yield within the specified processing volume, data needs to be collected from a minimum set of collection procedures. After the initial data is obtained, a modified processing prediction algorithm can be developed that can accurately predict cell yield based on the apheresis center processes. Unfortunately, limits on availability of flow cytometry at apheresis collection centers may limit use of these processing prediction algorithms.

That said, implementing this processing prediction algorithm is beneficial from the perspective of both patient/donor safety and operational cost. Apheresis procedures are relatively safe; however, the risk cannot be totally removed. Using the processing prediction algorithm to ensure the patient/donor are on the instrument for only the time needed to obtain the necessary target cell yield can potentially reduce safety risks related to patient tolerance, i.e., overcollection. Additionally, by preventing overcollection, this algorithm could impact storage capabilities in apheresis clinics. For clinics that juggle multiple types of procedures, using the processing prediction algorithm could affect their staffing models and capabilities, potentially affecting their return on investment.

Prompt response to alarms

Responding to apheresis instrument alarms is another important optimization technique. Every time an alarm condition is initiated, the pumps on the instrument are paused and the separation of cells is lost. The prevention and mitigation of potential alarm conditions become important to maintaining consistent cell separation. It is important to note that for most alarm conditions the pumps pause on the instrument. When the system restarts the pumps, it takes time to re-establish cell separation and thus decrease the time dedicated to pumping separated MNCs from the disposable tubing set to the final product

bag. This can often impact collection efficiency, and consequently product quality due to undesired blood components that can be collected during the pause, as well as lengthen the duration of the procedure. The clinical team needs to carefully evaluate pauses/delays in procedures and their impact on patient/donor safety.

For example, the top alarm received on apheresis instruments are inlet access or return access alarms. These alarm conditions are directly related to the vascular access device used for the procedure. Promptly addressing this alarm condition by flushing the access point, using repositioning techniques, or ensuring proper flow prior to attaching the vascular device to the disposable tubing, can help improve the overall efficiency of the collection procedure by minimizing pump pauses.

AC management

Monitoring for cell aggregation in the circuit is a key optimization technique to consider when conducting cell collection procedures. Monitoring the disposable tubing set for signs of platelet clumping will prevent clogging and, consequently, potential decreases in cell collection yield. Cell clumping prevents free flow of the blood and, consequently, the ability of the centrifuge to separate the cells properly is stunted.

During operation, the instrument will default to a set Inlet:AC ratio. There are times when these default values do not adequately maintain anticoagulant effects while the cells are in the plastic disposable tubing kit. When clumping is seen in the circuit, most manufacturers will instruct operators to decrease the Inlet:AC Ratio for a specific inlet volume processed to add more anticoagulant into the disposable tubing kit. In addition, an operator can re-evaluate the disposable connector or tubing after a specific inlet volume has been processed to assess whether the clump has been broken up. Operators always monitor patients or donors carefully for any side effects associated with machine adjustment.

Patient Preparation/Management

Considerations for patient preparation and procedural patient management are equally as important as technical instrument considerations. Developing strategies for preparing patients for a cell collection procedure, determining optimal timing for cell collections, vascular access needs, and monitoring the patient during the procedure are equally important and can vastly impact the collection outcome.

Patient preparation for a cell procedure and management of the patient or donor during the procedure are instrumental for a safe patient/donor-centric cell collection. Developing a process whereby the patient or donor can come see the apheresis clinic may help reduce anxiety that is experienced by patients/donors, particularly in pediatric patients. Many patients have not heard of apheresis and the instrument can be overwhelming for them. Some institutions have the patient or donor come to the apheresis clinic to be consented for the procedure. This gives apheresis physicians, nurses, or technicians an opportunity to discuss the collection risks and benefits, potential side effects, and how the donor or patient can prepare for the procedure.

Patient/donor preparation should focus on setting expectations for the procedure and equipping them with management tactics to help the procedure go more smoothly. Good information to communicate includes approximately how long they will be in the clinic for the collection, the need for proper diet and hydration the day before collection, type of anticoagulant used and potential complications, and preparation for the type of vascular access they will need to complete the procedure. Of course, this is all part of the consenting process, but meeting the collection center staff will aid the patient and donor in making a connection to mentally prepare for the procedure. Ultimately, the approach to preparing the patient for apheresis collection is multidisciplinary, involving the clinical team and apheresis team collaboratively.

AC INFUSION MANAGEMENT

The AC infusion rate, which is the rate at which the patient receives AC from the disposable circuit, is another critical concept in the Spectra Optia anticoagulation management. The AC infusion rate is the dose the patient receives in mL/min/liter of TBV processed. There is a safety range of 0.8–1.2mL/min imposed by the system. Operators can override the safety range of the instrument until they reach an ultimate hard stop range, but the instrument will alert the operator that they are operating out of the established safe ranges. This is called running in caution status.

The patient's hematocrit (HCT) is a crucial data input. In some instruments the HCT data is used to determine

the patient's red cell volume and plasma volume. These volumes feed into calculations that inform the setup of the interface in the separation connector. Based on the HCT, the device will determine the speed of the plasma pump controlling where the MNC layer resides in the connector, and thus how it aligns with the collection port on the tubing system. The white blood cell layer should align with the collection port in order to be removed from the connector to the product collection bag.

BLOOD COUNTS

The target cell count during collection varies between stem cell collections and immune effector cell collections. The target cells for a stem cell collection are the hematopoietic stem cells or CD34+ cells. These cells circulate in the peripheral blood at a low steady state, but their numbers can be increased dramatically with the use of cytokine stimulation (e.g., granulocyte colony-stimulating factor) to mobilize CD34+ cells into circulation. Additionally, plerixafor (Mozobil®, Genzyme Corporation) can also be used, most commonly for poorly mobilized autologous stem cell collections. The patient's or donor's pre-collection CD34+ cells can reasonably predict the quantity of stem cells collected. Typically, an efficient stem cell collection can be obtained if the patient's/donor's pre-collection CD34+ count is 20 cells/μL.[11] For most stem cell transplants, the minimum number of cells needed is commonly 2.0×10^6/kg, however a dose of 5×10^6 CD34+ cells/kg is associated with faster recovery of platelets and granulocytes.[12,13] Collection of peripheral blood lymphocytes or CD3+ cells for chimeric antigen receptor processing does not require cytokine or medicinal mobilization. For CAR-T cell product manufacturing, collections could target 2.0×10^9 total nucleated cells (TNC) and 1.0×10^9 CD3+ T cells.[14] An overview of the different CAR-T cell products, the varying requirements and procedures set by the manufacturers regarding leukapheresis product, apheresis, and product application are summarized in Korell *et al.*[14]

VASCULAR ACCESS

Vascular access is often a rate limiting step for apheresis procedures including both stem cell and immune effector cell collection. Adequate vascular access is key to maintaining the high venous blood flow through the apheresis device necessary for cellular collection. Blood flow through apheresis collection devices can range from 10 to 120 mL/minute and needs to be maintained potentially for several hours for efficient cellular collection procedures. It is important to know the projected inlet rate of the apheresis device so a catheter with appropriate maximum flow rate may be chosen. A flexible 22-gauge catheter can sustain maximum flow rates of up to 35 mL/minute while an 18-gauge can do 60 mL/minute, whereas a steel 17/18 gauge needle can sustain higher rates of approximately 75 mL/minute. Several factors to consider when choosing a venous access approach are: 1) type and duration of apheresis therapy; 2) the potential need for additional intravenous interventions (medications, chemotherapy, radiologic studies requiring dye injection); 3) patient age/size; and 4) diagnosis. Ultimately patient (and family) understanding, comfort, and preference are the most important considerations.

Each venous access approach has its own set of risks, benefits, and considerations. Stem cell and immune effector cell collections typically do not require long term vascular access solutions such as tunneled catheters and subcutaneous ports. Ideally peripheral access should be considered first for non-emergent, short-term procedures when patient clinical status and preference allow. Hospitals worldwide have tried to decrease reliance on central venous lines (CVLs) in healthy allogeneic blood donors undergoing peripheral blood stem cell collection. Ghazi et al. were able to decrease the rate of CVL usage for these donors at their institution from 72% to 0% over the course of a 16-month quality improvement program.[15] Autologous patients, however, represent a different population where two points of peripheral access may not be possible due to clinical status. If a central venous catheter is needed for a temporary situation like stem cell collection or immune effector cell collection, a non-tunneled line should be considered and can often be placed expediently. Care needs to be used as they do not have a cuff and are sutured in place. Length of use for temporary catheters should be less than 2 weeks due to higher infection rates as compared to tunneled lines. It is essential the patient, clinical team, and apheresis team collaboratively assess the vascular access options and chose the most effective approach.

DONOR CONSIDERATIONS FOR ALLOGENEIC CELL THERAPY PRODUCTS

Allogeneic therapies, including allogeneic CAR-T, are gaining traction due to several advantages over autologous products: immediate availability to patients, reduced cost, and more standardized product. However, this approach can be associated with safety risks to the patients, such as infection from adventitious agents in the donor blood, graft versus host disease (GvHD), and rejection of allogeneic cells, in addition to cytokine release syndrome and CAR-related gene modifications often observed with autologous products.

While a well-defined manufacturing process and a robust control and testing strategy are critical for all cell-based therapy product development, the allogeneic approach requires additional factors to ensure safe administration to patients. Donor screening and testing are critical factors that must be taken into account during the product manufacturing. It should be noted that even in an autologous setting where there are no regulatory requirements on donor eligibility-based human viral testing, contract development and manufacturing organizations (CDMOs) often require it to minimize the risk of bringing viruses into their GMP facilities. This section of the chapter discusses the principles and available guidance for suitable qualification and testing of starting material and components, donor eligibility determination, as well as appropriate product testing and characterization for human cells, tissues, and cellular and tissue-based products (HCT/Ps). Additionally, to establish histocompatibility for users of allogeneic-sourced HCT/Ps, human leukocyte antigen (HLA) testing and confirmation may be performed as part of the product identity tests. Discussion on HLA matching is presented in Chapter 2.

Adventitious agents

Adventitious agents are considered to be viruses, bacteria, fungi, mycoplasma/spiroplasma, mycobacteria, rickettsia, protozoa, parasites, and transmissible spongiform encephalopathy (TSE) agents. A major concern for developers of cell-based therapy products is the risk of contamination of cells by adventitious agents during the manufacturing process. This is reflected in 21 CFR 610.13, which states the following: "Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application." Most importantly, such contamination could result in detrimental clinical consequences for the eventual patients. Therefore, it is critical that developers establish thorough testing methods and protocols to ensure that manufacturing process-produced batches are free of adventitious agent contamination.

Possible sources for contamination include the patient that was the source of the initial agent isolate, the cell substrates involved in the manufacturing process, other raw materials used during manufacturing that were of biological origin, personnel handling, and improper maintenance of manufacturing equipment. Adventitious agent testing should utilize a combination of methods and strategies to provide assurance (to the extent possible) that products are free from adventitious agents and should be performed at various stages during the manufacturing process to maximize the chance of detecting contaminants. Tests for contaminants of cell-based products include electron microscopy, assays for retroviral reverse transcriptase, and detection of contaminants in cell culture and animal host systems. Contaminant detection *in vitro* can be achieved by observing for cytopathic effect, hemagglutination, or hemadsorption. *In vivo* assays involve the inoculation of animal species and subsequent observations for mortality and testing of tissue for the presence of hemagglutinins and production of antibodies. Polymerase chain reaction (PCR) is also a powerful tool that can be used to detect the presence of contaminant genomes. Tests should comply with the International Council for Harmonization (ICH) Q2 (R1) guideline. Further guidance is provided in FDA Guidance for Industry, *Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications.*[16]

Starting material qualification

In the November 2020 workshop on Allogeneic Adoptive Cell Therapies Donor Cell Test Requirements, held by the Alliance for Regenerative Medicine,[17] the FDA CBER recommended that starting material qualification for allogeneic CAR-T therapies include the following:

- Safety testing: sterility, mycoplasma, adventitious agents, as well as other relevant human pathogens not included in donor eligibility testing (discussed in subsequent sections);

- Established acceptance criteria, e.g., minimum cell number, % CD3+, viability;
- Additional characterization studies such as phenotypic analysis (e.g., % of CD4+ and CD8+ cells, % NK cells, % monocytes, % B cells) as these might inform the need for cell selection during process development.

Donor screening and testing

The FDA provides guidance on appropriate standards for cell donor screening and donor testing. In general, the FDA requires donor screening and testing for human cells, tissues, or cellular or tissue-based products (HCT/Ps) when source material is collected from allogeneic human donors (21 CFR 1271).[18] Donors are defined as individuals to be used as sources for HCT/Ps intended for implantation, transplantation, infusion, or transfer into a human recipient. In general, FDA guidelines for donor testing and donor screening are meant to address the following three areas:

- Limiting the risk of transmission of communicable disease from donors to recipients;
- Establishing manufacturing practices that minimize the risk of contamination;
- Requiring an appropriate demonstration of safety and effectiveness for cells and tissues that present greater risks due to their processing or use.

Donor screening

Donor screening determines whether a donor is either eligible or ineligible to donate cells or tissues to be used in HCT/Ps. According to 21 CFR, Parts 1270 and 1271, all donors of cells and tissue must be screened for the following medical conditions:

- Risk factors for, and clinical evidence of, Relevant Communicable Diseases and Disease Agents (RCDAD), including:
  - -Human immunodeficiency virus (HIV), types 1 and 2
  - -Hepatitis B virus (HBV)
  - -Hepatitis C virus (HCV)
  - -Human transmissible spongiform encephalopathy (TSE), including Creutzfeldt-Jakob disease (CJD)
  - -Treponema pallidum (syphilis)
- Communicable disease risks associated with xenotransplantation

In addition to the general requirements for donor screening above, medical records of donors of viable, leukocyte-rich cells must be screened for risk factors for and clinical evidence of relevant cell-associated communicable disease agents and diseases, including Human T-lymphotropic virus.

Donor testing

Donor testing for RCDADs must be performed as described in 21 CFR § 1271.80 and § 1271.85.[19] Testing must be performed in an FDA licensed, CLIA certified laboratory (Clinical Laboratory Improvement Amendments of 1988) or equivalent as determined by the Centers for Medicare and Medicaid Services (CMS), using appropriate FDA-licensed, approved, or cleared donor screening tests, according to the manufacturer's instructions for use (IFU). It should be noted that for diseases or disease agents where no FDA-approved tests are available, a risk-based approach should be taken to qualify the donor cells to ensure that the final cell-based product safety is not compromised.

According to 21 CFR 1271.80(b), general requirements for donor testing are as follows:

- Testing must be done for relevant communicable diseases. In the case of a donor one month of age or younger, a specimen from the birth mother must be tested instead of a specimen from the donor.
- In general, donor specimens must be collected for testing at the time of recovery of cells or tissue from the donor or up to 7 days before or after recovery. However, specimens from donors of peripheral blood stem/progenitor cells, bone marrow (if not excepted under Title 21 CFR, Parts 1271(d)(4)), or oocytes may be collected for testing up to 30 days before recovery.
- Appropriate FDA-licensed, approved, or cleared donor screening tests, in accordance with the manufacturer's instructions, must be conducted to adequately and appropriately reduce the risk of transmission of relevant communicable disease agents or diseases; however, until such time as appropriate FDA-licensed, approved, or cleared donor screening tests for Chlamydia trachomatis and for Neisseria gonorrhea are available, FDA-licensed, approved, or cleared tests labeled for the detection of those organisms in an asymptomatic, low-prevalence population must be used.

Donors must be deemed ineligible if found to be any of the following:

- A donor whose specimen tests reactive on a screening test for a communicable disease agent in accordance with §1271.85, except for a donor whose specimen tests reactive on a non-treponemal screening test for syphilis and negative on a specific treponemal confirmatory test.
- A donor in whom plasma dilution sufficient to affect the results of communicable disease testing is suspected unless: a) the specimen taken from the donor is tested before transfusion or infusion and is tested up to 7 days before recovery of cells or tissue, or b) an appropriate algorithm designed to evaluate volumes administered in the 48 hours before specimen collection is used, and the algorithm shows that plasma dilution sufficient to affect the results of communicable disease testing has not occurred.
- Clinical situations in which plasma dilution is suspected to be sufficient to affect the results of communicable disease testing including, but not limited to the following: a) blood loss is known or suspected in a donor over 12 years of age, and the donor has received a transfusion or infusion of more than 2,000 milliliters (mL) of blood (e.g., whole blood, red blood cells) or colloids within 48 hours before death or specimen collection (whichever occurred earlier) or more than 2,000 mL of crystalloids within 1 hour before death or specimen collection (whichever occurred earlier), or b) regardless of the presence or absence of blood loss, the donor is 12 years of age or younger and has received a transfusion or infusion of any amount of blood (e.g., whole blood, red blood cells) or colloids within 48 hours before death or specimen collection (whichever occurred earlier) or crystalloids within 1 hour before death or specimen collection (whichever occurred earlier).

Specimens from all donors must be tested for evidence of infection due to RCDADs listed in the Donor Screening section above. In addition to the general requirements for donor testing, testing requirements for specimens from donors of viable, leukocyte-rich cells or tissue are as follows:

- Specimens from donors of viable, leukocyte-rich cells or tissue must be tested to adequately and appropriately reduce the risk of transmission of relevant cell-associated communicable diseases, including Human T-lymphotropic virus, type I and Human T-lymphotropic virus, type II.
- Specimens from donors of viable, leukocyte-rich cells or tissue must be tested for evidence of infection due to cytomegalovirus (CMV) to adequately and appropriately reduce the risk of transmission. A standard operating procedure must be established and maintained for governing the release of an HCT/P from a donor whose specimen tests reactive for CMV.

It is important to note that donor eligibility screening and testing requirements can differ by country. For example, other countries may not use FDA licensed test kits, CLIA certified labs, or require all the nucleic acid and antibody-based testing. This is especially important in the case of allogeneic therapies that are being expanded for use by many patients.

Considerations for other transmissible agents

The COVID-19 pandemic has highlighted the necessity for some testing considerations for specific transmissible agents that are not covered in the published guidance. These can include infections that may be regionally endemic, associated with specific donor populations, or occur during a particular period of time. The FDA determines whether certain communicable disease agents and diseases are relevant based on the risk of transmission, severity of effect, and availability of appropriate screening measures or tests. For example, West Nile Virus (WNV), Sepsis, and Vaccinia, which were not specifically listed under 21 CFR 1271.3(r)(1), were determined to be relevant under 21 CFR 1271.3(r)(2).[20] Additionally, the Zika virus (ZIKV), which was recognized as a relevant transfusion-transmitted infection in August 2016 under 12 CFR Part 630, is no longer considered relevant.[21]

The general perspective is that manufacturers should perform risk assessment that identifies, evaluates, and mitigates factors that may allow for transmission of such disease agents through their product.

Additionally, when appropriate, the FDA publishes and updates disease-specific guidance for the industry. The recent COVID-19 pandemic shaped the FDA's thinking and expectations for cell and gene therapy product manufacturers as outlined in the 2021 Guidance for Industry.[22] At the time of the writing of this chapter, FDA does not recommend using laboratory tests to screen asymptomatic HCT/P donors for SARS-CoV-2[23] because SARS-CoV-2 has not been declared a relevant communicable disease agent and disease under 21 CFR 1271.3(r). However, if a manufacturer is considering incorporating donor testing for SARS-CoV-2 as a risk mitigation strategy for manufacturing a CGT product, then viral tests (molecular or antigen) approved, cleared, or authorized by the FDA should be used to diagnose current infection.

Final cell product release testing

Release testing of cell-based therapy products, including CAR-T, entails performing a series of assays that demonstrate product safety, identity, purity/impurity, quantity/strength, and potency. For detailed discussions on the development of these assays, recommended tests for autologous therapies, and challenges related to characterization for cell-based therapies, refer to Chapter 9.

In addition to the tests outlined in Chapter 9, allogeneic therapies require additional tests for adventitious virus. Similar considerations/recommendations apply to these tests, including:

- Final product must be tested using qualified tests with establish limit of detection (LOD)/limit of quantification (LOQ).
- Acceptance criteria must be set for lot release with detailed justification.
- Presence of active viral replication needs to be evaluated if virus is detected.
- Testing of incoming donor material and in-process samples are recommended to determine if there is correlation with DP test results and if there is amplification during the manufacturing process if a virus is present.
- Retain samples are recommended in case additional testing is necessary.

Perspectives on HHV Testing

In addition to the common virus testing listed in various guidelines,[24,25,26] FDA also recommends monitoring of HHV-6 and HHV-7 during the manufacturing process and in the final product. HHV is ubiquitous and latent infection in the general population is expected. Although latency is typically asymptomatic, HHV-6 reactivates in > 50% of allogeneic hematopoietic stem cell transplantation (HSCT) recipients and can produce clinically significant manifestations, including encephalitis, delayed engraftment, and an increased rate of graft-versus-host disease, substantially increasing mortality.[27,28.29] In organ transplants, horizontal transmission of HHV-6 in liver has been shown to result in a fatal outcome.[30]

In patients receiving CAR-T therapies, the risk of potential HHV reactivation is also inherent.[31,32] Unfortunately, HHV-associated encephalitis can be difficult to distinguish from neurotoxicity, a common adverse effect of CAR-T therapy. The FDA acknowledges that additional information is needed in the clinical trial setting to determine whether and to what extend the product contributed to HHV reactivation. Samples can be banked until additional information from CMC related product assays mature to initiate testing of CAR-T recipients. Considerations for appropriate testing of banked samples include next-generation sequencing (genotyping) to distinguish product-related vs. reactivation effects, and serological testing to understand the prevalence of baseline rates of latent infection, asymptomatic rising titers of Ab and qPCRs, and contribution of rising titers to clinical infection. In addition, real time assessments for rising titers and high DNA copy numbers may be needed in recipients experiencing neurotoxicity consistent with the practice of medicine to diagnose HHV infection.

Given the unknown risks from products, difficulty of differential diagnosis, and serious nature of HHV infection, the FDA currently recommends the following in regard to allogeneic CAR-T products:[17]

- Negative lots be released first;
- Positive lots be released in sequence (least to most positive) after sponsor submits preliminary efficacy and safety data in 3-5 subjects who receive negative lots;
- Protocols should mandate assessment and treatment of HHV infections with the onset of neurotoxicity symptoms.

Regulatory considerations

The regulatory guidance surrounding the identification of appropriate donors, collection of cells, and manipulation and preservation of cells varies around the world. In the U.S., the FDA mandates the requirements of not only donor selection and collection but also processing and storage. Particular to controls and quality management around cell collection, the FDA Code of Federal Regulations requires collection sites to establish and maintain procedures to control all documents required for the entire cellular therapy process. This includes identifying the appropriate individuals to review procedures for accuracy, approvals with dates/signatures, ensuring certain documents are available at the appropriate point of use, and removing obsolete procedures and procedure versions from the system. A well-established protocol for an effective document control is also critical, as this is the foundation of quality management systems.

Additional comprehensive evidence-based standards in cellular therapy for voluntary accreditation are published by the Foundation for the Accreditation of Cellular Therapy (FACT) and JACIE, the Joint Accreditation Committee of ISCT and EBMT (the European Society for Blood and Marrow Transplantation.)These standards on cellular therapy product collection, processing, and administration apply to hematopoietic progenitor cell transplantation/therapies as well as immune effector cell therapies and are accepted in many countries worldwide. Overall, it is important to review the regulatory and accreditation guidelines for the country where the cell collection process takes place. Refer to Chapter 2 for an overview of the regulatory landscape related to cell-based therapies.

Conclusions

Recent commercialization and ongoing development of cell-based therapies emphasize the criticality of high-quality starting cell material. For cell-based therapies, this starts with the apheresis process, which involves many variables that require detailed consideration to ensure the best possible outcome for the cell collection procedure and, ultimately, the patients.

This chapter covers various technical aspects of the cell collection process, clinical operation considerations including patient preparation and monitoring, as well as available regulatory guidance on donor screening and testing. As the industry evolves, efforts are constantly made on ensuring consistent, robust apheresis processes for clinical manufacturing through standardization of apheresis center training, equipment, and protocols to minimize variation and increase the quality of starting materials, as well as access to a large, diverse, and reliable donor network. Through process standardization and comprehensive quality management programs, cell therapy manufacturers can help ensure consistent quality of cellular starting material that optimizes and maintains product safety and efficacy, without sacrificing the safety and comfort of the donor and/or patient.

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CHAPTER 6

Ancillary Materials

CHAPTER LEADS

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Introduction

At the cutting edge of technology in medicine, we can find many products that involve the utilization of additional materials not intended to remain in the final consumable therapeutic. These materials are referred to as Ancillary Materials (AMs), which are defined as "material that comes into contact with the cell or tissue product during cell-processing, but is not intended to be part of the final product formulation."[1] The final therapeutic products commonly referred to in the literature include those that fall under broader categories such as advanced therapy medical products (ATMP), cell and gene therapies (CGT), and tissue engineered medicinal products (TEMP). Throughout this chapter we will commonly refer to all therapies herein mentioned as CGTs.

In the rapidly changing landscape of CGTs, a lack of common terminology leads to a lack of global regulatory oversight for AMs. For example, the European Union (EU) commonly refers to these materials as "raw materials," a term that could easily be misconstrued within this document's context. To make matters worse, CGTs typically use many unique and diverse AMs with different levels of quality control and availability (refer to Table 6-1 for common definitions of AMs). This diversity of AMs, along with their unique and complex characteristics, makes them difficult to qualify. Generally, the AM qualification process involves identification, selection, and suitability for use in manufacturing, characterization, vendor qualification, and quality assurance and control; however, it depends on many factors, including the type of CGT product being manufactured and the stage of manufacture in which the AM is used. As a result, there is no "one-size-fits-all" qualification program, and many, if not all, regulatory concerns fall within AM users and suppliers. Hence, there is a clear need to globally standardize terminology and regulations to ensure higher quality and consistent CGT products.

In this chapter, we give the reader an in-depth understanding of the landscape by defining the many nomenclatures utilized, review different regulatory bodies by country of origin, and explore specific AMs commonly used during CGT manufacturing. Where appropriate, principles and examples specific to cell-based therapies are presented. In this document, the authors follow the general definitions and principles of AMs presented in the ISO/TS 20399 standards, which exclude non-biological consumables and plasticware (e.g., tissue culture flasks, bags, tubing, pipettes, needles) and feeder cells, but include consumables that can have a biological

component (e.g., coated dishes or beads). Finally, we introduce some case studies to further strengthen the case for AM standardization, which aims to drive improvement to the consistency and quality of AMs through an appropriate level of documented lot-to-lot consistency and best practices for AM suppliers and users.

Principles of Ancillary Materials

During the manufacturing process, AMs come into contact with the cellular therapeutic product, however are not intended to be in the final product. Examples of AMs used in CGT applications include, but are not limited to:

- Monoclonal antibodies: used in cell selection/depletion
- Cytokines, growth factors, and other supplements: used to regulate/activate/differentiate cells in culture
- Antibiotics, serum culture media, and enzymes: used to passage cells
- Substrates/scaffolds (e.g., Matrigel): used for cell expansion and differentiation

These materials come in direct contact with cells destined for clinical use and require the utmost safety, potency, and purity. As a result, AM manufacturers utilize labels such as "For Research Use Only," "Laboratory Grade," "CGMP," and "GMP" amongst others in an attempt to categorize and distinguish their products with little to no global guidance. The most common nomenclature found in product labels are presented in Table 6-1.

So, the question arises, what exactly is the difference between one label and another? To answer this question, we must understand the current state of global regulation in the AM space as well as regional regulatory agencies and how they regulate or lack regulation of these imperative materials utilized in the manufacturing process of CGTs.

Table 6-1: *Definitions for commonly used nomenclature by AM manufacturers in product labels*

Term	Meaning	Alternative Terms
Animal Derived Component Free (ADCF)	A term to describe products or materials that do not contain an ingredient that is an animal tissue or body fluid, or is isolated or purified from animal tissue or body fluid. May contain recombinant proteins produced in animal cell lines or by fermentation processes. Does not necessarily limit the use of animal-derived components used in the manufacture of AM raw materials (secondary materials) or materials used further downstream unless indicated.	Animal Component Free (ACF) Animal Origin Free (AOF)
Chemically Defined	A term to describe products or materials that have known chemical structures (defined by a chemical formula) and high purity, for example, small molecules, salts, carbohydrates, amino acids, fatty acids, and steroids (cholesterol, dexamethasone). This is evaluated on a case-by-case basis. Does not contain proteins or other ingredients with a complex structure. Ingredients may be synthetic or biologically derived (e.g., cholesterol from sheep's wool grease).	ACF Serum Free
Clinical Grade	A term to describe products or materials that are suitable for clinical use, for example, injectable grade. Material shown to be safe and efficacious for human use through appropriate clinical trials and regulatory approvals. Usually, clinical-grade products are approved as drugs by regulators, and labeling or product documentation should state sterility and safety profile. Suitable for clinical use for a specific intended use only. Clinical-grade AM for a specific and approved intended use does not mean that the AM is approved for other "off-label" processing uses without qualification and approval from the appropriate regulatory agency.	Pharmacopeial-grade Infusible-grade Active pharmaceutical ingredient (API)-grade Approved for human use

The authors acknowledge that some terms listed under "Alternative Terms" are not direct synonyms of the terminology, however represent common use of these definitions in the industry.

continued on next page

Table 6-1: *Definitions for commonly used nomenclature by AM manufacturers in product labels*

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Term	Meaning	Alternative Terms
Good Manufacturing Practice (GMP)	Refers to the current GMP (CGMP) regulations requiring manufacturers, processors, and packagers to take the necessary steps to ensure that their products are traceable, safe, pure, and effective. When associated with a product or material, this term denotes that the product has been prepared under some or all of the CGMP requirements to ensure proper design, monitoring, and control of the manufacturing processes, facilities, and the final product. The CGMP regulations can be considered under a phased approach, depending on the phase and intended use of a product within a clinical application.[2] All users should review intended use statements on product documentation. Intended uses of GMP products vary and are not necessarily qualified or intended for applications outside of "research use only." Regional differences exist, where some national regulatory agencies provide GMP certificates to manufacturers of GMP AMs and others do not provide them. Term is universally recognized by regulatory authorities.	CGMP Manufactured under GMP GMP-compliant GMP-grade Compliant to 21 CFR 210, 211, 820
Laboratory Grade	A term to describe products or materials that are intended to be used within the laboratory or for research use and not intended for food, drug, or medicinal/clinical use.	Research-grade Research use only Laboratory use only Non-pharmaceutical–grade
Research Use Only (RUO)	A term to describe the intended use of materials generally limited for use in research or preclinical applications only and not for clinical trials unless qualified appropriately and approved for use in a given application by applicable regulatory authorities. RUO products may be manufactured under various quality management systems, including ISO-certified or GMP.	Laboratory use only Research-grade Non-pharmaceutical-grade Laboratory-grade
Serum-free	A term to describe products or materials that do not contain serum or plasma as an ingredient. May contain processed or derived ingredients from blood, serum, or plasma such as albumin, transferrin, low-density lipids, hormones, and platelet lysate. May contain other undefined ingredients that are not serum or plasma (e.g., tissue extracts such as bovine pituitary extract, platelet lysate, growth factors, hormones, and carrier proteins). Serum-free media (SFM) allows researchers to grow specific cell types or perform specific applications in the absence of serum.	Defined media
Xeno-free	A term to describe products or materials that do not contain components derived from other species. May contain serum or serum-derived components from the native species (e.g., human cell lines can be cultured using human-derived components such as human serum). Xeno-free media (XFM) allows researchers to grow specific human cell types in the absence of animal serum (e.g., fetal bovine serum).	ACF
TSE/BSE free	Declaration that products or materials are fully chemically synthesized or free from transmissible spongiform encephalopathy (TSE) and bovine spongiform encephalopathy (BSE) risk materials. Country of origin of material or product is essential in assessing BSE/TSE risk, because some countries are categorized by the World Organization for Animal Health (OIE) as BSE-negligible risk or controlled for BSE risk. In the U.S., USDA lists and updates list of country at risk. Testing for BSE/TSE on raw materials does not currently exist. The term is universally recognized by regulatory authorities.	

There is currently no AM-specific legislation within the United States or the European Union that provides a clear legal framework to guide AM qualification practices. Compliance requirements are not made explicit, and regulators depend on the judgement of product developers to justify and validate the use of each AM within each specific application.[3,4,5,6] Despite the dearth of legislative text specifically addressing AM use, with the number and breadth of AMs used in CGT, regulators across the globe approve and generally prefer the use of standards in AMs. Guidelines are available from various national and international organizations to guide best practices in qualifying AMs, including from the United States Food and Drug Administration (FDA) through the United States Pharmacopeia (USP), the International Council for Harmonization (ICH),[7] the International Organization for Standardization (ISO),[8] the European Medicines Agency (EMA),[9] and European Pharmacopoeia (Ph. Eur.).[10] This chapter draws on U.S.- and EU-based sources and other well-established regulatory agencies[11,12,13,14] with the understanding that regulators across these jurisdictions share a common understanding of AM qualification through their subscription to ICH principles. The following is a list of the more relevant ones.

International

- World Health Organization (WHO) GMP for Biological Products
- ISO/TS 20399-1:2018 Biotechnology — Ancillary materials present during the production of cellular therapeutic products
- ISO 9001: Quality Management Systems: requirements
- ISO 13485: Medical Device Quality Management System: requirements for regulatory purposes
- ISO/TC 276
- ICH Q5A/D Quality of biotechnological products
- ICH Q5E: Comparability of biotechnological/biological products subject to changes in their manufacturing process
- ICH Q6B Specifications: Test procedures and acceptance criteria for biotechnological/biological products
- ICH Q7: GMP Guide for Active Pharmaceutical Ingredients
- ICH Q8 (R2): Pharmaceutical development
- ICH Q9: Quality risk management
- ICH Q10: Pharmaceutical quality system

Australia

- Australian Regulatory Guidelines for Biologicals (ARGB)
- Therapeutic Goods Administration (TGA)

Japan

- Pharmaceuticals and Medical Devices Agency (PMDA) and Ministry of Health, Labor and Welfare (MHLW) Public Notice No. 210, 2003 - Standard for Biological Ingredients
- Standards for manufacturing and quality control for medical device and *in vitro* diagnostic reagents (MHLW No. 169, 2004)
- General principles for the handling and use of cellular/tissue-based products (MHLW No. 266, 2001)
- Guidelines on ensuring quality and safety of products derived from processed human stem cells (MHLW No. 1314, 2000)
- Points to consider on manufacturing and quality control (MHLW notification No. 0327025, 2008)

Europe

- ATMP Regulation (EC) No. 1394/2007
- Ph. Eur 5.2.12 Raw Materials of Biological Origin for the Production of Cell-Based and Gene Therapy Medicinal Product
- EudraLex Volume 4 GMP guidelines

USA

- USP <1043> Ancillary Materials for Cells, Gene and Tissue-Engineered Products
- USP <92> Growth Factors and Cytokines used in Cell Therapy Manufacturing (limited to rh-IL4)
- FDA Chemistry, Manufacturing, and Controls (CMC) guidance
- 21 CFR 1271.210 - Current Good Tissue Practice (GTP)
- Part 211 CFR Part 11 subpart E - Control of Components and Drug Product Containers and Closures

In an IND submission, sponsors can reference Drug Master Files (DMFs) to provide information on the ancillary materials used in their product manufacturing. DMFs are submitted directly to FDA by the AM manufacturers, and provide confidential, detailed information on the AM that the agency can review in an Electronic Common Technical Document (eCTD) format, including the facilities, processes, or articles used in the manufacturing, processing, packaging, and storing of these AMs. There are 4 types of DMFs; AMs generally use Type II DMFs. DMFs allow sponsors to reference material without the MF holder (the AM manufacturer) having to disclose the contents to the sponsors.

Risks Associated with Ancillary Materials

Risk can be defined as a metric for the amount of danger posed by a given situation or variable. With respect to quality-related risk in CGT product manufacturing, as is the case for other biopharmaceutical manufacturing contexts, risk should always be assessed with the potential for diminishment of safety to the patient as the primary concern. To that end, regulatory guidelines emphasize the need for a risk-based approach in CGT manufacturing,[15] which includes the selection and implementation of AMs. During early phase discovery and development, it may be acceptable to assume higher risk and use research-grade AMs with lower requirements for quality testing and documentation. This will allow AMs to be used early that are less costly, available from more manufacturers, and not restricted to the highest quality standard. Furthermore, this will also allow more comprehensive screening of sources for critical reagents that may not have been thoroughly evaluated in the process. As development progresses into preclinical and early phase clinical trials, a CGT manufacturer may select intermediate grade AMs produced using additional manufacturing and testing processes included in the product quality documentation. In late phase clinical trials and commercial manufacturing, CGMP grade AMs are preferred to reduce risk by ensuring the AMs are traceable, consistent, safe, pure, and effective. In the absence of products used for manufacturing ATMPs that follow current regulations on AM quality from either ISO or Pharmacopeia, a scheme to bring AMs into CGMP manufacturing can be implemented, where each subsequent step in that path is associated with an increasingly stringent set of quality principles, culminating in the production of an AM that is suitable for commercial use.[16]

RISK MANAGEMENT

The general approach for mitigating risk is to follow a strategic framework endorsed by all ICH harmonized countries. These principles underpin GMP concepts implemented through a quality management system (QMS). The latter is defined as a set of well-documented business practices and procedures responsible for assuring quality policies. While ISO 9001:2015 provides an exemplar QMS that AM manufacturers can follow and be certified against, it does not define the QMS, hence other models may be implemented. More specific for the manufacture of medical devices is ISO 12485:2016, which is generally considered a higher level of quality compliance than ISO 9001. Risk management strategies for AM users are covered in ISO/TS 20399-1:2018 Part 3. *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products* (USP <1043>) describes the application of the same risk-based approach to AM qualification and shares much of the same risk mitigation as Ph. Eur. 5.2.12 *Raw materials of biological origin for the production of cell-based and gene therapy medicinal products.*

Risk assessment

Risk management's first stage is risk assessment, wherein individual hazards are identified, analyzed, and evaluated. Recommended modalities for risk assessments are failure mode effects analysis (FMEA), or hazard analysis and critical control point. In the context of AM qualification, a number of different grades of AMs, certificates, terminologies, and compliance with QMS can be applied or used, potentially posing different levels of risk/hazard. Hazards to the product specification are most apparent, but patient safety issues that are not directly covered by the product specification are also paramount. Irrespective of the AM grade or quality standard that is claimed by the AM supplier, a number of factors should be considered by AM users when evaluating a biological material for its suitability in the manufacture of cellular

therapeutic product.[17] Examples of hazards to identify are those that threaten identity, functionality, purity, sterility, and other specification components; supply chain risks such as continuity of supply should also be considered. Each identified hazard should be analyzed to estimate the likelihood of occurrence and severity of harm. A risk evaluation is then performed to determine the strength of evidence for each of the risk assessment stages (identification and evaluation), an essential step to understanding the risk assessment output quality. A quantitative, semi-quantitative, or qualitative output can be produced depending on the exact risk assessment method used. The output can then provide guidance on how far down the process streams these AMs are acceptable for use, and/or guide risk control activities (e.g., the appropriate levels of testing depending on the point of use in the process) by prioritizing those that score highest.

It should be noted that changes in the AM manufacturing can occur, potentially causing changes to the composition of the biological AMs, which can impact the quality of the final cellular therapeutic product. It is important that the AM user is aware of these changes prior to them being effected. Additionally, the user should identify and qualify an alternative AM supplier in the case that the preferred supplier ceases its manufacture. As such, it is critical to establish a Quality Agreement mandating the supplier to provide sufficient notice for any change, and include a clause relating to the continuity of supply.[18]

From the sponsor's perspective, a risk assessment should be performed on the impact of such change, and comparability studies might be warranted to evaluate the product quality pre- and post-change. Principles of comparability studies are covered in Chapter 2.

Risk classification for ancillary materials

In an attempt to aid manufacturers and developers in designing their qualification programs for a variety of AMs, tiers of sample risk categories are presented below. Nonetheless, risk also depends on the amount and the stage that AMs are introduced into the manufacturing process. The following sample tiers do not address the impact on quantity or time of use, which is left up to both the AM supplier and the CGT manufacturer.

Tier 1—AMs are low-risk, highly qualified materials that are well-suited for use in manufacturing. The AM is a licensed biologic, an approved drug, an approved or cleared medical device, or it is intended for use as an implantable biomaterial. Generally, these components or materials are obtained as a sterile packaging system or dosage form intended for their label use but are instead utilized "off label" in the manufacturing process for the cell, gene, or tissue-engineered product.

Tier 2—AMs are low-risk, well-characterized materials that are well-suited for use in manufacturing. Their intended use is for drug, biologic, or medical device manufacture, including cell, gene, and tissue-engineered products as AMs, and they are produced under relevant CGMPs. Most animal-derived materials are excluded from this category.

Tier 3—AMs are moderate-risk materials that require a higher level of qualification than previous tier materials. Frequently, these materials are produced for *in vitro* diagnostic use and are not intended for use in the production of cell, gene, or tissue-engineered products. In some cases, an upgrade of AM manufacturing processes may be necessary to employ the AM in manufacturing these products (e.g., modification of the production process for a diagnostic grade monoclonal antibody to include robust viral removal steps in purification).

Tier 4—Highest risk level for AMs and extensive qualification is necessary prior to use in manufacturing. The materials are not produced in compliance with CGMP and are not intended for use in the production of cell, gene, or tissue-engineered products. This risk level includes highly toxic substances with known biological mechanisms of action, and also includes most complex, animal-derived fluid materials not subjected to adventitious viral removal or inactivation procedures.

Risk control

After risks have been assessed and stipulated, steps can be implemented to manage them appropriately. AMs that are particularly important to the manufacturing process or are not sufficiently qualified by the supplier may require additional in-house testing before they can be used within an acceptable risk tier. AMs that do not meet the required specification may need to be removed or replaced, or an alternative supplier may be found that is able to provide the AM with a suitable quality. If process modifications are implemented, the risk assessment

procedure may require revisiting to assess the new practices. For each hazard, the acceptable level of risk should be decided to guide the degree of risk reduction measures required (i.e., risk acceptance).

CURRENT SELECTION CRITERIA AND QUALIFICATION PROGRAM OUTLINE

AM qualification is defined by USP <1043> as "the process of acquiring and evaluating data to establish the source, identity, purity, biological safety, and overall suitability of a specific AM."[19] AM qualification is, in effect, a risk control measure that sits within the risk management plan of a product supply chain and life cycle. A product's QMS should consider in its design the specific application of each AM to the production process; the qualification activities of any given AM may therefore be different across two instances of use. A well-designed qualification framework becomes more comprehensive throughout clinical development and should be constantly reviewed and updated as new scientific understandings and analytical capabilities become available.

In general, as a good starting point for qualifying AMs for use in the production of CGTs, we recommend the following list of action items:

- Verify the vendor's Certificate of Analysis to ensure material meets acceptance criteria
- Perform audits of suppliers and partners
- Verify that purification of material does not use untested components or components that can risk the end-user
- Verify adequate viral inactivation/removal techniques are implemented to ensure material is free of adventitious agents
- Verify the Certificate of Origin to determine human or animal-derived components and confirm BSE-free countries of origin for the materials
- Analyze and verify identity, purity, and performance of crucial AMs in manufacturing procedures

Reagents Considerations

Reagents that fall under the AM classification include well-characterized chemicals, complex compounds (e.g., antibiotics, anticoagulants, density gradients, toxins), multi-component mixtures (e.g., buffers, culture media), and complex biological compounds or mixtures (e.g., enzymes; blood-, plasma-, or serum-derived products; biological extracts; cytokines; antibodies; and conditioned media from cultured cells). These components pose an augmented risk to the end-user if removal is inadequate or incomplete.

SAFETY, EFFICACY, CONSISTENCY, AND STABILITY

Reagents are defined as materials used for cell growth, differentiation, selection, purification, or other manufacturing steps but are not intended to remain in the final product. Reagents have the potential to affect the safety, potency, and purity of the final cell-based therapy product, and therefore the quality of reagents should be tested for these attributes prior to their introduction into the cell therapy manufacturing process. The variability of reagents should be controlled by implementing vendor qualification strategies built into the process. It is recommended to utilize FDA-approved or FDA-cleared reagents, or clinical grade reagents when possible. It is common during the early stages of development to opt for research-grade reagents; however, if therapies use research grade materials due to the inability to procure or unavailability of clinical grade reagents, the FDA recommends including a certificate of analysis provided by the supplier and verified in-house if possible. Additional tests may be needed to verify safety if the reagent contains human-derived or animal-derived material. Testing for these attributes should also be done on the cell therapy product during various stages of manufacturing to continuously ensure high quality of the final product. Example testing timepoints are presented in Chapter 9. As the cell therapy manufacturer gains experience during development, there should be increasing understanding of the reagent attributes that could impact activity, purity, and stability of the cell product.

Quality parameters and critical product attributes of the reagent must be identified to ensure lot-to-lot consistency and the desired clinical effect of the final cell product. Therefore, to ensure safety before final product testing, AMs should include assessments for sterility, mycoplasma, and adventitious viral agents. Microbiological testing should be performed on starting raw materials, ancillary materials, in-process intermediates, and final products as appropriate depending on the clinical phase.

Microbiological testing should include sterility testing for bacterial and fungal contamination as well as mycoplasma testing as described in 21 CFR 610.12 and in the United States Pharmacopoeia (USP) <71>.[20,21] In-process sterility testing is recommended at critical points during manufacturing, such as during purification, *ex vivo* gene modification, extended culture periods, and other timepoints as identified by the sponsor to ensure safety. A negative or no-growth result is an accepted criterion to move forward with the utilization of tested materials or intermediate after primary processing. Additionally, when animal- and/or human-derived biological materials are used in the production or formulation of AM, and depending on the risk assessment of the AM's exposure to a cellular therapeutic product, step(s) for removal or inactivation of viruses should be included.[22] These processes require validation studies and documentation so that they are available to the AM user.

Sponsors need to appropriately perform and describe adventitious agent testing as specified in *Points to Consider in the Characterization of Cell Lines Used to Produce Biological Products*[23] and ICH guidance Q5A: *Guidance on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin.*[24] Viral testing is recommended on all cell banks, viral banks, and final vector products. A number of well-stipulated and effective assays have been developed to detect many known viruses in ancillary materials, such as polymerase chain reaction (PCR), quantitative PCR, antibody detection, and others. If human- and animal-derived materials (cells, blood derivatives, etc.) are used in the manufacturing process, donor testing for human pathogens and human viral agents should also be performed, as described in any global regulatory guidance.[25]

In addition, assessments of other product characteristics such as identity, purity (including endotoxin), and potency are needed. It is recommended to perform these tests throughout the manufacturing process to evaluate manufacturing and ensure the quality and consistency of AMs utilized in the production of the CGTs. It is critical to determine the specifications used for intermediate acceptance criteria and AM acceptance beforehand. Specifications are the quality standards or tests, analytical procedures, and acceptance criteria that confirm the quality of AM products, product components, and other materials used in the production of the final therapy. Specifications should be appropriate to the product development stage and should be refined and tightened as product development progresses.

Purity testing is important to understand the safety risks associated with impurities that are either product-related or process-related components and the ability of the process to remove such impurities. Purity testing should include assays for pyrogenicity/endotoxin, residual proteins, DNA, RNA, and others as determined by the producer. Purity testing should also include assays for solvents used during production and purification, and reagents used during manufacture. For further detailed guidance please refer to ICH Q3 on Impurities.[26,27,28]

CASE STUDIES TO SUPPORT AM STANDARDIZATION

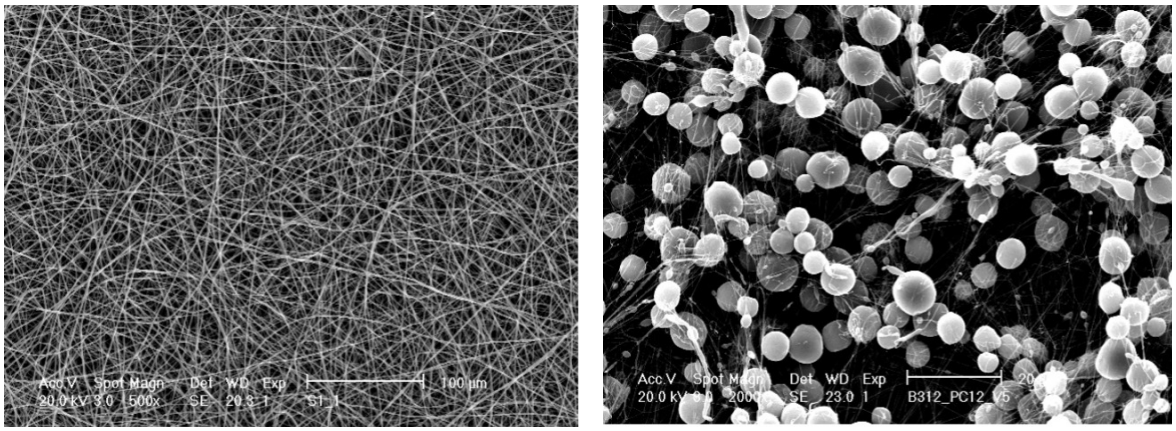
In this section, we introduce a few case studies involving various types of AMs. These examples are meant to reflect best practices considerations for AM suppliers and users for successful implementation of AMs in cellular therapeutic products.

Case study 1: Research-grade vs. CGMP-grade substrates and scaffolds

Cell/tissue-based products rely on cell-substrate interactions to properly integrate *in vivo*. This interaction, which entails cell adhesion to the substrate, is influenced by the substrate mechanics, as shown by the effect of substrate rigidity on the stability of adhesion complexes and motility.[29,30,31] Other cellular processes, such as proliferation, stem cell differentiation, cell growth, and apoptosis are also governed by the mechanical and geometrical properties of the cells and their environments.[32,33,34] It is therefore crucial to engineer the substrate to allow for proper cell functionality.

This case study presents an example of a product developed using research-grade raw materials that failed to translate into CGMP raw materials. The project consisted of creating a hydrogel containing nanofibrous structures to give the injectable gel structural integrity and optimize cellular infiltration and propagation. The raw material in question was polycaprolactone (PCL) synthetic polymer at 80K molecular weight. The project required electrospinning PCL nanofibers in a random deposition pattern of a specific fiber diameter (Figure 6-1). The constraints

Figure 6-1: *(Left) Nanofibrous construct electrospun with recipe A, research-grade PCL; (Right) Construct electrospun with recipe A, CGMP-grade PCL*



included tight fiber diameter distribution, lack of bead formation, and no webbing or fusion.

The fibers were expected to be as smooth as possible (i.e., no pores or any other imperfections that would render roughness on the fiber surfaces). The contract development and manufacturing organization (CDMO) was well aware of the driving parameters that require close monitoring to ensure a successful electrospinning process to produce the final nanofibrous product. Close attention and monitoring were performed on voltage potential, needle-to-collector distance, rotating collection mandrel speed (RPM) based on mandrel outer diameter, polymer extrusion flow rates, polymeric solution concentration, and environmental condition (temperature and humidity). The CDMO advised the client to utilize the final CGMP-grade material even during development, especially for the electrospinning process, knowing that while highly reproducible, subtle changes in the parameters mentioned above could have profound effects on the final product.

Despite the CDMO recommendation, the client elected to use research-grade PCL during development. The CDMO evaluated the PCL raw material and attempted to get as much information as possible from the vendor. Not surprisingly, as research-grade material, there was very little documentation aside from the standard safety data sheet (SDS) and a bare Certificate of Analysis (CoA). Per the customer's instructions, the CDMO herein moved forward, developing an electrospinning recipe to produce the customer-specified fiber product. While the CDMO successfully procured a recipe, the final fibers did not meet the user-specified criteria when the polymer grade switched to CGMP grade.

Case study 2: Enhancing the safety of virus-inactivated ancillary materials through neutralizing antibodies

The presence of adventitious agents in biologics manufacturing, particularly viruses, can impose severe consequences on patient safety. Cell therapy manufacturers minimize the risk of virus presence in their final product by selecting starting and raw materials with low risk of containing adventitious virus, and performing in-process testing at appropriate timepoints during the manufacturing process to ensure the material is free from detectable viruses.

To that end, the use of virus-inactivated AMs in CGT manufacturing helps produce optimal end drug products while overcoming regulatory hurdles in the pathway towards commercialization. AM manufacturers can incorporate steps to remove and inactivate potential viral contaminants; for example, there are a few providers in the advanced therapy market with a virus-inactivated human serum to be used as a safe and effective media supplement.[35] This virus inactivation effect can be achieved through immune neutralization, which is one of the three main functions of antibodies.[36]

Immune neutralization

Immune neutralization results when a bound antibody interferes with an essential function of its target antigen molecule, neutralizing the normal effect of the target.

It has been a powerful ally for humans attempting to understand and fight infectious diseases. Antiserums containing neutralizing antibodies against viruses and bacteria have been used since the early twentieth century and are still being used today (e.g., against the Ebola[37] and SARS-CoV-2[38] viruses). After someone has been infected and recovers from a pathogen, their body fluids (blood plasma) will contain antibodies specific to that pathogen. This convalescent plasma can be collected and infused into another person, sometimes creating what is known as passive immunity. It is possible for the neutralizing antibodies present in this antiserum to provide both effective prophylaxis against their target and a therapeutic intervention. This same neutralization phenomenon is responsible for what has been achieved via purified antibodies and monoclonal antibody production technology.[39]

In the age of purposefully engineered immune cells, we find immune neutralization working to our benefit within some of the ancillary materials currently available that support these advanced cellular therapies. There are a few providers in the advanced therapy market with a virus inactivated human serum to be used as a safe and effective media supplement, helping to produce optimal end drug products while overcoming regulatory hurdles in the pathway towards commercialization.[40] Several products undergo a robust solvent detergent treatment and benefit from immune neutralization through the pooling of numerous single donor units. This results in a significant reduction of enveloped and non-enveloped viruses.

Since the validated virus and pathogen reduction methods have already been approved by the FDA, drug and biologic manufacturers can move forward with confidence knowing the material they incorporate into their manufacturing process is maximizing the ultimate safety profile for the end user. The consistency achieved by pooling so many donor units enables greater control over a therapy's manufacturing process compared to conventional human-derived media supplements.

Furthering this consistency is immune neutralization and dilution of harmful interfering biomolecules. This is evident in pooled plasma products that minimize the risk of a leading cause of transfusion-related deaths in the U.S.—Transfusion-related acute lung injury (TRALI).[41] A large number of TRALI cases have been shown to be connected to the harmful antibodies Anti-HLA and Anti-HNA.[42,43] Despite comprising both male and female donations, this reduction in immunization risk is also seen with the absence of anti-D immunization and the total lack of inhibitor formation during clinical trials.[44]

Case study 3: Variability in the industry – recombinant protein example

Ensuring the quality and consistency of ancillary materials used in the manufacture of cell and gene therapies is critical to both controlling costs and ensuring the integrity of the therapeutic product. It is the responsibility of the ancillary material manufacturers to reduce the variability in the biomanufacturing of recombinant proteins intended for further manufacturing of therapeutics. Because these products are biologics in and of themselves (in addition to the therapeutic products they enable), they represent critical materials that must be controlled and released in a way that ensures consistency. The primary means by which recombinant protein manufacturers can ensure consistency in therapy developers' manufacturing processes is by ensuring a reproducible process and a reproducible means of measuring and reporting specific and biological activity.

Activity values

Cell therapy companies often rely on the lot-specific activity values (reported in International Units) on their supplier's CoA to measure the amount of material being used in their manufacturing processes (Figure 6-2). However, it is critical to realize that the values reported by different CGMP recombinant cytokine manufacturers cannot be directly compared with confidence if they do not adopt the same approach to measuring activity and analyzing the results. As an industry, it is important to align around standard

Figure 6-2: *Example of lot-specific activity values reported on a CoA*

Test	Result
Specific Activity	14.38 MIU/mg
Biological Activity	13.23 MIU/vial

reporting backed by an accurate and reproducible consensus-based process for analyzing and reporting activity values. This limits variability and ultimately increases the consistency of the final therapeutic product.

Specific activity: Expressed in IU/mg. Specific activity is inherent to the molecule and does not change, regardless of the concentration of the protein in the solution.

Biological activity: Expressed in IU/ml or IU/vial. Biological activity changes when the concentration of the protein in the solution changes.

Relative potency and parallel line assay

Relative potency is defined as the specific activity value (IU/mg) obtained for a test sample as a relative value compared to a reference standard's reported specific activity (IU/mg), by directly comparing the difference in concentration needed by the reference standard and the test sample to result in the same biological response for both.

To measure and report activity on Recombinant Human Interleukin-2 (rHu IL-2), one manufacturer utilizes a parallel line concentration-response model to estimate a relative potency compared to the National Institute for Biological Standards and Control (NIBSC 86/500) reference, applying the principles outlined in USP <1032> *Design and Development of Biological Assays* and USP <1034> *Analysis of Biological Assays.*

In comparison, other CGMP recombinant cytokine manufacturers take an alternative approach when calculating activity values. This, along with other differences in assay methodology, can result in reported values that are not directly comparable. We caution against using reported activity values to measure and dilute samples for comparability assays between different manufacturers.

It is important for the industry to align with best practices around the determination and reporting of cytokine activity. Successfully identifying best practices and implementing standards can streamline the raw material qualification process, resulting in a more robust and resilient supply base, ensuring that therapy developers can assess suppliers accurately, and onboard secondary suppliers without the need for extensive and costly comparability assessments.

It is worth noting that the World Health Organization also chose to use a parallel-line approach when determining potency for the current International Standard for IL-2 (NIBSC 86/500).[45] Using this method, the reported rHu IL-2 specific activity levels are comparable to the specific activity of the current international standard (NIBSC 86/500), which is known to be approximately 13.73 MIU/mg.[46]

See Figure 6-3 for a simplified example of the model the authors recommend to compare relative potency by plotting biological response vs. concentration (log scale) for a test sample and a reference standard. The horizontal

Figure 6-3: *Example of concentration-response model showing region applied in parallel line assay (left); and relative potency displayed within linear portion of parallel line model per USP<1034> (right)*

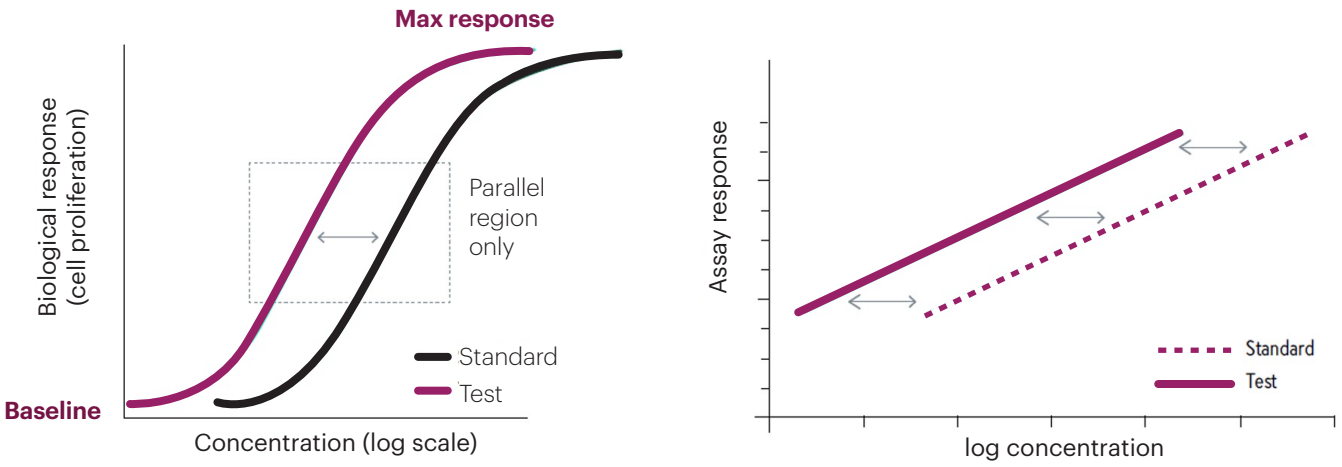


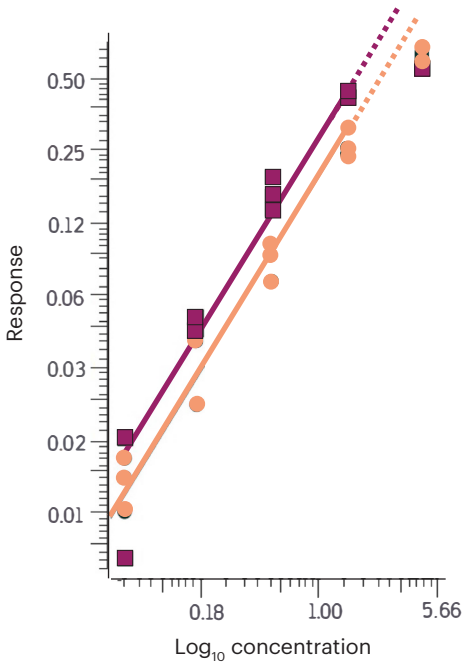
Figure 6-4: *Example tests of statistical validity for data points used in parallel line model.*

Test of regression (F-test)		
$F_{regression}$	454.249	This test passes if $F_{regression} > F_{critical}$
$F_{critical}$ (95.0%)	4.600	**Test Passed**
Test of linearity (F-test)		
$F_{non\text{-}linearity}$	1.065	This test passes if $F_{non\text{-}linearity} < F_{critical}$
$F_{critical}$ (95.0%)	3.112	**Test Passed**
Test of parallelism (F-test)		
$F_{non\text{-}parallelism}$	2.663	This test passes if $F_{non\text{-}parallelism} < F_{critical}$
$F_{critical}$ (95.0%)	4.600	**Test Passed**

distance between these graphs represents the difference in concentration needed by the reference standard and the test sample to result in the same biological response for both. This defines relative potency.

The parallel, linear regions for the test sample and reference standard in this model are compared to estimate a relative potency if, and only if, they first pass statistical validity tests for regression, linearity, and parallelism (Figure 6-4). Per USP <1032>, all biologically similar compounds will produce statistically similar results, but just because something has statistically similar results, does not ensure that it is biologically similar. Failure to produce statistically similar results, however, can be taken as evidence that they are not biologically similar.[47] Once found to be statistically valid, we assume biological similarity and parallelism, and a relative potency is calculated by comparing the concentration-response function of the test sample to the concentration-response

Figure 6-5: *Relative potency determination using parallel line model via direct relationship between test sample's and reference sample's concentration-response functions*



FR(z) = concentration-response function for Reference (NIBSC)
FT(z) = concentration-response function for Test Sample
FT(z) = FR(ρz) ρ = relative potency z=concentration

NIBSC Standard: $FR(z) = \alpha R + \beta \log(z)$
$FR(z) = \alpha R + \beta x$

IL-2 test sample: $FT(z) = \alpha R + \beta \log(\rho z)$
$FT(z) = \alpha R + \beta \log(\rho) + \beta \log(z)$
$FT(z) = \alpha T + \beta x$

If parallel: $\alpha T = \alpha R + \beta \log(\rho)$
relative potency: $\rho = \text{antilog}\ [\ (\alpha T - \alpha R) / \beta]$

function of the reference standard, as shown in Figure 6-5, adapted from USP <1034>.[48]

Alternative method for calculating activity levels

The commonly employed alternative method for calculating activity levels, used by many cytokine manufacturers, is to determine the ED50 using the entire concentration-response graph and convert that directly into specific activity as shown in the graph and equation in Figure 6-6.

ED50 is the concentration (ng/mL) that results in 50% of the maximum biological response. By applying the equation, one can calculate a specific activity reported in U/mg. This then needs to be normalized against a reference with known potency so that specific activity in IU/mg can be reported, which is supposed to be a universally comparable value.

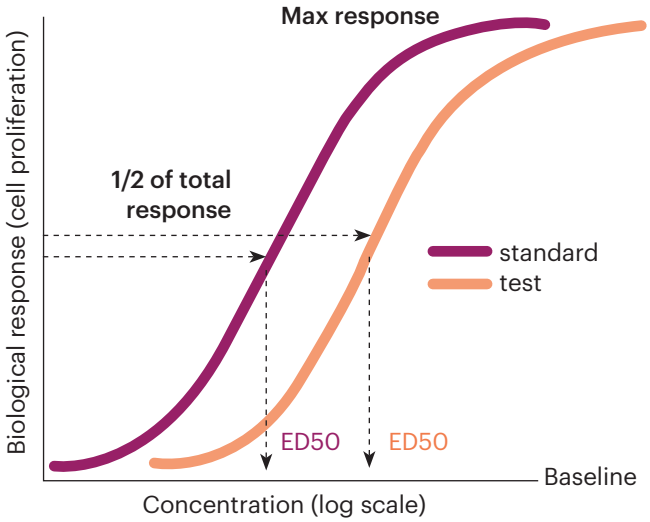
In our experience, for cytokine activity, there is a bigger error involved when calculating a relative specific activity using the ED50 value rather than using a parallel-line model. This is supported by the following guidance in USP <1032> regarding variance:

> "Simple analysis of quantitative bioassay data requires that the data be approximately normally distributed with near constant variance across the range of the data.[47]
> "In practice, many bioassays have relatively large variation in log EC50 (compared to the variation in log relative potency) among assays (and sometimes among blocks within assay). If not addressed in the variance model, this variation in log EC50 induces what appears to be large variation in response near the mean log EC50…"[47]

If manufacturers use different methods to measure and calculate specific activity, there will be completely different sources of error, resulting in activity values that should not be directly compared. Relative activity values can only be compared relatively, which means they must result from the same comparability testing protocol and data analysis methodology.

One simple option available for companies looking to compare cytokines from different manufacturers via comparability assays is to measure and dilute the test samples according to mass concentration, rather than the reported specific activity. If a consensus-based reference standard is included during this comparison, the relative specific activities of the test samples can be recalculated and compared using these results.

Figure 6-6: *Concentration-response model showing example ED50 comparison and equation used to convert ED50 value directly into specific activity (U/mg)*



Specific activity (U/mg) = $1x10^6$ / ED50 (ng/ml)

Conclusions

The authors have long been at the forefront of the efforts to coordinate and accelerate the development and diffusion of industry standards. Through the Standards Coordinating Body (SCB)'s working group, the authors co-lead a standards advancement project on Ancillary Materials, resulting in a three-part series of documentary standards on ancillary materials: ISO/TS 20399: *Ancillary materials present during the production of cellular therapeutic products*. It is in our interests, as a community of manufacturers seeking to accelerate the development and commercialization of cell and gene therapies, to standardize the assays that most directly impact our customers' manufacturing processes and ultimately the end patient experience. Only through greater standardization can we hope to achieve the resilient supply chains, cost of goods reductions, and manufacturing robustness that define a mature industry.

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CHAPTER 7

Lentiviral Vector Manufacturing Process

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Introduction

Lentiviral vectors (LVs) represent an important means of transferring and integrating transgenes of interest into cells. They are capable of transducing dividing and non-dividing cells including T-cells, making them particularly suitable for the *ex vivo* generation of CAR T-cell therapies. In fact, LVs are being used to manufacture several recently approved CAR T-cell therapies (Kymriah®,[1] Breyanzi®,[2] and Abecma®[3]), and at least 50 ongoing or planned clinical trials of CAR T-cell therapies involve the use of LVs according to ClinicalTrials.gov.[4] Importantly, LVs can also be used as drug products for *in vivo* gene therapy. Given their broad applications, the LV production market is expected to grow to $800 million by 2026.[5]

Lentiviral vectors are typically based on HIV-1, a well-studied virus pathogenic to humans.[6] Different generations of LV systems have been developed, with early generations being fine-tuned to remove unnecessary or safety-concerning elements, while ensuring both efficient gene transfer and absence of replication-competent lentivirus (RCL). Currently, third generation LVs are most widely used for clinical applications. They are typically produced using a 4-plasmid transient transfection system comprised of 3 packaging plasmids[7] and 1 self-inactivating gene transfer plasmid (Figure 7-1). The transfer plasmid consists of the lentiviral backbone containing the transgene expression cassette flanked by HIV-1 long-terminal repeats essential for packaging the viral RNA genome into viral particles, reverse transcription, and integration into the recipient cell.[8,9] The 3 packaging plasmids contain *gag-pol* (encoding for structural proteins and viral enzymes), *rev* (encoding for a post-transcriptional regulator), and *env* which encodes the pseudotype of the viral vector (typically the vesicular stomatitis virus [VSV]-G envelope gene is used).[6,7,10] Though LVs can be pseudotyped with different envelope glycoproteins, the VSV-G glycoprotein enhances stability during downstream processing and features a large transduction spectrum due to its interaction with low-density lipoprotein (LDL) receptors that are ubiquitous among most cell types.[6,10,11] However, resting lymphocytes used for CAR T-cell therapies have low expression of the LDL receptor, leading to inefficient transduction in the absence of cell activation.[12] Though VSV-G is the most common envelope protein pseudotyped onto LV particles, other envelope glycoproteins such as RD114 (derived from a feline endogenous retrovirus) have found application in the transduction of lymphocytes for CAR T-cell therapies.[13,14]

Lentiviral vectors can be viewed as either drug substance (DS) or drug product (DP) depending on their application (Figure 7-2). When used *in vivo* to directly

Figure 7-1: *Plasmids used in traditional third-generation LV production*

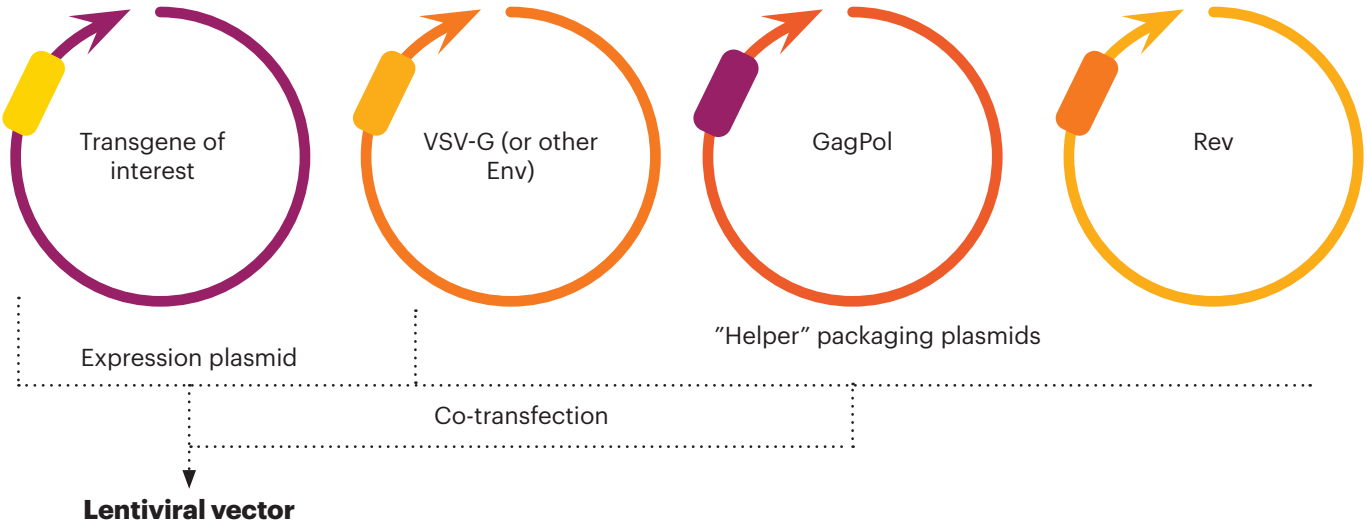

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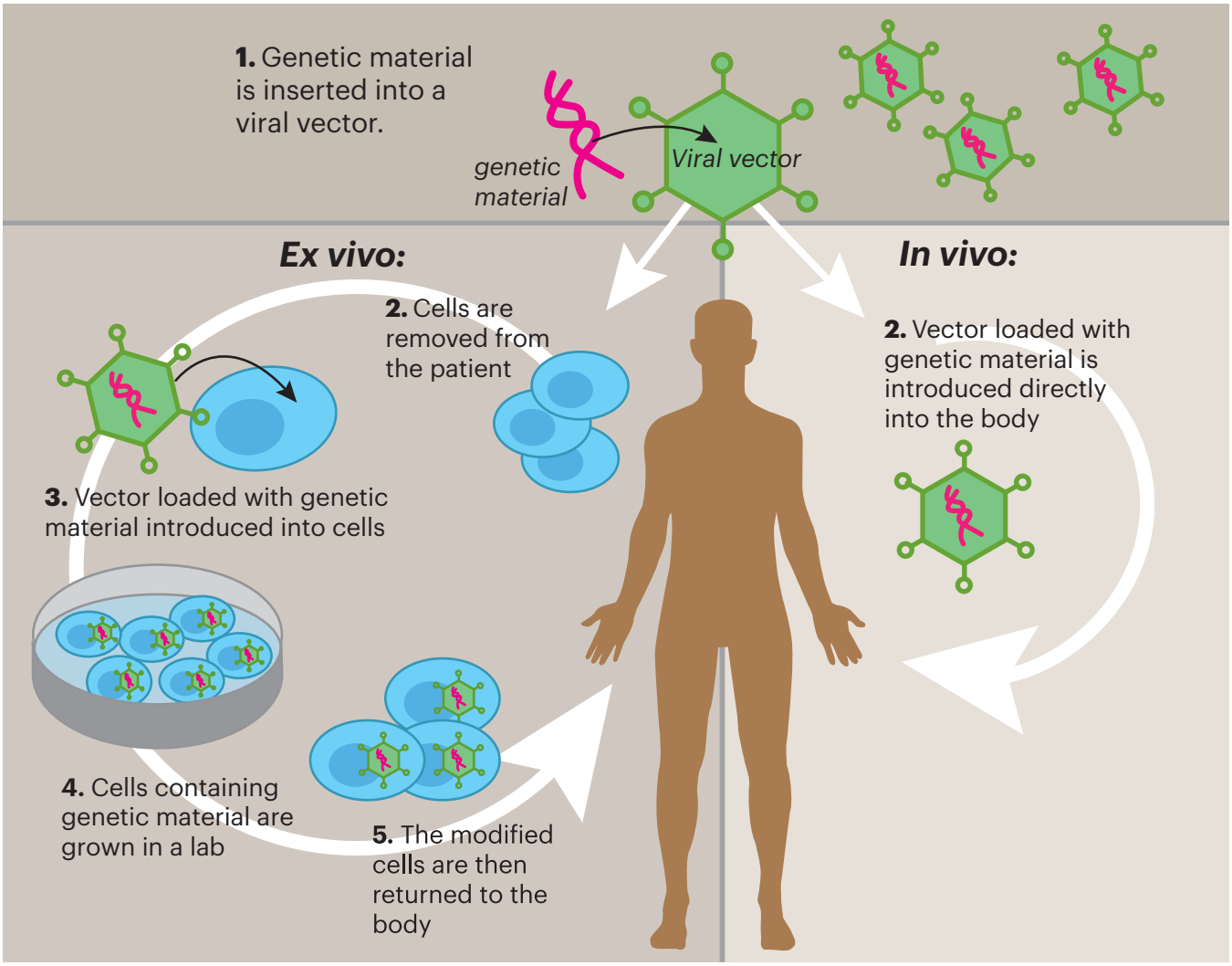
Figure 7-2: *Vector can represent drug substance or drug product depending on the application*



insert genetic material into human cells, LVs take on the role of the final drug product. However, when used to transduce human cells *ex vivo*, such as in the generation of CAR T-cell therapies, LVs may be more appropriately classified as drug substance. For CAR T-cell products and other *ex vivo* applications, separate quality target product profile (QTPP) and critical quality attributes (CQAs) should be developed for the vector material. Additionally, vector manufacturing process residuals, impurities, and multiplicity of infection (MOI) should be included in the QTPP of the final drug product. This speaks to the criticality of the vector in the eyes of regulatory bodies: whereas the European Medicines Agency[16] views LVs as starting material, the U.S. Food and Drug Administration (FDA)[17,18] simultaneously describes vector used for *ex vivo* genetic modification of cells as bulk drug substance, an active pharmaceutical ingredient, and a critical component of the final drug product. Regardless of classification, LVs must be appropriately characterized and all assays used for quality control during its manufacture should be properly qualified and validated in a phase-appropriate manner. Within any investigational new drug application (IND), a separate drug substance section should be provided for vectors used for *ex vivo* modification of cells, as outlined in Module 3 of the Common Technical Document (CTD).[19]

The processes for development of the final drug product are often unique to the individual sponsor, however

most LVs in clinical and commercial use are supplied by contract development and manufacturing organizations (CDMOs), where the development and manufacturing process is already defined, often requiring only an adjustment of the transgene expression cassette within the gene transfer plasmid. The essential role of LV in the production of CAR T-cell therapies (and other cell and gene therapies) speaks to the importance of coordination and communication between the sponsor and the CDMO. Helper plasmids are becoming increasingly available as off-the-shelf products, the proprietary nature of which may be protected under a drug master file (DMF). Similarly, gene transfer plasmid templates and cell bank systems are becoming more widely available as "off-the-shelf" products. As such, the CDMO must procure these essential components from qualified vendors that can demonstrate consistent quality of their products and solid control of risks associated with supply chain logistics. While the sponsor is ultimately responsible for filings to the health authority, obtaining all essential information for the filings will require significant collaboration with the CDMO.

Lentiviral Vector Manufacturing

Manufacturing of LV, like all biologics, can be divided into upstream and downstream processes, with upstream production steps aiming to produce high-titer vector that is conducive to downstream purification. Upstream processes vary depending on the cell line used for production and whether the cells are transiently transfected or developed into stable producer cell lines. Adherent cell cultures tend to accommodate the smaller-scale needs typical during developmental stages of product development, whereas suspension cultures are more suited for large-scale production. Supplementation of cell media (e.g., with serum or sodium butyrate) can improve producer-cell viability and vector stability but may present challenges to product safety and ease of downstream processing. Downstream processing steps seek to maximize viable vector recovery while reducing impurities that could compromise the efficacy or safety of the eventual drug product. Downstream steps follow the general order of purification, enrichment/concentration, sterile filtration, and storage.

STARTING MATERIALS

According to the European Commission Directive 2001/83/EC,[16] in the case of products consisting of viral vectors, the starting materials shall be the components from which the viral vector is obtained (i.e., the plasmids used to transfect the packaging cells and the master cell bank [MCB] of the packaging cell line). Additionally, in the case of genetically modified cells (which would include CAR T cells), the starting materials shall be the components used to obtain the genetically modified cells (i.e., the starting materials to produce the vector, the vector, and the human or animal cells). The principles of good manufacturing practices (GMP) shall apply from the bank system used to produce the vector onwards.[18]

The cell bank system used to produce LV traditionally consists of HEK 293 or HEK 293T packaging cell lines. The HEK 293 cell line was developed in 1977 by transfecting human embryonic kidney cells with sheared adenovirus type 5 DNA.[20] The gene encoding the SV40 T-antigen was then introduced into the 293 cell line to create the 293T cell line. Today, HEK 293T cells are generally preferred over HEK 293 cells due to their increased cell growth and transfection efficiency, at least partially owing to the presence of the SV40 T-antigen.[21,22]

As discussed previously, the third-generation LVs used most commonly utilize a 4-plasmid system: one plasmid contains the gene(s) of interest and 3 refined "helper" plasmids contain viral sequences necessary for production.[6,10] The need to transiently transfect 4 different plasmids with each vector manufacturing batch can be a source of variability in manufacturing processes; thus, sponsors must ensure that plasmids are appropriately and consistently manufactured. Though plasmids have historically been manufactured in custom batches, commercial production of "off-the-shelf" lentiviral helper plasmids is becoming increasingly common. In addition, plasmid production companies can often provide plasmids of varying grades, ranging from research-grade plasmids for process development purposes to those suitable for commercial production.[15]

Though the FDA's *Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications*[23] does not specifically address plasmid DNA products intended for noninfectious therapeutic indications, information applicable to plasmids used in

LV manufacturing can be drawn from its contents. As such, all components used during LV production should be sufficiently described in the manufacturing summary included in an IND application. This includes detailed descriptions of the plasmid construct generation and the DNA sequence of the entire plasmid(s). In its 2020 *Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)*,[17] the FDA describes bacterial MCBs as the starting material used to generate plasmid DNA, which can then be used as a manufacturing intermediate to generate LVs. The FDA recommends that the bacterial MCB be appropriately qualified and that sufficient information on that qualification be submitted in the IND.[17] Information submitted to support qualification should include certificates of analysis for cell banks, executed batch records, and evidence of passed release tests that adequately establish the safety, identity, purity, and stability of the microbial cell preparation used in the bank.[17] Interestingly, a plasmid construct is used to generate the bacterial MCB (usually *E. coli*-derived). Often, this plasmid construct is synthesized de novo under R&D manufacturing conditions. Within the IND, the sponsor should include the full sequence of the plasmid used to construct the MCB, and the sponsor should be aware of any safety and cross-contamination issues.

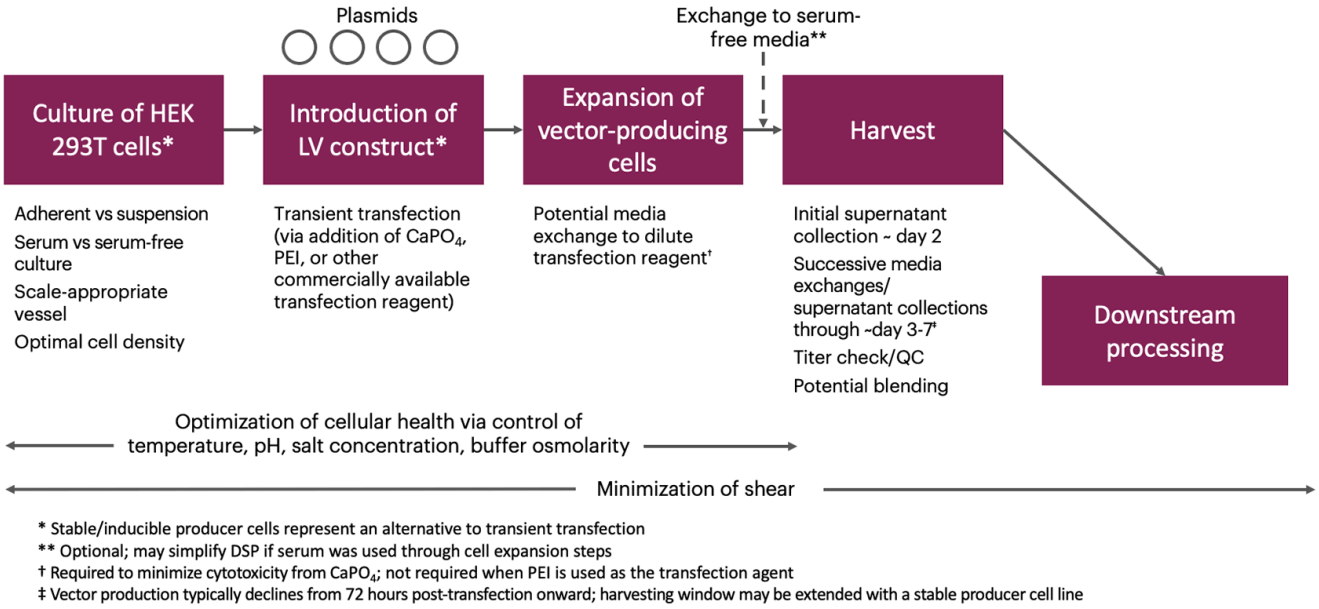
The genotype and source of the mammalian cell line (e.g., HEK 293, HEK 293T) used to construct the vector production or packaging MCBs and working cell banks (WCBs) must also be described in the IND. Additionally, both the MCBs and WCBs should be tested to ensure that they are free from contamination with adventitious bacterial and viral agents; all testing should be well documented and controlled. Viruses are generally more difficult to detect than bacterial contaminants and can be of greater consequence should they be present in cell culture. As such, sponsors should be careful to select starting and raw materials with a low risk of containing adventitious virus. Process-specific raw materials should be obtained from reliable sources that can demonstrate adequate control of their production. Raw materials can include cell culture media and additives, reagents (e.g., transfection reagents such as polyethylenimine [PEI]), endonucleases, and consumables (e.g., cell culture units, membranes, buffers, filters, resins, and cryovials). Starting and raw materials should be subjected to a proper assessment of risk they could impose to vector production; principles of risk assessments and criticality determination are discussed in Chapter 4. Additional guidance on viral evaluation of starting materials is found in ICH Q5A (R1)[24]; guidance on viral testing throughout LV production stages as well as specific testing methods will be discussed later in the chapter.

Considerations for viral contamination and clearance

The Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) recently published insights for gene and cell therapy developers regarding the most common viral contaminants in cell cultures, the sources of these contaminants, the cell lines affected, and corrective actions that can be taken.[25] Their findings demonstrated that viral contamination in cell-based manufacturing, though relatively rare due to existing standards, can be extremely costly and detrimental to manufacturing operations. Overall, human or primate cell lines were more likely than rodent cell cultures to be contaminated by viruses pathogenic to humans. Only one case of HEK 293 cell-line contamination was reported; human adenovirus type 1, which is pathogenic to humans, was implicated in that case and the suspected source was the operator rather than the cell source or materials used in cell culture (e.g., serum or media component).

Unexpected changes in cell culture behavior can be an indication of viral contamination but many viral contaminants are not apparent. Virus-specific testing (e.g., polymerase chain reaction [PCR]) is often required for detection, though even PCR may fail to detect low-level viral contamination in raw materials prior to viral amplification. Because viral vector production generally starts with fully characterized MCBs, they have a relatively low risk of viral contamination from their starting cell sources. However, given the detrimental effects of potential viral contamination, testing for adventitious virus should be conducted at multiple timepoints in the LV manufacturing process (see Figure 7-7 in the *Safety* section of this chapter).[25] Notably, testing for contaminants is required at the unclarified harvest stage, as they are most

Figure 7-3: *General upstream processing strategy for LV production via transient transfection of HEK 293T cells*



likely to be detected prior to any downstream processing. Again, guidance on viral testing throughout production stages as well as specific testing methods are discussed in greater detail in the *Safety* section of this chapter. Because lentivirus is enveloped as well as temperature- and pH-sensitive, implementing known viral clearance methods (e.g., detergent/solvent, high heat, or extreme pH) for LV processing is not possible.[25,26] Therefore, the LV manufacturing industry must rely heavily on prevention and vector characterization methods to ensure patient safety. Care must be taken to prevent viral contamination via thorough testing of raw materials and host cells used in upstream processing.[27] Downstream processing methods (e.g., chromatography, filtration) can also act as clearance methods that will separate contaminating virus from lentiviral particles without harming the activity profile of the LV particles.[25-27]

UPSTREAM MANUFACTURING PROCESSES

Multiple factors must be considered in order to optimize bulk production of LV during upstream processes.[10] The production mode (e.g., transient transfection vs stable or inducible cell line, adherent vs suspension) must be considered early in process development. Several considerations impact the scalability of cell expansion, where viability of cells must be maintained while achieving suitable cell density to meet yield demands. Such considerations include the type of cell line used (adherent vs suspension), the equipment used for cell expansion (flasks/bottles for small batches vs bioreactors for large batches), and the type of cell media and supplementation. The general upstream processing strategy for LV is shown in Figure 7-3; individual steps will be discussed further in subsequent paragraphs.

Transient transfection vs stable cell line

The predominant mode of LV production involves transient transfection of cells with optimized plasmid systems (Figure 7-1) that maintain critical elements for vector expression and packaging but eliminate unnecessary genes that compromise safety and yield. Co-transfection of the gene transfer and "helper" plasmids can yield titers in the 10^6 to 10^8 transducing units (TU)/mL range, though larger transgenes upward of 7.5kb may affect yield.[6,10,28] Titers are also impacted by cell density, incubation time, temperature, pH, and plasmid concentration.[10] In fact, the optimal plasmid ratio can be so impactful on titer that it is often considered proprietary information.

A wide variety of transfection reagents can be used for LV production, each with unique implications in terms of scalability, cost, and efficiency. Calcium phosphate offers an inexpensive option that is most suitable for

Table 7-1: *Examples of inducible and stable cell lines for LV production*

Cell line	LV generation	Induction system	Envelope	Reported titer	Adherent or Suspension	Reference
GPRG-TL20-IL2RG	3rd	Tet-off	VSV-G	5x10^7 TU/mL	Adherent	Throm 2009[35]
293SF-PacLV	3rd	Tet-on, Cumate	VSV-G	3.4x10^7 TU/mL/day	Suspension	Broussau 2008[36]
293TsaGLOBE	3rd	Tet-on	VSV-G	1.4x10^7 TU/mL	Suspension	Chen 2020[37]
LentiPro26	3rd	NA	MLV Amphotrophic Envelope	10^6 TU/mL/day	Adherent	Tomas 2018[31]
WinPac	3rd	NA	RD114-PR	1x10^6 TU/mL	Adherent	Sanber 2015[34]

small-scale use. Calcium phosphate and plasmid DNA form precipitates that attach to the cell surface and are endocytosed. The need for constant mixing to ensure consistent precipitate formation, the requirement to change medium after transfection, and the sensitivity of the process to temperature, pH, and component concentrations makes this method difficult for large-scale production.[10] The use of lipofectamine, a cationic lipid that entraps plasmid DNA within liposomes that fuse with the cell membrane, is limited by cytotoxicity, cost, and the requirement for media exchanges post-application.[10,29,30] Polyethylenimine, which forms polyplexes with plasmid DNA that are endocytosed by cells, is less sensitive to pH alterations and its lower toxicity precludes the need for media exchanges following transfection. It requires less plasmid DNA than calcium phosphate, is relatively inexpensive, and can be used in both adherent and suspension cultures, making it more suitable for large-scale LV production.[10,29]

Transient transfection offers fast and dependably consistent LV production but is suboptimal for large-scale production due to the cost of plasmid and reagents, significant batch-to-batch variation in titers, and the need to clear both transfection reagents and residual plasmid DNA.[29] As such, a stable cell line that constitutively produces high-titer, high-quality, homogenous LV would be ideal for large-scale use. Unfortunately, the generation of stable cell lines for LV production has proven challenging, at least partly due to the VSV-G envelope featured in most second- and third-generation plasmid systems. Though VSV-G offers broad tropism and the potential for high titers, its inherent cytotoxicity does not permit constitutive expression and, therefore, its use is limited to transient transfection modalities.[10,31] Expression of *gag*, *pol,* and certain transgenes can also introduce cytotoxicity.[6] Through the addition or removal of tetracycline, inducible systems such as Tet-on and Tet-off can limit cytotoxicity by triggering gene transcription during only key phases of LV production.[6,10] Such inducible systems have been used to produce LV used in studies of investigational therapies for Wiskott-Aldrich syndrome[32] and X-linked severe combined immunodeficiency.[33] However, the use of tetracycline requires removal downstream, and inducible systems carry the risk of "leaky" expression that can lead to uncontrolled LV production.[10] Constitutive producer cell lines successfully used for LV production utilize an alternate envelope protein,[31,34] offer lower titers than those produced by transient transfection methods, and have yet to be adopted into mainstream practice. Though details of producer cell lines are often proprietary information, examples of inducible and constitutive producer cells used for LV production are provided in Table 7-1. More complete listings and additional information can be found in reviews by Perry et al.[10] and Merten et al.[6]

Adherent vs suspension production

During early development stages, adherent cultures are commonly used due to the innately adherent nature of HEK 293T cells, the higher LV titers and cell densities achieved, and the desire for flexible manipulation of cells within small culture flasks and bottles.[10] However, adherent cultures can be limiting during scale-up. Though

multilayer flasks provide additional surface area for cell attachment and growth, they are bulky and require extensive open manipulation during upstream and downstream processing steps. The use of microcarriers, which can be porous or solid, fails to achieve high LV titers due to cell clumping, inconsistent delivery/removal of nutrients and oxygen, and compromised removal of toxic metabolites.[10] Fixed-bed bioreactors may be best-suited and most economical for large-scale expansion of adherent cultures, allowing for cell expansion on a 3D matrix that provides a balance of higher surface area with less bulkiness.[10,38] Though plagued somewhat by poor cell distribution, optimized fixed-bed bioreactors such as the iCELLis® and scale-X™ are able to achieve LV titers close to 10^6 TU/mL.[39] Examples of vessels used for adherent cultures include HYPER*Flask*® and HYPER*Stack*® (multilayer, Corning); iCELLis® (fixed bed, Pall); and scale-X™ (fixed bed, Univercells).

Suspension cultures are theoretically more ideal for large-scale LV production, as they can achieve greater production volume without the need for attachment surfaces.[29] HEK 293T cells can be adapted to suspension culture, though adaptation is generally accompanied by a loss of productivity, with titers around 10^6 TU/mL or lower. Shear forces are higher for suspended cells and they are inherently more difficult to transfect, as they represent moving targets for transfection complexes. Nevertheless, a gradual adaptation of HEK 293T cells to suspension media has recently achieved LV titers of 10^8 TU/mL using 5-Liter shaker flasks.[40]

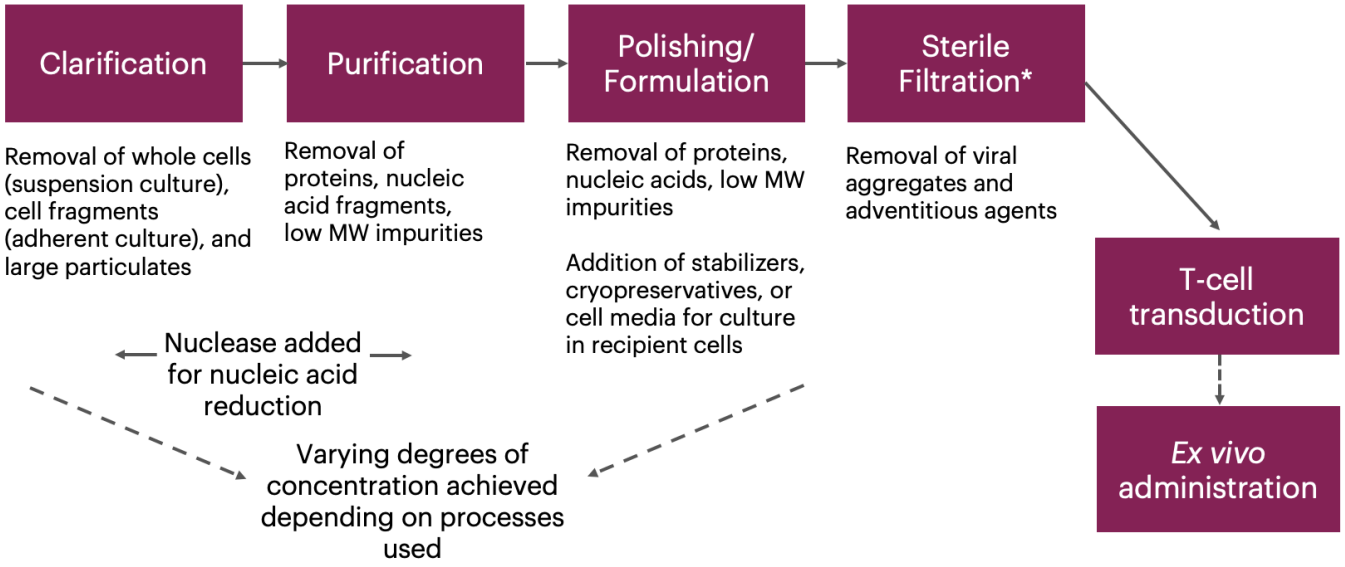
Suspension cultures minimize manual handling and enable both automation and in-line monitoring. As with all steps of CAR T-cell manufacturing, closed bioreactors with weldable connections can minimize contamination potential throughout much of the LV production process. The potential for shear damage to cells introduced by shaking or stirring can be difficult to control during scaled-up production, particularly within large bioreactors, and media replacements required for transient transfection modalities can be more challenging. As such, it remains to be seen whether the high titers accomplished in 5-L shaker flasks can be achieved in larger-scale production. Stirred-tank bioreactors can scale up to thousands of liters, whereas rocking bioreactors offer a lower-shear environment at a lower scale (10-200 L).[10] According to Comisel et al., single-use stirred tank bioreactors may be the most cost-effective vessel type for LV production in suspension-adapted cell lines, though capacity limits are restrictive in high-dose, high-demand scenarios.[38] Examples of bioreactors used for suspension cultures include Xcellerex™ XDR (stirred tank, Cytiva); Biostat® (stirred tank, Sartorius); and WAVE (rocking platform, Cytiva). Additional discussion of technologies used for both adherent and suspension cultures is found in Chapter 8 of this document and in the review by Perry et al.[10]

Serum vs serum-free production

Many LV production modalities utilize serum to optimize both cell growth and vector stability. Major detriments to serum addition include the need for removal of higher protein loads in downstream processes and the risk of immunogenicity.[10] Additionally, as the majority of serum used for LV production is bovine-derived, the risk of bovine-related disease transmission is of utmost concern. The bovine spongiform encephalopathy (BSE) epidemic in the United Kingdom during the 1990s brought heightened attention to the dangers of prion-mediated transmissible spongiform encephalopathies (TSEs) for human health. As no laboratory tests are currently available to screen live cattle for the prions causing BSE infection, the sourcing of bovine serum (country/herd/animal) is an important aspect of overall risk mitigation for any cell therapy product.[41] In addition to concerns related to TSEs, the process of treating fetal bovine serum (FBS) with gamma radiation to achieve viral inactivation is important for making FBS GMP-compliant for use in LV manufacturing.[17]

To minimize risk of animal-related disease, there is a drive to remove serum from the production of LV. Though serum is required to mitigate the cytotoxicity introduced by calcium phosphate, PEI can be used in the presence or absence of serum.[10,29] Although adherent cells are difficult to grow in serum-free media, HEK 293T cells can be adapted to suspension growth in serum-free media.[6,40] Stable cell lines have also been successfully adapted to serum-free media.[29] The detailed comparison between adherent and suspension cell platforms has been described in an earlier section of this book chapter.

Figure 7-4: *General downstream processing strategy for LV intended for ex vivo therapies*



* Can be excluded if manufacturing process certified as fully aseptic.

DOWNSTREAM MANUFACTURING PROCESSES

Downstream processing (DSP) of LV seeks to maximize vector recovery while minimizing impurities that could negatively affect the efficacy or safety of the genetically engineered cell therapy drug product (i.e., the CAR T cell). The degree of purity and concentration required depends on the intended use of the product; purity is of utmost importance during clinical trials where human safety and CAR-T manufacturing performance could be jeopardized by residual components. Typical process-related impurities include process residuals such as PEI, plasmid DNA, host cell nucleic acids, host cell proteins, serum, and leachables.[10]

Enveloped viruses such as lentiviruses are inherently susceptible to physico-chemical stress such as changes in temperature, pH, other culture conditions, and shear stress.[42] As such, steps used in downstream LV manufacturing must prioritize the stability of the viral particle. Downstream purification techniques continue to advance; yet, LV manufacturing typically results in overall DSP yields of roughly 10-25%.[43] Because of the short half-life of LV (~3-18 hours at 37°C[38]), multiple harvests are commonly implemented in LV adherent processing, necessitating batch-style downstream efforts. A move toward continuous processing and purification is evolving and is yet to be broadly implemented in LV manufacturing.

Key uncertainties about continuous DSP remain, including whether upstream batches would be individually tested, pooled, and then submitted to downstream processing, or whether harvests would be processed continually upon removal from the bioreactor and then pooled at the end of downstream steps.[43] Regardless of approach, consistent control of upstream processing must be demonstrated and all processes used during pooling should be properly validated. Sequential harvests from the same bioreactor can be pooled as one sub-lot of a batch, but defined release criteria (e.g., titer) and appropriate safety testing should be in place for each individual sub-lot. ICH Q7 specifically states that out-of-specification batches of active pharmaceutical ingredient (e.g., LV, according to the FDA definition) should not be blended with other batches for the purpose of meeting specifications (e.g., titer). Additionally, blending processes should be adequately controlled and documented, and the batch record of the blending process should allow for traceability back to the individual batches contained within the blend.[44]

Continuous DSP also requires a solid understanding of LV purification principles. Steps such as depth filtration, ultrafiltration, and chromatography can be employed in a variety of sequences throughout the downstream process and often serve to clarify, concentrate, and buffer exchange the LV harvests. The general DSP strategy for LV is shown in Figure 7-4; individual steps will be discussed further in subsequent paragraphs.

Clarification/cell removal

Clarification occurs as the first step in DSP and typically involves microfiltration and/or centrifugation. The complexity of clarification steps is highly influenced by upstream manufacturing processes. Because newer technologies (e.g., fixed-bed bioreactors) used for adherent culture perform a baseline level of cell removal (cells stick to the reactor material itself), simple polymer-based filters are often sufficient for removing leftover cell fragments.[10] More complex clarification strategies are required when using suspension culture for LV production, as whole cells must be removed.

Cells can be sedimented by low-speed centrifugation, which also serves as a pre-filtration step to prevent clogging of the filter(s) in subsequent steps, though centrifugation subjects LV to shear force and requires open manipulation. Membrane filters feature an inert polymer with pores of a specific diameter; use of progressively finer filters can accomplish significant clarification. Due the size of LV (80-120 nm in diameter), using filters with exceedingly small pore diameter can result in product loss. For this reason, the pore size of the clarification membrane filter is generally 0.45 μm.[45] Depth filters retain cell particles throughout sponge-like material composed of polymer, binder, and a filtration aid (typically diatomaceous earth [DE]). The use of DE can lower LV titer; however, Valkama et al. reported functional recovery close to 100% using a depth filter with DE to clarify LV pseudotyped with VSV-G.[45] Through design-of-experiment approaches, Labisch et al. recently found that a DE concentration of 9 g/L achieved the optimal balance between clarification and recovery.[46] Recovery of >95% of titer via a depth filter without DE has also been reported.[47] The optimal filtration technique for clarification clearly remains to be seen, but in most cases, adsorption onto the filter or onto a component excluded by the filter results in at least some loss of LV titer. Notably, some LV can be recovered by flushing the filter with buffer.[10,45]

Two technologic advances are worthy of mention when discussing clarification. Tangential flow filtration (TFF), though commonly employed during concentration steps, can be used with both membrane and depth filters to separate suspension cells from LVs; yields as high as 90% have been reported recently with 2-5 μm depth filters used in TFF.[10,48] Another option is low-shear centrifugation (i.e., kSep® by Sartorius), accomplished through a careful balance of centrifugal and fluid-flow forces. Capital cost is very high, but advantages include a closed system, scalability, and high product recoveries.[49]

An important step in DSP is nucleic acid reduction, which is generally facilitated via the addition of nuclease (e.g., Benzonase®, Denarase®) during the clarification stage or early purification stages. Nucleic acid impurities can originate from either the plasmid DNA or producer cell line DNA. Nuclease serves to reduce the size of any residual DNA to no greater than ~200 base pairs to prevent the risk of any deleterious effects in recipient cells.[10] The amount of nuclease required will be higher if added during clarification vs after an initial purification/concentration step. Nuclease will be removed from the LV during purification and polishing steps. An alternative to nuclease addition is the SecNuc™ platform developed by Oxford Biomedica, which features either (1) co-transfection of producer cells with both vector components and a plasmid that encodes a nuclease enzyme, or (2) co-culture of vector-production cells with nuclease-expressing helper cells.[50]

Chromatography

Chromatography, which is amenable to scalability and automation, can be used after initial clarification to further separate viral particles from contaminants and impurities by exploiting differences in their interactions with stationary and mobile phases. These properties can include size, charge, hydrophobicity, and binding affinity.[10] Inherently, capture chromatography also achieves some degree of bulk product concentration, as the virus is eluted in a smaller volume. Traditional size exclusion chromatography (SEC) is somewhat limited by the size of LV particles, as high molecular weight contaminants and residual plasmid/cell DNA may not separate from viral particles. Additionally, SEC is difficult to scale and may be more suitable for final "polishing" of the vector rather than bulk purification.[6] Affinity chromatography (AC) requires the modification of LV envelope protein with a "tag" (e.g., histidine, heparin, or biotin) that binds to an immobilized ligand.

Segura et al. reported 53% recovery of VSV-G pseudotyped LV particles with removal of 94% of impurities using heparin AC.[51] Though having potential to yield highly pure, concentrated product, AC is limited by the need to modify the LV envelope, which could theoretically affect the viral biologic activity or introduce (in the case of heparin) a product of animal origin.[10,42]

Anion exchange chromatography (AEX), whereby negatively charged vector particles bind to positively charged chromatographic matrix, is a promising technology for large-scale LV purification. Early reports by Scherr et al.[52] and Kutner et al.[53] highlighted LV recovery of 68% and 76%, respectively, using AEX. Separation is performed in bind-elute mode, utilizing stepped concentrations of salt buffer (up to a final concentration of ~1 M NaCl) for elution steps. As LV is destabilized by high-salt solutions, dilution of the LV bulk immediately after final elution must be performed to prevent envelope degradation.[10,42] Novel stationary phases are being developed, including a cellulose nanofiber-based stationary phase that produces functional vector yield of roughly 90%.[54]

Concentration

Some degree of LV concentration is accomplished during initial clarification and purification steps, though additional concentration is generally required. Concentration can be accomplished by sequential rounds of centrifugation/resuspension or via SEC, but tangential flow filtration (TFF), which allows for reduced manual handling and high vector concentration, is particularly useful for large-scale virus concentration.[42] When run in ultrafiltration/diafiltration mode, TFF not only allows for volume reduction and buffer exchange, but also removes low molecular weight impurities.[10] TFF systems can utilize hollow fiber membranes or cassettes comprised of various membrane material (e.g., cellulose- or polyethylene sulphone). All approaches offer high surface area to volume ratios, though the use of flat cassettes can subject LV particles to higher shear forces. As discussed previously, TFF can be used for multiple purposes (clarification, purification, and concentration), and is therefore employed at multiple steps of the overall downstream manufacturing process.[10]

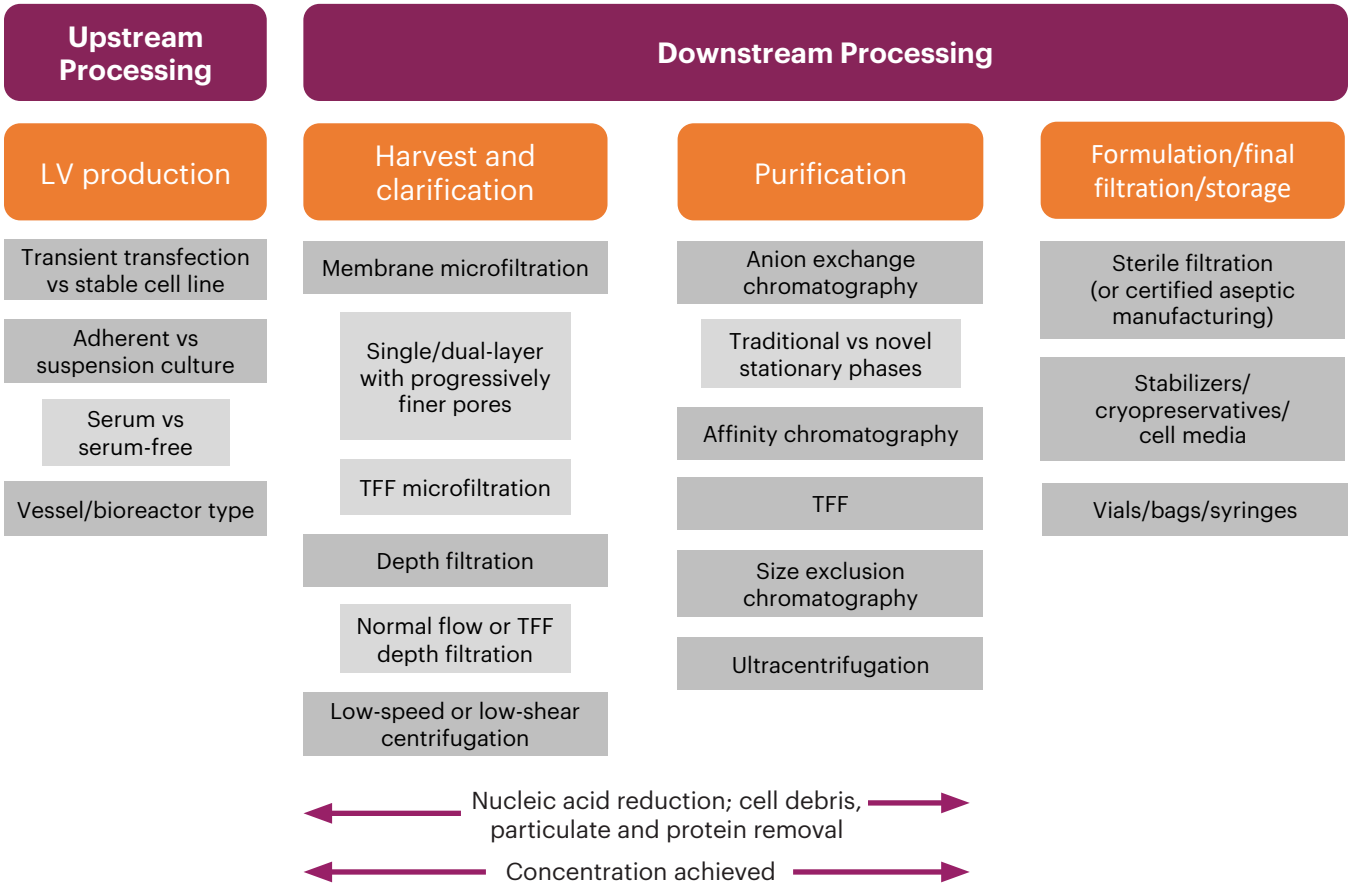
Final formulation

Formulation of LV can be performed via either chromatography (SEC) or TFF, with SEC being more limited to small-scale operations. Using TFF, the final buffer exchange step can be used to introduce a buffer system that provides maximal LV stability. Buffers commonly employed include HEPES, HIS-HCl (both resistant to pH drift with temperature change), TRIS, or phosphate-buffered saline. Buffer excipients are often added, such as recombinant human serum albumin (to promote stability) and sugars (as cryopreservatives and osmolarity regulators).[10,42] Alternatively, for *ex vivo* applications such as CAR T-cell therapies, vector can be formulated into the cell media used for culture of target cells.[55] Though proteins and sugars found in traditional cell media may help to stabilize the vector, formulating in cell media may not allow for optimal stabilization and could result in LV aggregation or adsorption onto container material. This can be particularly problematic if the CAR T cell product requires short-term freezing during transport.[10,42]

Sterile filtration or aseptic processing

Sterile filtration, commonly the final DSP step, utilizes a 0.2 or 0.22 μm filter to remove any aggregates or adventitious agents. Sterility assurance is a GMP regulatory requirement, but terminal sterile filtration is generally associated with LV loss of 30-50%.[10,45] Altering the timing of sterile filtration within the overall DSP sequence may improve LV recovery. For example, the downstream processing protocol developed by Oxford BioMedica has placed the sterile filtration step after the first TFF-based concentration step and before the second (final) TFF step to reduce vector losses due to adsorption to membrane material at very high vector concentrations.[56] Alternatively, the sterile filtration step can be skipped, provided that the manufacturing process is certified as being fully aseptic. Validation of aseptic processing (via media process tests) and use of a clean room (ISO 5) for all manipulation steps is required,[57] and processes that forego sterile filtration are likely to be examined closely by regulatory agencies. Incorporation of closed or semi-closed systems increases the feasibility of aseptic processing,[6,42,55] and a successful large-scale, semi-closed protocol that skips the sterile filtration step has been reported.[21] The

Figure 7-5: *Options for upstream and downstream processing of LVs*



alteration of DSP steps described by Oxford BioMedica and the option to omit sterile filtration altogether speak to the variability possible within DSP protocols. Options for technology and procedures to employ at various steps of upstream and downstream processing are shown in Figure 7-5.

Final vector product container closure

Equipment and materials used for final LV storage must be compatible with the vector and any excipients used to stabilize the vector, contain low amounts of leachables/extractables that could alter the quality of the vector, and be amenable to freeze/thaw cycles (when cryopreservation is likely). Physical adsorption to surfaces of syringes, bags, and glass vials has been reported with LV, with the suggestion that temperature, protein concentration, and hydrophobic interactions between vector and material surfaces can affect the rate and degree of adsorption.[58] Notably, careful choice of excipients can help to minimize adsorption to container surfaces, as demonstrated by Kumru et al. with the use of glass vials.[58] Rigid polymer vials and flexible bags are options for LV storage, and either may be suitable when using vector material as drug substance, as in the case of CAR T-cell production. Though tested with expanded T-cell cultures rather than LV material itself, ethylene vinyl acetate (EVA) bags and cyclic olefin polymer (COP) vials with rubber stopper-aluminum seal closures performed comparably in terms of container cryopreservation performance.[59]

Importantly, vector filled into bags with sterile weldable connections (e.g., those reported by Schambach et al. using Baxter Cryocyte™ Bags, Transfer Packs, and Plasma Transfer Sets) can enable closed processing and conformance with GMP standards during transduction of human cells for CAR T-cell products.[60,61] Bags that enable closed processing may be particularly useful during large-scale allogeneic CAR T-cell production. The choice of bags vs vials could also be influenced by the required dosing volume and whether vector is being used as drug product for direct injection/infusion. Optimization of fill volume

may help to reduce waste during transduction.[38] Similarly, larger bag sizes could have implications for the amount of product sacrificed during required sterility testing.

PROCESS CHARACTERIZATION

LV process validation is composed of the aggregate studies contributing to process knowledge (process design, process characterization, process qualification, and ongoing commercial monitoring). LV and cellular drug product process validation steps should be conducted at similar phases of clinical development for the overall program. Proper characterization of the LV manufacturing process is essential for minimizing risk and ensuring consistent quality of the vector. Throughout clinical development, process design is refined and critical process parameters (CPPs) are identified; the end goal is a consistent process that minimizes potential sources of process and material variability. Process characterization requirements may vary depending on the intended application of the LV (as drug product or drug substance), with formal process characterization studies implemented once the final commercial process is established. Vector used for *ex vivo* cell therapies such as CAR T-cell products is more appropriately defined as drug substance. As described in ICH Q8(R2)[62]:

"At a minimum, those aspects of drug substances… and manufacturing processes that are critical to product quality should be determined and control strategies justified. Critical formulation attributes and process parameters are generally identified through an assessment of the extent to which their variation can have impact on the quality of the drug product."

Process characterization is enabled by the application of design-of-experiment (DOE) approaches to determine how factors affecting the process impact or interact with the output of the process (with outputs generally representing the CQAs of the LV). Examples of where DOE approaches can be applied to optimize LV manufacturing steps include the following:

- Raw material inputs
- Temperature, pH, and time of manufacturing and hold steps
- Clarification and/or sterilization steps (e.g., filter material, pore size)
- Chromatographic approaches to purification
- TFF pressure and flow rates and/or centrifugation time and speed
- Choice and concentration of stabilizers and/or cryopreservatives
- Container closure systems

During process characterization, knowledge of CPPs generated from DOE studies are used to define the operating ranges for various process parameters that ensure consistent performance of the process. Analytical methods used in characterization studies should be scientifically sound (e.g., specific, sensitive, and accurate) and provide reliable results.[63] Stability of manufacturing intermediates, which may include material from collection or hold steps (e.g., temporary storage of bulk harvest, purification intermediates, concentration steps) should also be assessed. This helps to determine the maximum hold times and facilitates the establishment of process limits.[17]

Analytical data obtained from comprehensive process characterization are used to develop in-process controls (IPCs), consolidation of which serves as the controlling document for the manufacturing process. A well-characterized process, though time-consuming, not only lays the foundation for process qualification and validation, but also reduces the risk of lost batches (and wasted time and money) moving forward. A description of the DS manufacturing process and process controls must be included in Module 3 of the CTD; any changes or updates to the manufacturing process/controls as product development proceeds should be submitted as an amendment to the IND.[17]

PROCESS QUALIFICATION

Process qualification represents the stage at which the ability to repeatedly produce in-specification LV at commercial scale is verified through process performance qualification (PPQ). As defined in ICH Q7,[44] performance qualification represents: "documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly based on the approved process method and specifications."

Process qualification represents an important stage of overall process validation, which is executed by a written protocol that specifies the manufacturing conditions,

controls, testing, and pre-defined success criteria. Prior to performing PPQ runs, all utilities and equipment used in production should be shown as suitable for their intended use and able to perform properly. All raw materials used in PPQ must meet predefined specifications and the personnel, equipment, facilities, and manufacturing processes used must reflect those expected during commercial production. Additionally, all methods used to evaluate PPQ lots should be validated.

Process qualification and validation for LV manufacturing follows the general approach outlined in ICH Q7[44] and the FDA guidance *Process Validation: General Principles and Practices*.[63] Most original IND submissions will not include PPQ data, but demonstration of a protocol-driven PPQ with as many commercially representative lots as possible is required prior to filing a BLA. Given that the intended applications of LV can vary, early discussions with regulatory agencies can help to determine the level of validation required for a particular product and the degree of conformance to CGMP standards required.

Comparability

Changes in process parameters for LV manufacturing may require comparability studies to ensure that the pre- and post-change LVs are similar in terms of identity, purity, safety, and efficacy. In addition to showing equivalency of manufactured LV, comparability exercises may need to include a comparison of processes themselves. Depending on the clinical development phase of the product and the risk imparted by the change in the process parameter, comparability may require evaluation of the cell therapy product attributes in a paired-arm approach using the same cellular starting material transduced (e.g., splitting the leukapheresis starting material from the same donor) with both pre- and post-change LVs.[18] This may be particularly relevant when a new manufacturing site, scale, or strategy (e.g., automation) is introduced.[18] Data from CPPs and results of in-process controls should be examined to better understand the impact of introduced changes on the final LV preparation.[64] Guidance on comparability studies for LV can be found in ICH Q5E.[65] Again, early discussion with regulatory agencies can help to determine expectations for comparability studies.

Internal Versus External Manufacturing and Testing

As thousands of sponsors develop new cell and gene therapies (CGTs), there will likely be more reliance on contract development and manufacturing organizations (CDMOs) for production of therapies or of key components such as plasmid DNA and viral vectors. As such, CDMOs are key stakeholders in the production of viral vectors, and sponsors and CDMOs should create close partnerships.[66] Depending on the CGT sponsor's internal manufacturing expertise and capacity as well as the novelty of a therapy or component, a sponsor may choose to outsource production at any early or late stage of development (i.e., from process development, through process validation and ultimately commercial production). Because CGT sponsors generally seek to compress development timelines, it is essential that the sponsor and CDMO streamline the technology transfer process.[67]

Inherently, knowledge transfer between sponsor and CDMO is more complex for a CAR T-cell therapy than it is for an intermediate vector component. Many manufacturing processes for CAR T-cell therapies are unique to the sponsor, making the knowledge transfer to a CDMO more detailed and complicated. Conversely, the majority of CDMOs have well-defined vector production processes, and sponsors may request only a few simple modifications to certain parts of the established process (e.g., plasmid constructs) to maximize output. The VSV-G gene is subject to export control and associated licensure requirements, which is an important logistical consideration for transfer of plasmids between the CDMO and sponsor.[68] Exporting licenses may also be required when certain other transgene components (e.g., porcine teschovirus-1 2A [P2A], foot-and-mouth disease virus 2A [F2A] sequences) are included in the plasmid construct. Coordination between sponsor and CDMO is critical for LV production, particularly in the context of ensuring that all Bureau of Industry and Security (BIS) Export Administration Regulations (EAR) requirements[68] are met.

TRANSFER OF MANUFACTURING PROCESS AND ANALYTICAL METHODS

Technology transfer between sponsor and CDMO

Successful technology transfer may be facilitated by alignment of expectations early in the sponsor/CDMO relationship. BioPhorum recently published a set of recommendations, from a panel of sponsor and CDMO representatives, for ownership of various production-related responsibilities.[67] Their recommendations in five key areas relevant to technology transfer and process validation are listed below and can be incorporated in kickoff meeting documents or checklists:

- Process design (e.g., identification of CQAs, definition of the manufacturing process and control strategy, design of process characterization studies)
- Analytical methods (e.g., definition/qualification of in-process and release testing, design, and ownership of comparability studies)
- Supply chain (e.g., critical material sourcing, testing, and supply strategy; raw material variability assessment and qualification)
- Facility and equipment (e.g., process maps for equipment and personnel, assess CDMO facility and procedures against sponsor process design and requirements)
- Regulatory and quality (e.g., final product release strategy and discussion with regulatory agencies, regulatory filing preparation, definition of ongoing validation strategy)

Assay qualification and validation

Responsibility for identifying assays for product or component release and characterization (e.g., potency), in-process testing, and comparability studies appropriately lie with the sponsor, as they should define product requirements. However, given the CDMO's intricate knowledge of their own facility design/operations and previous experience with manufacturing similar products, they may be most qualified to recommend assays for adventitious agents and/or residual plasmid/producer cell DNA. The sponsor and CDMO often share responsibility for execution of assay qualification and validation. Unique product-specific assays may be best qualified and validated by the sponsor since those methods may be performed by the QC unit of the sponsor. Qualification of standard, compendial assays could be the responsibility of the CDMO.[67]

PROPRIETARY PROCESSES

Either the sponsor or CDMO (or both) can protect proprietary LV manufacturing steps within a Drug Master File (DMF). By definition, a DMF is a submission to the FDA that "may be used to provide confidential detailed information about facilities, processes, or articles used in the manufacturing, processing, packaging, and storing of one or more human drugs."[69] By design, the non-holding party can refer to material contained in the DMF within their IND. While the FDA will be able to reference material contained in the DMF, the non-holding party will not have access to the material contained within. As such, DMFs can represent a barrier to knowledge and technology transfer. Protection of proprietary information may be challenging in the EU—most sponsors want to conduct clinical trials and achieve commercialization in the EU but filing of DMFs is not allowed/afforded there. Additional regulatory guidance on DMFs can be found in Chapter 2.

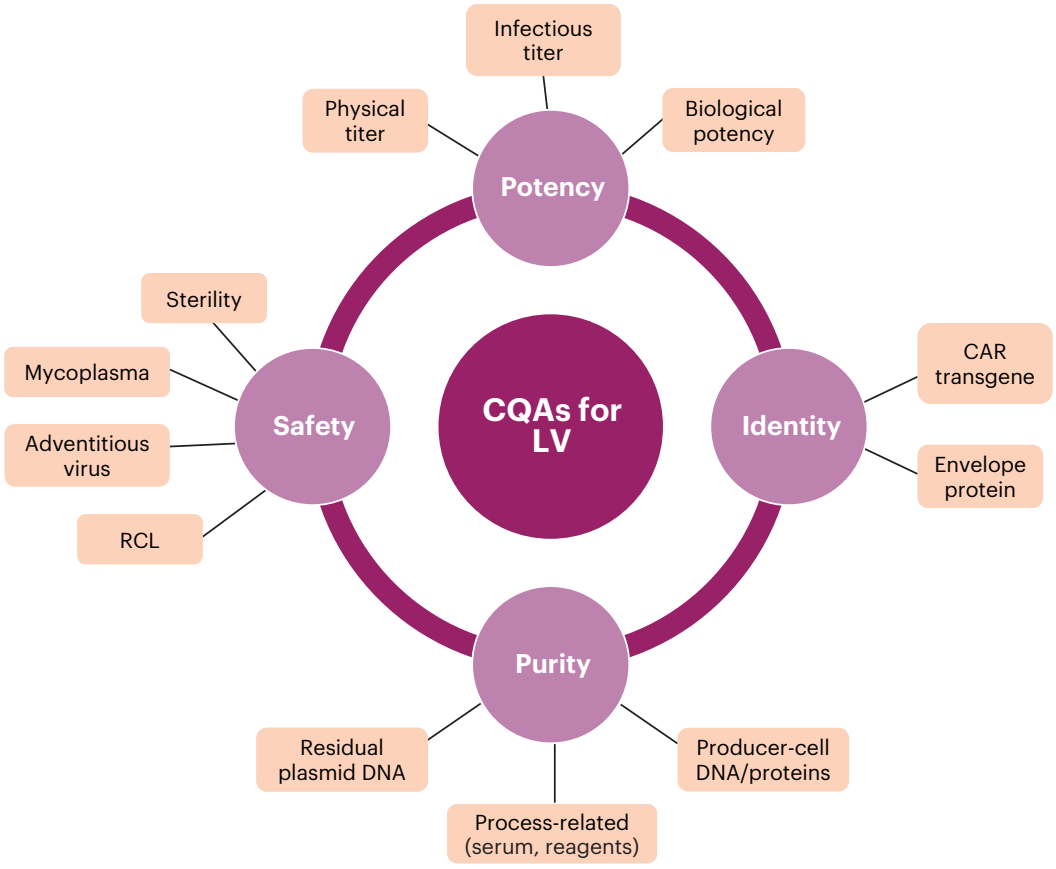
Product Testing and Characterization

Requirements for drug substance characterization to be reported within Module 3 of the CTD are outlined in the 2020 FDA Guidance: *Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)*.[17] Requirements for characterization generally increase as products move through different phases of development. Additional information can be found in the 2003 FDA Guidance: *INDs for Phase 2 and Phase 3 Studies: Chemistry, Manufacturing, and Controls Information*.[70]

CRITICAL QUALITY ATTRIBUTES OF LV

As a critical component of the final product, viral vector CQAs are important to consider early in the development of any CAR T-cell therapy. Information on identifying and defining CQAs can be found in Chapter 4. Examples of CQAs specific to LV are found in Figure 7-6.

Figure 7-6: *Examples of CQAs for LV to be used in the development of CAR T-cell therapies*



RELEASE TESTING

Testing is performed on every LV lot to confirm its quality and alignment with specifications set for each key attribute. The understanding of appropriate testing, and corresponding testing limits, grows as LVs progress through stages of clinical development and as the LV field matures. During early clinical development, a set of assays for LV quality should be performed after each process step to help determine which steps are most critical and which assays are best-suited to pick up on process changes that impact the final LV product. As the LV product moves toward commercialization, assay selection, assay timing, and specifications will be refined based on increased product knowledge. Examples of assays used for release testing and common references used to justify CQA specifications for LVs are included in Table 7-2.

ASSAY QUALIFICATION AND VALIDATION

According to ICH Q2(R1), the objective of any assay used to characterize a drug substance or drug product should be clearly understood.[94] Qualification and validation of the assay should center on the following:

Accuracy
Precision
Specificity
Detection limit
Quantitation limit
Linearity
Range

The main objective of assay qualification (performed during development to enable clinical release testing, typically with a reduced number of lots available that are fully representative of the clinical LV) and validation (performed in preparing commercial process validation with multiple lots of the final, fully representative product) is to robustly demonstrate that the assay will serve its designated purpose.[94] If changes in LV production or analytical procedures occur at any phase of development, revalidation of assays may be required. The need for revalidation will depend on the

Table 7-2: *Release testing for key LV attributes.*[10,24,71,72]

Attribute class	Specification attribute (determined by CQA)	Potential assay(s)	Common reference(s) for justification of CQA specification*
Identity	Transgene presence	PCR, ddPCR, NGS, Sanger Sequencing	21 CFR 610.14[73]
	Envelope	SDS-PAGE, MS, immunoblotting, ELISA	21 CFR 610.14[73]
Safety	Adventitious virus (human, bovine, and porcine if animal-derived materials used)	*in vivo* and *in vitro* assays	ICH Q5A (R1)[24] 9 CFR 113.53[74] 9 CFR 113.46[75] 9 CFR 113.47[76]
	Replication-competent LV	qPCR, PERT, cytopathology	Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. FDA Guidance for Industry, January 2020[77]
	Mycoplasma	PCR, RT-PCR, cell culture-based assays	USP <63>[78]
	Sterility	Culture-based assays	USP <71>[79]
	Endotoxin	LAL method: gel-clot, chromogenic, and turbidimetric	USP <85>[80]
General	pH	pH meter (potentiometry)	USP <791>[81]
	Osmolality	Osmometer	USP <785>[82]
	Appearance (color and clarity)	Visual	USP <631>[83]
Purity	Residual plasmid	qPCR	Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology: Replacement of Annex 3 of WHO Technical Report Series, No. 814. (2013)[84]
	Residual host cell DNA, total DNA	qPCR, Picogreen, DNA Threshold assay	Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology: Replacement of Annex 3 of WHO Technical Report Series, No. 814. (2013)[84] USP <509>[85]
	Host cell protein	ELISA, MS	USP <1132>[86]
	Residual serum/ nuclease/ transfection reagent/ solvent	ELISA, MS, chromatography	Serum: 21 CFR 610.15(b)[87] Nuclease: ICH M7(R1)[88] Transfection reagents: ICH M7(R1)[88] Solvent: USP <467>[89]
	Product-related impurities: Interfering particles, non-infectious particles	ELISA, MS	Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17]
	Visible particulates	Visual inspection**	USP <788>[90] USP <790>[91] USP <1790>[92]

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Table 7-2: *Release testing for key LV attributes.*[10,24,71,72]

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Attribute class	Specification attribute (determined by CQA)	Potential assay(s)	Common reference(s) for justification of CQA specification*
Strength/ potency	Physical/genomes titer	ELISA (p24), qPCR, RT-PCR, HPLC	Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17]
	Infectious/functional titer	Transduction of cells followed by quantification of the pro-viral DNA copy number by qPCR or by immunofluorescence with flow cytometry	Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17]
	Physical titer: infectious titer ratio	Calculation	Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17]
	Functional/biological potency (transduced primary cells)	Cell proliferation, cytotoxicity, cytokines	Potency Tests for Cellular and Gene Therapy Products. FDA Guidance for Industry, January 2011[93]

* Harmonization based on filing jurisdictions
** Visible inspection of all filled LV vials is a manufacturing criteria, captured on the batch record. However, some sponsors may include a visible particulate evaluation as part of the appearance method. Additional characterization methods for identifying inherent and intrinsic particulates include light microscopy, DLS, SEC-MALS, TEM, AUC, and FFF-MALS
AUC, analytical ultracentrifugation; ddPCR, digital droplet polymerase chain reaction; DLS, dynamic light scattering; ELISA, enzyme-linked immunosorbent assay; FFF, field flow fractionation; HPLC, high-performance liquid chromatography; LAL, Limulus amebocyte lysate; MALS, multi-angle light scattering; MS, mass spectrometry; NGS, next-generation sequencing; PCR, polymerase chain reaction; PERT, product-enhanced reverse transcriptase; qPCR, quantitative polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEC, size exclusion chromatography; TEM, transmission electron microscopy

extent of the change and its potential for impacting the final LV product. All relevant data collected during validation (and/or revalidation) should be submitted within regulatory filings.

Examples of assays used to confirm various identity, safety, purity, and potency attributes of the LV product are discussed in the sections that follow and listed in Table 7-2. Where applicable and established, Table 7-2 also provides common references used to justify attribute specifications; these may be of use when initially defining specifications and the QTPP in early-phase development. Chapter 9 of this document thoroughly discusses analytical technologies.

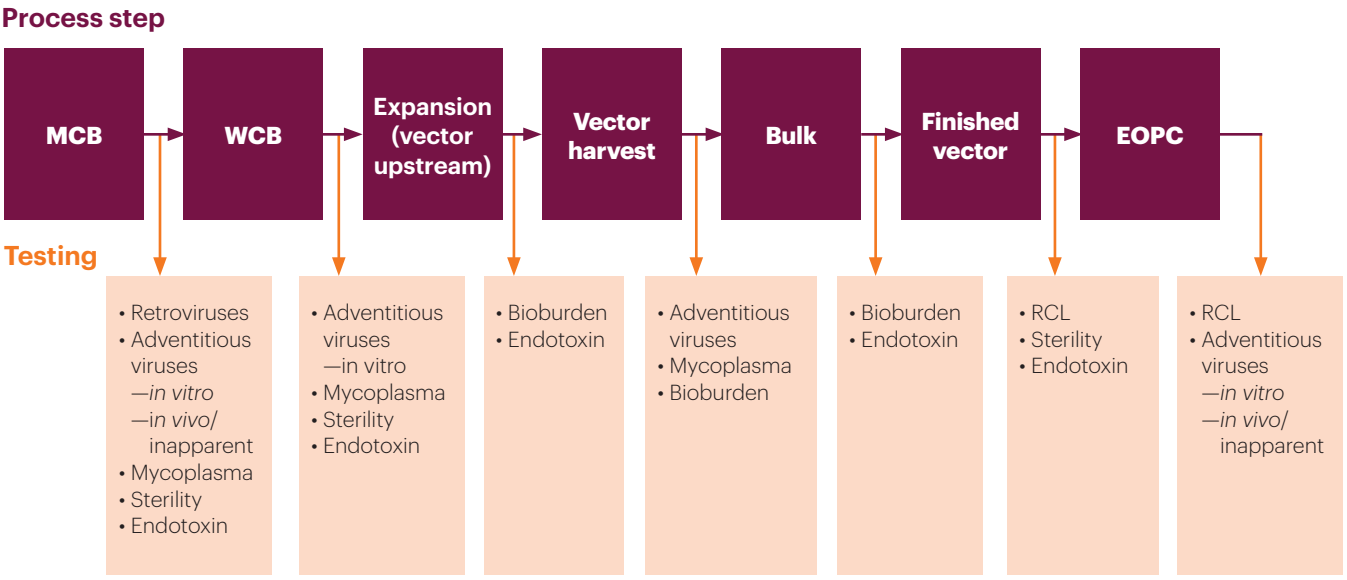
IDENTITY TESTING

Testing of LV is performed to ensure the identity of the transgene insert and to distinguish viral pseudotype (e.g., VSV-G, MLV envelope) if multiple vectors are manufactured in the same facility. Assays can be nucleic acid-centric and protein-centric. Examples of assays used to confirm the vector genome and quantify viral protein are listed in Table 7-2.

SAFETY TESTING

Testing LV preparations for adventitious virus, Mycoplasma, bacteria, and endotoxin follows procedures common to any sterile biological product (Table 7-2).[24,78,80] A safety concern specific to LV relates to

Figure 7-7: *LV production process timepoints for safety testing.*



the potential for replication-competent lentivirus (RCL). Though the plasmid systems used in traditional third-generation LV vectors make recombinant virus generation more of a theoretical than practical concern, regulatory agencies require extensive testing for RCLs in vector products.[77,95] Additionally, risk assessment and mitigation strategies to prevent the transmission of SARS-CoV-2 via cell and gene therapy products is recommended by the FDA.[96] Typical timepoints for safety testing during LV production are outlined in Figure 7-7.

RCL testing

Theoretically, RCL could develop at any step during manufacturing of the LV product or during the expansion of *ex vivo*-transduced cells in culture due to recombination of viral genes carried within the helper and gene transfer plasmid. Testing for RCL at each step of the drug substance (i.e., LV) and drug product (i.e., CAR T cell) manufacturing process would result in significant loss of material and time. The latest FDA guidance on RCL testing reflects the accrued evidence of safety associated with advances in LV design and testing. As such, the FDA no longer recommends RCL testing on WCBs and has revised recommendations regarding the amount of vector that should be tested.[77] Current recommendations for RCL testing are summarized in Table 7-3; techniques used for RCL testing are listed in Table 7-2.

Table 7-3: *Material, frequency, and type of testing recommended to ensure RCL-free vector and cell product.*[77]

Material	Frequency of testing	Testing for RCL
MCB (stable cell line)*	One-time	Cells and supernatant
Vector harvest material -End-of-production cells -Vector supernatant	At product release	Cells and supernatant
Ex vivo transduced cells	At product release	Cells only**

* Non-transduced packaging cell lines (MCB) are tested for retroviral activity of contaminating viruses
**If accumulated manufacturing and clinical experience demonstrates that the transduced cell product is consistently RCL-negative, an agreement may be reached with the FDA to reduce or eliminate testing of *ex vivo* genetically modified cells.

When cell testing is required, the FDA recommends testing 1% or $1x10^8$ (whichever is less) pooled vector-producing cells or *ex vivo* transduced cells by co-culture with a permissive cell line. When supernatant testing is required, the FDA recommends testing at least 5% of the total supernatant by amplification on a permissive cell line. The FDA also considers current manufacturing evidence, which indicates that <1 RCL/dose equivalent is an achievable level for vector preparations intended for clinical use. As such, sufficient vector supernatant should be tested to ensure a 95% probability of RCL detection if present at a concentration of 1 RCL/dose equivalent (i.e., the volume of vector that would be administered in a CAR-T dose based on LV volume used at T-cell transduction). The sponsor should detail the amount to be tested and provide a justification for the proposed testing volume within the IND.[77] Since RCL would theoretically co-purify with the LV product, the guidance could be interpreted to indicate that RCL testing can be performed on the final LV, which would confer additional material savings and ease of testing a smaller volume than the vector harvest.

SARS-CoV-2 testing

In its 2021 Guidance for Industry, the FDA outlined expectations for cell and gene therapy product manufacturers to perform a risk assessment that identifies, evaluates, and mitigates factors that may allow for transmission of SARS-CoV-2 via their products.[96] The FDA added that the risk assessment and mitigation strategies should be included in the appropriate regulatory submissions (e.g., IND, BLA, or DMF). The guidance notes that SARS-CoV-2 has been shown to be capable of infecting and replicating HEK 293 cells.[96,97] Considerations specific to LV production could include processes used to control viral spread (e.g., minimizing open manipulation steps) and contamination risk during manufacturing (e.g., employee screening practices). The FDA does not provide specific testing recommendations for source material, cell banks, in-process intermediates, or final drug products, though such testing may be included in a risk mitigation strategy based on the assessment of potential risk. The risk mitigation strategy could potentially include screening EOPC cells for SARS-CoV-2 RNA.

PURITY TESTING

Purity tests seek to ensure that the LV product is free from physical, biological, and chemical contamination that could affect the quality and safety of the final LV product. Generating a good understanding of impurities, and the toxicities they could confer, early in vector and process development will aid in developing acceptable tolerances in the final LV product.[66,71] Purity expectations set forth by regulatory agencies must be satisfied, and will continue to evolve as the field of biologic therapies advances.[72]

Residuals

Sources of impurities include the host cell system used to generate the LV product (e.g., residual host cell DNA and proteins), residual plasmids used for transfection, residual transfection reagents, non-functional vector (e.g., broken, immature, with insufficient envelope protein, or those that have failed to package the vector DNA[98]), residuals from culture medium (e.g., serum), and residuals from downstream purification processes (e.g., nuclease, chromatography resin components). Host cell and plasmid impurities in the LV product may interfere with the analytical methods used to test both the LV and the CAR T-cell product. The ability of the manufacturing process to reduce the amount, size, or activity of residual host cell and plasmid DNA should be demonstrated. In general, reduction of host-cell DNA to <10 ng per dose with fragments smaller than 200 bp is recommended.[84] Examples of technologies used to detect impurities and common references used for justification of allowable impurity levels are listed in Table 7-2.

Aggregates/Particulates

Biologically derived products, particularly those derived from viruses, viral vectors, or proteins, may contain inherent (those that are expected from the product itself) and intrinsic (generated within the manufacturing process, potentially including silicone oil, rubber, glass, or stainless steel) particles or agglomerates. In such cases, limits for visible particulates, which are generally considered a safety concern for patients, must be specified in the approved regulatory application. Drug products for parenteral administration, including difficult-to-inspect cell-based medicinal products, should be "essentially free of visible particles."[91,92,99] A position paper by Mathonet et

al. reviews best practices for visual inspection processes of biotechnology-derived products, including associated operator training, quality control sampling, testing, and setting of acceptance criteria that correspond to "essentially" or "practically" free of visible particles.[100]

Aggregates of DNA (host-cell or plasmid-derived) can form during various stages of LV production[72] and downstream processing steps (and their order) can influence the formation of aggregates. For example, if nuclease is applied late in DSP, large-sized residual nucleic acid contaminants present after harvest and during early DSP steps can capture vector particles, forming aggregates that could result in vector loss.[6] Particulates should be characterized to confirm identity of inherent and intrinsic nature.[101] Methods and technologies used for particle and aggregate detection, identity, and common references used for justification of allowable levels are listed in Table 7-2. Testing for visible particles that may arise from LV aggregates is also part of stability testing (briefly discussed later in this chapter), as their formation can be influenced by storage containers and conditions.

POTENCY TESTING

Potency is the quantitative measure of biological activity (generally expressed in units) resulting from a particular quality attribute. Potency or strength measures for LV include physical titer and infectious titer. Physical titer is expressed as the number of viral particles per mL and reflects the total number of viral particles present (active and inactive), whereas infectious titer measures how much vector is available to transduce a cell and is expressed as transducing units per mL. Infectious titer is always lower than physical titer, but provides a more accurate representation of the transducing ability of the vector.[71]

An emerging expectation from regulatory agencies relates to biologically relevant potency.[18] In the case of CAR T-cell products, biological potency centers around how the LV (e.g., titer, transduction) contributes to CAR expression and cytotoxicity. Expectations for demonstrating a solid understanding of LV contribution to biological potency grow as the CAR T-cell product proceeds through product development stages. By the time of pivotal clinical trials, characterization of the biological potency conveyed by LV should demonstrate that its contribution to the eventual CAR T-cell therapy is well understood. Examples of technologies used to assess potency of a LV product are listed in Table 7-2.

Multiplicity of infection

In *ex vivo* applications, Multiplicity of Infection (MOI) is a parameter used to predict how LV titer (typically measured with an *in vitro* assay, and/or in a non-primary cell line) correlates to infectivity of the primary target cell. Since transducing titer may range between LV lots, determination of the MOI allows for a variable volume of LV to be used during CAR-T manufacturing based on corresponding LV titer where:

MOI = LV titer (Transducing units/mL) × Transduction volume (mL) / number of target cells

In theory, MOI should have a linear relationship between titer and infectivity of primary cells.[102] In reality, determination of MOI is complicated by factors such as target cell health, LV stability, transduction reagent, incubation time, media exhaustion, and batch contamination.

Because of the inherent variability of the behavior between LV and donor T cells, special care should be taken to determine MOI during LV and CAR-T process development. Like other LV and CAR-T parameters, establishing an accurate MOI depends on product knowledge and process consistency. The ultimate goal is to set an MOI in the CAR-T batch record that will produce consistent CAR-T product quality attributes. Examples of MOI application for CAR-T manufacturing purposes are provided in Table 7-4.

MOI as a CAR-T manufacturing process parameter is determined during CAR-T process development by testing a titrated range of LV on primary cells[103] in a scale-down model and confirming the candidate MOI in full-scale development runs.[18] Due to LV batch-to-batch variation, MOI often needs to be determined experimentally for each lot of LV by transduction with serial dilutions.[18] Titration data from multiple LV lots can be combined to determine the final MOI, a manufacturing unit operation, for the CAR-T process. In CAR-T, characterization studies to titrate LV transduction efficiency to determine response curves show that increased MOI correlates to increased vector copy number and potency of the primary (i.e., CAR-T) cell, with an eventual plateau.[104] The approaches listed here reflect the industry

Table 7-4: *Examples of MOI application for CAR-T manufacturing purposes.*

MOI (set parameter)	10		
Viral titer (variable)	$1x10^8$ TU/mL	$1x10^8$ TU/mL	$1x10^7$ TU/mL
Number of target cells (may be variable due to donor cell growth)	$100x10^6$ cells	$75x10^6$ cells	$100x10^6$ cells
Transduction volume (input to manufacturing process)	10 mL	7.5 mL	100 mL

standard to date. However, the *Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products Draft Guidance for Industry*[18] provides additional clarity on MOI determination, such that a lot release test (i.e., qualified and listed on vector specification) to determine the vector concentration that can be used to normalize the amount of vector used for CAR-T transduction is recommended.

The ideal MOI will allow maximal transduction of target cells, with minimal toxicity[105] and little LV waste. Employing a characterization study to titrate MOI during development is beneficial in the long-term, as an MOI set higher than necessary for GMP production would result in excessive use of expensive vector product.

STERILITY TESTING

Principles of sterility testing for LV products generally follow those outline in USP <71>.[79] However, LV used as a drug substance (e.g., destined for use in an *ex vivo* CAR T-cell product) may have some latitude with sterility testing because it's not a direct injectable.

USP <71> states that, for liquid products with a final volume of >100 mL per container, a minimum of 10% of the contents of the container (but not less than 20 mL) must be used for sterility testing on at least 2 different media. USP <71> also states that, for batches containing more than 100 but less than 500 containers, a minimum of 10 containers must be tested. Adhering to these requirements, particularly for LV packaged in bags (to enable closed processing during transduction of human T-cells), could result in substantial loss of product and could substantially delay production of the final CAR T-cell product. Importantly, the minimum quantity and number of containers to be tested are qualified in USP <71> as being subject to other justification and authorization, suggesting that some latitude in sterility testing may be afforded. The sponsor's sterility testing schema should be outlined in the LV Justification of Specification and aseptic control strategy (if aseptic processing is implemented in lieu of sterile filtration).

Stability

Stability testing is conducted throughout the lifecycle of LV development and serves to provide key information on how the vector is influenced by temperature and storage container. According to ICH Q1A (R2), "stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes."[106] As such, stability assays focus on assessing key CQAs of the LV product, including those related to identity, purity, and potency.[107] Results of stability studies help to establish the appropriate shelf-life of the vector and optimal storage conditions.[17]

As with drug substance characterization, regulatory requirements for drug substance stability studies (and the level of result detail) generally increase as drug products move through advanced phases of development. A summary of available stability data, and an outline of ongoing study design for the DS, should be included in each DS-related section of Module 3 of the CTD. Results from ongoing stability studies should be updated in the IND on a regular basis. Information on these requirements can be gleaned from FDA guidance (Table 7-5). The nature of the particular drug substance

Table 7-5: *FDA guidance on stability program requirements for drug substance*

Guidance document	Applicable guidance
Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). January, 2020[17]	The types of stability studies (either conducted or planned) that will be used to demonstrate that the DS is within acceptable limits are recommended to be described in the original IND submission. Stability studies may evolve with product development. As product development progresses, consideration of stability studies required to determine an expiration date is warranted and these items should be discussed at late-phase IND meetings. Information on qualification of analytical procedures used to generate stability data should be included in the original IND.
INDs for Phase 2 and Phase 3 Studies: Chemistry, Manufacturing, and Controls Information. May, 2003[70]	Any changes to the DS stability program from that described in earlier phases should be provided in an information amendment. Stability data from clinical trial materials used in Phase 2 and Phase 3 studies should be provided in annual reports as data become available. During Phase 3, a stability protocol to be used for formal stability studies should be developed to ensure appropriate stability data are generated for filing at the NDA stage.

will determine which tests should be included in the stability program,[70] and expectations for the DS stability program should be discussed during early meetings with the FDA to ensure smooth transition through different phases of development.

A variety of analytical tools can be used to conduct stability studies. Thorough discussion of analytical methods can be found in Chapter 9. The stability-indicating properties of methods can be assessed during assay development by testing with vector treated by forced degradation conditions (such as multiple freeze-thaw cycles).[17] Special considerations for vector stability include maintenance of infectious titer and functional potency. The potential for viral protein aggregates, which can create immunogenicity issues when the final drug product is administered to a patient, must be assessed during long-term storage. Aggregation detected in the stability studies, as evident by visible particulates, indicates unstable formulation of the final vector drug substance during long-term storage. Acceptance criteria for real-time stability studies should be derived from the LV release specification and available stability data. As release specification acceptance criteria are refined after accumulation of product knowledge, stability study acceptance criteria can also be updated to match the release criteria. However, differences between the acceptance criteria of the stability studies and the release specification may be justified based on the observations from the real-time stability studies.[106] For instance, increased degradation products (impurities) may be acceptable in stability studies with sufficient justification.[108]

VECTOR BULKS

For drug substance bulk material that is to be stored after manufacture but prior to formulation and final manufacturing, ICH Q5C[107] states:

> "…stability data should be provided on at least 3 batches for which manufacture and storage are representative of the manufacturing scale of production. A minimum of 6 months stability data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug substances with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis."

The quality of DS batches used in the stability program should be representative of the quality of material used in preclinical and clinical studies as well as that to be made on a production scale.[106,107] In essence, your bulk stability protocols should provide data to cover

Table 7-6: *Examples of stability studies for bulk vector (IND to commercial).*

Phase	Study type	Temperature	Quality	Lots	Method Status	Study duration
Preclinical (IND-FIH)[106]	Long-term	Nominal	GMP (Dev OK)	3	Qualified	12 months[17,109]
Phase 1[106]	Long-term	Nominal	GMP	1 to 3	Qualified	12 months[17,109]
Pivotal (registration lots)[110]	Long-term	Nominal	GMP	All PPQ	Validated	12 months[17,109]
Pivotal (registration lots)[110]	Stressed & accelerated	˚C -20, +5	GMP	3	Validated	12 months
Commercial	Long-term	Nominal	GMP	Up to 3	Validated	12 months[109]

the period between manufacturing of the bulk to the initiation of fill finish (Table 7-6).

FINISHED VECTOR

ICH Q5C states that "stability information should be provided on at least 3 batches of final container product representative of what will be used at manufacturing scale."[107] Though this recommendation is specific to drug product, even vector intended for final use as a drug substance (as in the case of LV utilized in CAR T-cell therapies) should likely be held to the same standard. Similarly, a minimum of 6 months stability data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested.

Retest periods and shelf life for stability should be based on real-time data obtained at the long-term storage condition. According to ICH Q1A(R2), for drug substances intended for storage below -20°C (as is the case with cryopreserved LV, which is often stored at a nominal condition of -80°C), the retest period for stability should be "treated on a case-by-case basis."[106] Stability protocols for finished vector should provide data to cover the full development period (Table 7-7).

STRESSED AND ACCELERATED STABILITY STUDIES

In its Q5C and Q1A(R2) guidance, the ICH strongly suggests that studies be conducted on the DS (in this case, finished vector) under stressed conditions.[106,107] To perform accelerated (conditions that should cause only a small amount of product degradation) and stressed (exaggerated storage conditions that introduce significant degradation to the finished vector) studies, temperature increases are applied to the vector to intentionally degrade it. Results of these studies help sponsors to understand how and to what extent the vector is degraded, what degradation products result, and can inform adjustments to vector formulation that could potentially minimize degradation and provide a data set to evaluate product risks for storage or shipping and handling temperature excursions.

CONTAINER CLOSURE SYSTEM

Stability studies on vector bulk substance should be conducted within containers used during production, whereas studies on finalized vector should be performed on DS packaged in a container closure system that is the same as (or simulates) the packaging proposed for storage and distribution.[106] Smaller-scale vessels may be used for vector bulk and finished vector stability studies, but the containers should be fully representative of the final container construction materials and closure system. Small-scale stability samples should also be filled in a manner proportional to that of the final container surface area and headspace. The suitability of the container closure system must be discussed in part 3.2.S.6 of Module 3 of the CTD. Specifically, "the suitability should be discussed with respect to, for example, choice of materials, protection from moisture and light, compatibility of the materials with the drug substance, including sorption to container and leaching, and/or safety of materials of

Table 7-7: *Examples of stability studies for finished vector (IND to commercial)*

Material	Phase	Study Type	Temperature	Quality	Lots	Method Status	Study Duration
Finished vector	Preclinical (IND-FIH)[106]	Long-term	Nominal	GMP (Dev OK)	3	Qualified	3 to 5 years[17,106,109]
Finished vector	Phase 1[106]	Long-term	Nominal	GMP	1 to 3	Qualified	3 to 5 years[17,106,109]
Finished vector	Pivotal (registration lots)[107]	Long-term	Nominal	GMP	All PPQ	Validated (PPQ)	3 to 5 years[17,106,109]
Finished vector	Pivotal (registration lots)[106,107]	Stressed & accelerated	˚C -20, +5, room temp, +36	GMP	3	Validated	72 hr to months
Finished vector	Pivotal (registration lots)[106,107,111]	CCIT	Nominal	GMP	3	Validated	3 to 5 years
Finished vector	Pivotal (registration lots)[107]	In-use conditions: Stability of manufacturing intermediates during process characterization		GMP	3	Validated	In-use conditions and holds
Finished vector	Commercial	Long-term	Nominal	GMP	Up to 3	Validated	3 to 5 years[110]

construction." Additionally, container closure integrity evaluations are required throughout the development of sterile products. During clinical stages, sterility testing of samples held at nominal conditions until the end of the study can be employed to confirm the continuing sterility throughout the product's shelf life or dating period.[111] In preparation for commercial filing, container closure integrity testing with validated methods to assess container ingress (such as dye, microbial, and gas) can be implemented in the stability studies.[111,112]

EXTRACTABLES AND LEACHABLES

The assessment of extractables and leachables is important for any DS or DP that will be maintained in a container during production hold steps or final storage/shipping. Depending on the exact means and extent of DS integration into the final DP, and the time that the DS spends in contact with container material, full extractable and leachable studies could be warranted, particularly if a novel container closure system is implemented (such as flexible bags, etc). That decision should be based on a thorough assessment of the risk that any leachables contained in the DS could pose to the final drug product. Extractables are compounds that can be extracted from the container closure system in the presence of a solvent. Extractables are tested for in the container material and represent a *potential* impact to the DS. Leachables are compounds that leach into the final formulation from the container closure as a result of direct contact with the formulation. Leachables are tested for in the final formulation itself (contained within the closure system) and represent *actual* impact to the DS.[113] Areas of concern with extractables and leachables relate to vector CQAs of efficacy and safety. At a minimum, discussion of the DS container closure system in Module 3 of the CTD should include suitability of the system with respect to leachables, and every company should evaluate and provide a profile of potential substances that could leach into the bioprocess. In the case of CAR T-cell therapies, it's possible that leachables present within DS may be sufficiently diluted during media-exchange and other steps of the CAR-T manufacturing process.

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CHAPTER 8

Manufacturing of Cell-Based Therapies

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Introduction to Cell-Based Therapy Manufacturing

Cell-based therapy products can be categorized as being either autologous or allogeneic products. Autologous products are patient-specific and are derived from the patient's own cells. Allogeneic products are non-patient specific, often sourced from cells of donors, and may be genetically modified, for example by knocking out the T-cell receptor (TCR) complex, to mitigate against adverse immune responses. Allogeneic and autologous products each present their own unique challenges for manufacturing products that meet the required standards for safety, efficacy, and quality.

While a lot can be learned from small molecules and biologics manufacturing (e.g., monoclonal antibodies), the fundamental difference in the product and value chain between cell-based therapies and traditional biopharmaceutical products is in the different manufacturing paradigms related to the drug substance (DS) and drug product (DP). In cell-based therapies, the DS contains the active cell product (manipulated or nonmanipulated) that

holds the therapeutic potential, and DP is the formulated DS that is filled into final containers that are ready for delivery to patients as therapy. For traditional biopharmaceuticals, separate manufacturing facilities, staff, and schedules for manufacture are typically employed for the DS and DP. As such, storage and transport between the DS and DP location can be critical. On the other hand, for cell-based therapy, while there is often a clear distinction between DS and DP, the same staff and facility are often used for the full process. Fill and finish may be performed separately in a contiguous portion of the facility, where careful considerations should be made on the hold times between the process portions to ensure minimum impact on product quality. In addition, for autologous therapies in particular, other manufacturing distinctions apply, such as a batch size of 1 and therefore implications for release testing and exceptional release, variability and limited availability in starting materials, and lack of terminal sterilization. Many of these concepts are discussed in this chapter.

This chapter will cover the principles related to cell-based therapy manufacturing, in particular for a CAR-T example, including the acquisition of starting materials, activating and engineering the cells to produce the desired targeting moieties (e.g., chimeric antigen receptor [CAR] or TCR), expanding the cell population to achieve the desired clinical dose, harvesting the cells, formulation of the DS, and finally, cryopreservation and storage of the final product. Many additional considerations are highlighted, such as the source of the starting materials from patients (e.g., autologous) or from healthy donors (e.g., allogeneic), the connectivity between each unit operation, and the requirements for strict aseptic processing, as there is no final filtration or terminal sterilization step in manufacturing cellular therapy products.

Additional considerations are discussed at the end of the chapter regarding operational components of producing CAR-T products. These include the consideration of electronic systems for manufacturing execution (MES), quality management (QMS), release testing and laboratory information management (LIMS), document management (DMS), and other systems designed to ensure track and trace abilities, enhanced documentation, and robustness of the manufacturing and testing processes.

STARTING MATERIALS

Quality therapeutic products start with quality raw materials, whether those materials are a set of chemical or biologic reagents, or the living cells that form the basis of a CAR-T cell therapy. Whether the cellular starting material originates from the patients themselves (autologous) or healthy donors (allogeneic), cells are usually collected through a process called apheresis, where blood is separated into its components as it passes through an instrument. The cell type of interest (i.e., white blood cells in the case of CAR-T therapies) is collected into a bag, while the other blood components are returned to the patient. Other cell sources and collection methods are available for other types of cell-based therapies.

Technical and engineering challenges related to starting materials arise as cell-based therapy products progress towards commercialization. The increased number of patients/donors and collection sites driven by the increased commercial demand result in the increased variability in cellular starting material, which is cited as one of the major reasons cell therapy products fail manufacturing runs,[2,3] therefore driving standardization efforts on the apheresis process.[4] Implementation of donor eligibility criteria as well as technical patient consideration ensures safe administration to patients and successful collection procedure, mitigating the risks in manufacturing the therapeutic product. Further discussion on the apheresis process and its challenges, technical considerations related to the instrument and protocol optimization techniques, patient considerations, and donor screening and testing are covered in Chapter 5.

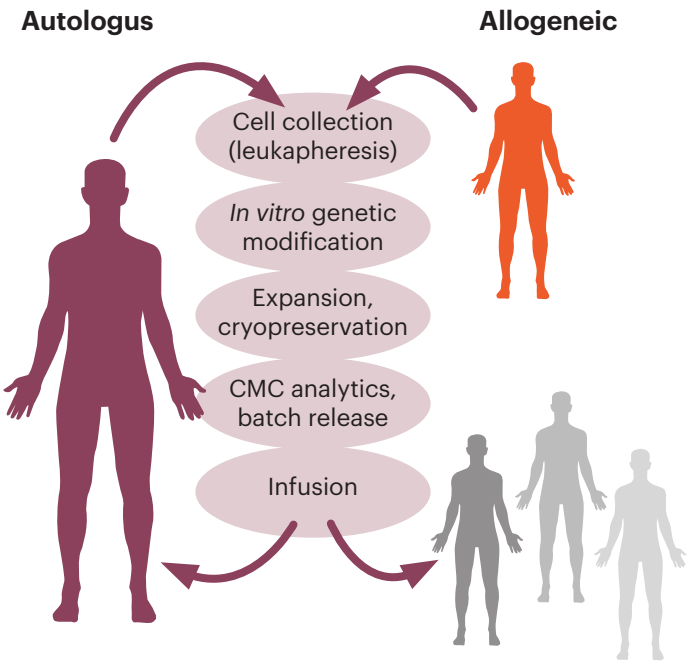
ALLOGENEIC vs. AUTOLOGOUS CONSIDERATIONS

Autologous and allogeneic cell-based therapy manufacturing process differences are reflected in the following simplified descriptions:

An autologous cell therapy requires on-demand manufacture, beginning with apheresis collection from a patient and ending with release of the product for imminent use in that same patient.

An allogeneic cell therapy is manufactured by beginning with apheresis collection from a donor and ending with release of the product for off-the-shelf use in many patients.

Figure 8-1: *Autologous and allogeneic generation of cell therapy products*



While both approaches share common challenges (e.g., cell sourcing, raw material qualification, product testing and stability, cold chain shipping logistics), each approach presents its own opportunities and challenges. The patient-specificity of the autologous therapies has the primary advantage that the eventual DP is native to the patient's body. The DP, therefore, should naturally avoid rejection by the patient's immune system. Additionally, it should not actively reject the patient's body (i.e., no risk of graft versus host disease, GvHD). However, these potential efficacy and safety advantages are met by the inconvenient reality that patients can vary significantly in age, health, weight, disease stage and burden, prior treatment history, etc., all of which can impact the quality or quantity of the starting material for the manufacturing process (i.e., the patient's own T cells). Variability in the starting material naturally presents the potential for significant variability in the cell therapy manufacturing process. But this inherent batch-to-batch process variability is not necessarily a significant issue when the process only needs to produce a single dose for a specific patient.

Allogeneic therapies, on the other hand, can in theory provide the opportunity for greater economic viability and impact a larger number of individuals by producing batches capable of treating many patients. Accordingly, the allogeneic process must provide more consistent production of these larger batches. In contrast with the autologous paradigm, the necessary optimization of process and product performance within allogeneic cell therapy production benefits from the ability to better control the donor population for the starting leukapheresis material. Donors may be selected by simple assessment of age and health status or by a more complex consideration of cell characteristics (e.g., phenotype) that are theorized to result in the desired high batch yields and consistent product quality.

Process development and characterization efforts also benefit from this improved control, since the starting material for these development activities will more naturally resemble that for the subsequent clinical manufacturing process. As a final note, the potential immunogenicity of the allogeneic DP does present a greater potential safety risk (i.e., acute rejection and via GvHD) for the patient. Therefore, additional genetic modification and cell selection/depletion steps during production are necessary to minimize this immunogenic risk, and further release testing is necessary to demonstrate sufficient clearance of this risk.

In summary, an autologous cell therapy process must overcome greater variability in the starting material due to the inherent variability among individual patients. The allogeneic cell therapy process must overcome the potential challenges associated with a more complex, larger scale process, a more complex safety profile due to the risk of GvHD, and the demand of generating consistently large batch yields. Specific disease prevalence and patient population are factors to take into account when deciding an autologous vs. allogeneic approach. For diseases with a small patient population, an autologous approach may still be favorable. On the other hand, the upside potential of developing an in-theory economically favorable, off-the-shelf product that can positively impact a much larger number of patients per batch encourages the allogeneic pursuit.

Aseptic Processing

Traditional biopharmaceutical manufacturing processes use terminal sterilization and/or filter sterilization to destroy or remove contaminants and ensure final product sterility. However, a specific challenge for cell therapy products is that terminal sterilization or filtration cannot be used. This is because viable cells or cell-derived preparations, which are the critical elements of final cell therapy products, cannot withstand terminal sterilization procedures without being killed and are too large to pass through sterilization-grade filter membranes. Additionally, due to the timing and logistics of treatment delivery, product sterility test results are not always available before administration of the product to patients. Thus, in cell therapy product manufacturing settings, all process materials, equipment, and surfaces are pre-sterilized using validated sterilization methods, and aseptic processing must be used during production to prevent the introduction of contaminants during all steps of the manufacturing process.

Aseptic processing can be defined as the handling of materials in an environment where the air supply, materials, equipment, and personnel are regulated to control microbial and particulate contamination. Aseptic processing is required from the first process step where sterility is considered necessary, to when the container system with the final product is closed, through the product delivery to the patient. Successful aseptic processing can be achieved by implementing the following measures during operations:

- Provisioning personnel controls (gowning and aseptic technique training and protocols)
- Environmental monitoring for quality and control
- Validated cleaning and sterilization procedures of containers, equipment, disposables, tools, and surfaces that come into contact with a DS or DP

A powerful tool for evaluating aseptic process robustness is aseptic processing simulation (APS), in which a process is run and analyzed under the worst-case contamination risk conditions. Unlike in traditional biopharmaceuticals where the focus of APS is in the final fill process (due to the use of terminal sterilization prior to the final fill), the burden of APS in cell therapy manufacturing rests on a more complete representation of handling throughout the process. Cell therapy product developers must also consider manufacturing process boundary conditions unique to the context of cell therapy. For example, developers must make sure that both initial cell/tissue harvesting and, if required, transport to the patient's bedside are done under aseptic conditions. By conducting operations using aseptic processing, developers can maximize their chances to avoid the high cost of reprocessing or producing replacement lots in cases of substandard product quality. Ensuring a successful lot is critical as many cell-based therapies are used to treat acute, malignant indications where the patient often doesn't have the luxury or health to wait for a replacement batch. Aseptic processing also assures patients and regulatory authorities that products are manufactured with safety as a top priority.

OPEN vs. CLOSED SYSTEMS

Cell therapy product manufacturing must, at all times and in all ways, account for the possibility of contamination introduced during steps in the manufacturing process. This is because contaminants can undermine the safety and efficacy of the product and introduce risks for patients. While all biopharmaceutical processes are subject to such concerns, it is extremely important for cell therapy product manufacturing processes to minimize contamination because they involve operations upon living cells. As such, cell therapy product developers must carefully consider whether to implement their manufacturing steps as open operations or closed operations.

In open operations, more common for traditional biopharmaceutical manufacturing and earlier phases of clinical trials, production occurs with process materials exposed to the room environment. By contrast, closed operations utilize facilities and equipment designed to ensure that DS, intermediates, and DP are not exposed to the room environment at any point during production. In recent years, due to cost and efficiency considerations, and to good manufacturing practice (GMP)-related guidance from regulatory agencies, there has been an industry-wide transition toward implementing closed

operations wherever possible. Cell therapy product developers benefit greatly by learning from this trend and supporting technologies that increase the adoption of closed operations. Additionally, technology developers are introducing new equipment to enable closed cell processing steps at an increasing rate.

Implementing closed operations, at any stage of research or production, reduces contamination risk by adventitious agents. This is because closed systems, by design, use components that minimize the need for human contact with the operating system, and provide physical barriers that serve both to reduce the risk of contamination by preventing contact with the room environment and contain the product or intermediate in a location away from sources of possible contamination. In addition, a closed operating system allows materials to enter or leave a system via predetermined control paths. As an additional benefit, because closed operations are often achieved by using specifically designed "plug and play" equipment that can easily be set up and launched by personnel, there is often a significant reduction in time and steps required for validation and operations. Given the high costs for space in biopharmaceutical manufacturing settings, dedicating too many production areas for separate cleanrooms (which are essential for minimizing contamination risks in open operations) and gowning areas can lead to unnecessarily excessive cost of goods. Closed operations can allow for more efficient use of space, because closed-systems allow for multi-patient batches to be produced in the same room, and fewer rooms needing to be designated for components or equipment (which are designed to have their own physical barriers) used in the system process. For more details on facility design considerations for cell-based therapies, refer to Chapter 11.

In practice, closed systems are often more precisely described as being functionally closed. This is because process systems may be routinely or occasionally opened to perform a maintenance procedure, add a reagent (e.g., media addition in a bag inside a BSC), or install a new component, after which the system is returned to a closed state through sterile welding or aseptic connections to the subsequent unit operations, or a sterilization step prior to use in the manufacturing process (subject to follow-up validation). Also, there may be situations where open processes are housed within isolators that comprise the closed systems. These situations are also functionally closed and demonstrate the ongoing transition from open to closed operations as a general feature of evolving industry practices. Process closure can be implemented within the context of a larger operating framework that involves multiple cleanroom suites that each constitute modules of the overarching production process (with the aim of maximizing productivity of each module). Since these modules are separated, the manufacturing process as a whole is comprised of both open and closed aspects. Developers must balance individual goals for productivity against the risk-reducing features of system closure on a case-by-case basis. An example closure analysis of a CAR-T process has been performed,[5] which includes assessment of contamination risks in each unit operation as well as mitigation strategies.

Several factors can pose challenges for developers to implement maximally closed operations, in large part because the use of closed manufacturing processes across the industry is still in its relatively early days. To begin with, open operations have been an established part of biopharmaceutical manufacturing. A number of facilities may require major redesigns to eliminate open operating procedures and will, therefore, require developers to contend with potentially high upfront costs and logistical challenges. And, if developers require parts from multiple suppliers, cases might arise where components sourced from different suppliers are incompatible and developers may need to establish custom guidelines for use across suppliers or may need to arrive at other solutions. Furthermore, since a lot of closed system equipment is relatively new to the manufacturing space and is designed with very particular functions in mind, personnel will need proper training to handle unfamiliar or sensitive process elements such as tubing, valves, and connectors. Finally, with current GMP (CGMP) guidelines put forth by regulatory authorities such as the FDA and the EMA, developers that establish closed systems must provide evidence that their systems are fully closed to the extent that satisfies the definitions set by the relevant guidelines. This will require developing testing protocols and validation steps that adequately demonstrate closure.

PHYSICAL CONNECTIVITY

Single-use connection technologies exist wherever a connection is made that allows materials to flow through during the manufacturing process or during final product delivery. Connectors are an essential part of any technology to facilitate the flow of materials from one unit operation to another without any leaks or breaks in sterility, which could jeopardize product sterility or loss of product that will affect patients' safety. Connectors and proper connectivity are vital to ensure that the integrity of every point of the process is always maintained. Connector type selection is also important. An aseptic, fully closed connection can be made within a process through aseptic connectors, such as AseptiQuik®, Opta®, Kleenpak™, or sterile welding. Aseptic connection could be a repetitive process; therefore, picking the right aseptic connector that is easy to use, genderless, and allows for an effective, quick, accurate leak-free connection is critical.[6] A challenge that remains is that while sterile connections are growing in availability, the disconnection process is not always sterile and still requires controlled space to disconnect materials. Alternatively, open style connections, such as luer and MPC, can be used, but developers must take into consideration that these connections have to be made in a highly classified environment, such as a positive displacement laminar hood, to avoid contamination during material transfer. Developers must consider the pros and cons of each connector to ensure they use the best connector for each process,[7] so the process must be designed ahead of time with consideration of process scaling for large scale manufacturing to meet patient demand.

Testing of critical components such as connectors must be performed both upstream, to protect the cell line, and downstream, especially in critical operations after filtration, to ensure these components do not introduce particulates, bioburden, leachables, or extractables into the final product, which could affect patient health.[8] Two organizational bodies have created guidelines for extractables and leachables on single-use assemblies: US Pharmacopeia[9] and BioPhorum.[10] Smaller volume samplings are performed during the cell therapy manufacturing process (for discussion on in-process sampling, refer to Chapter 9); therefore, connectors must be carefully selected when used for in-process sampling (e.g., sampling manifolds) to maintain process sterility while minimizing product waste. Connectors must always maintain integrity and sterility; therefore, selecting the right connector is essential to safely and efficiently transfer therapy products to patients where every drop counts.

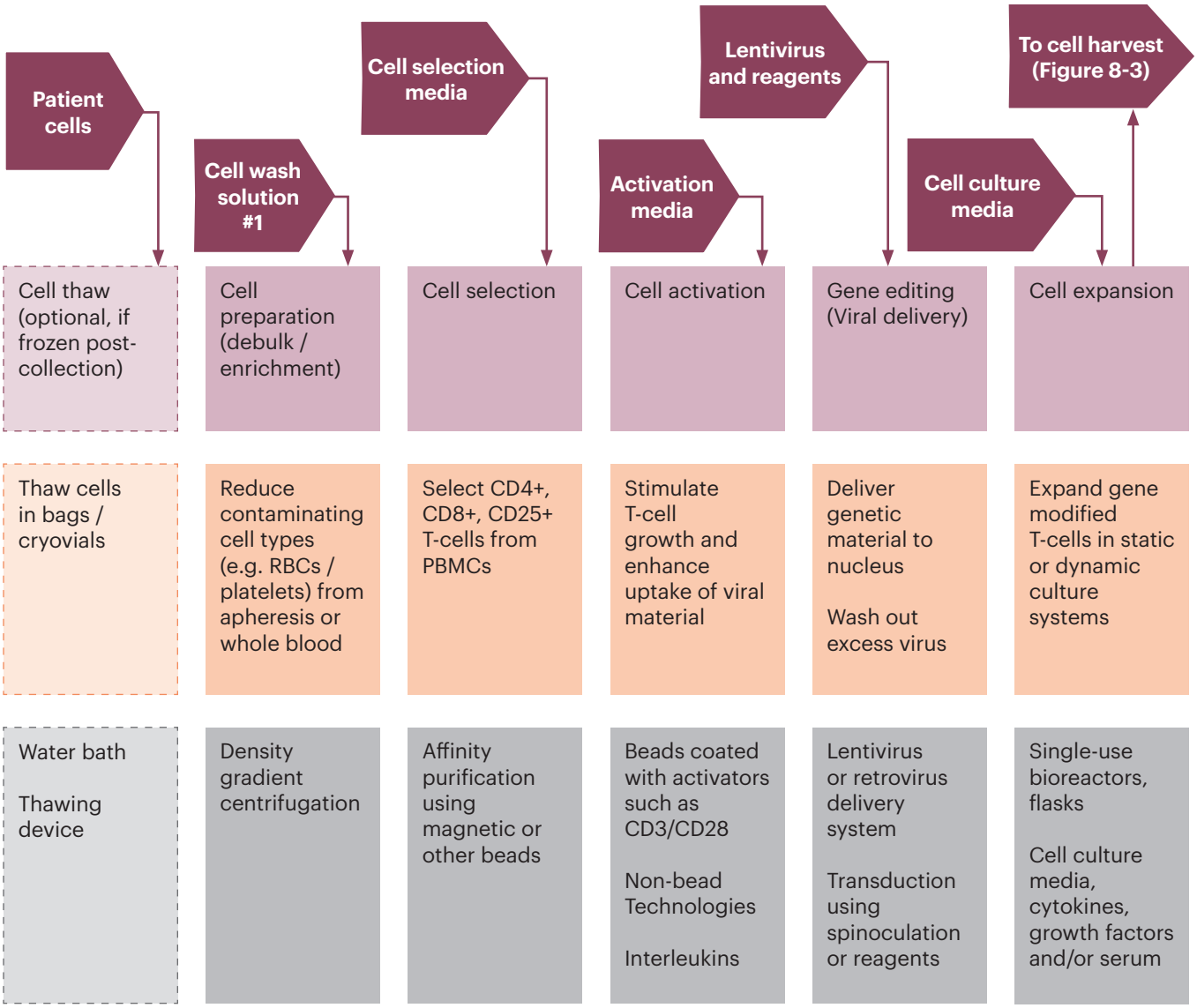
Unit Operations for an Example CAR-T Manufacturing Process

The main manufacturing steps in CAR-T therapy production are:

- Cell isolation (leukapheresis)
- Activation
- Transduction and/or genetic editing
- Expansion
- Harvest and formulation
- Cryopreservation
- Reinfusion (post-chemo depletion)

The following figures (Figures 8-2 to 8-5) show an overview of the manufacturing steps. Resting peripheral blood mononuclear cells (PBMCs) are collected from the donor. This material may be cryopreserved within 24 hours and stored at -120°C. Afterwards, a controlled thaw and wash step removes the cryopreservation material. Activation is achieved by ligating CD3 and CD28 with antibodies attached to magnetic beads or similar reagents, followed by transduction with self-inactivating lentivirus that codes for the gene of interest (e.g,. chimeric antigen receptor (CAR)). After transduction, residual materials are washed out. The cells are then expanded to achieve yields necessary for the final dose. The engineered cells are de-beaded (as needed), washed, formulated, and placed in bags or vials for infusion or cryopreservation. Release testing is done following the manufacturing process to ensure product quality and safety. Sterility testing is often done prior to cryopreservation while additional critical quality attribute (CQA) testing is performed on the final product (e.g, cryopreserved material). Considerations for each of the unit operations are discussed in the following sections. Note that most of the discussions are focused on autologous processes, with additional considerations for allogeneic products.

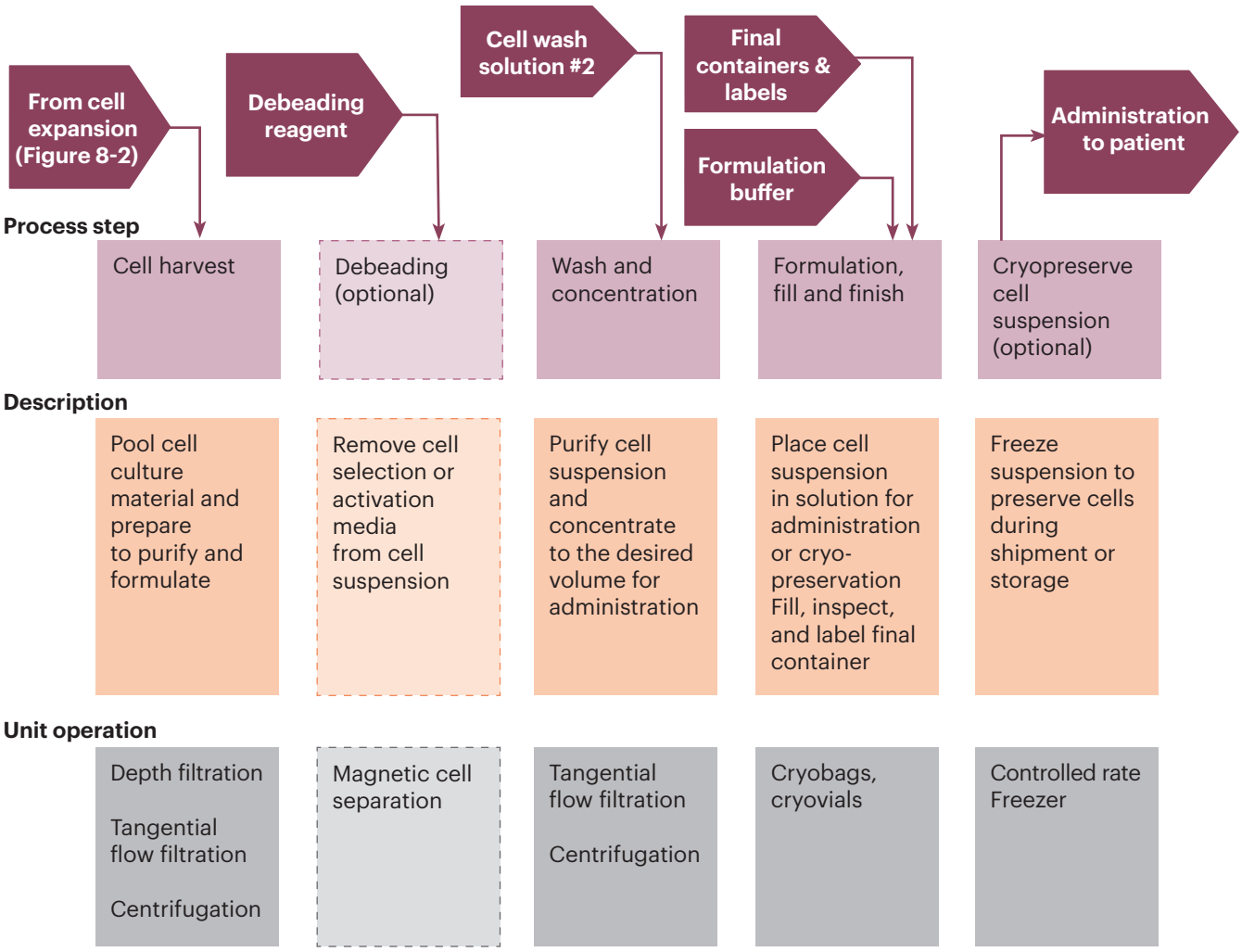
Figure 8-2: *Autologous cell therapy manufacturing process (cell preparation to expansion)*
(Adapted from BioPhorum Cell therapy manufacturing maps.)[11]



THAW (FOR PROCESSES INITIATING FROM A CRYOPRESERVED STARTING MATERIAL)

Thawing is a critical processing step in cell therapy that, if performed incorrectly, can lead to significant cell damage via mechanisms such as intracellular recrystallization or solute effects.[12] Generally speaking, independent of the container in which the starting material was frozen (e.g., cryobag, cryovial), thawing should be performed in a controllable and rapid manner, so that extracellular crystallization can dissolve rapidly to enable proper cell rehydration. As such, within cell therapy GMP manufacturing, thawing is typically performed utilizing a temperature-controlled warming apparatus such as a water bath or, more preferably, one of the many water-free (semi-)automated thawing systems now available in the market (e.g., Plasmatherm by Barkey, ViaThaw by Cytiva). These systems are most often composed of pre-heated components (typically pre-warmed at temperatures ≥37°C) that are placed into contact with the frozen container to rapidly increase the sample temperature via (mostly) conductive mechanisms. Depending on the thawing device of choice, thawing can be controlled by

Figure 8-3: *Autologous cell therapy manufacturing process (cell harvest to fill and finish)*
(Adapted from BioPhorum Cell therapy manufacturing maps.)[11]



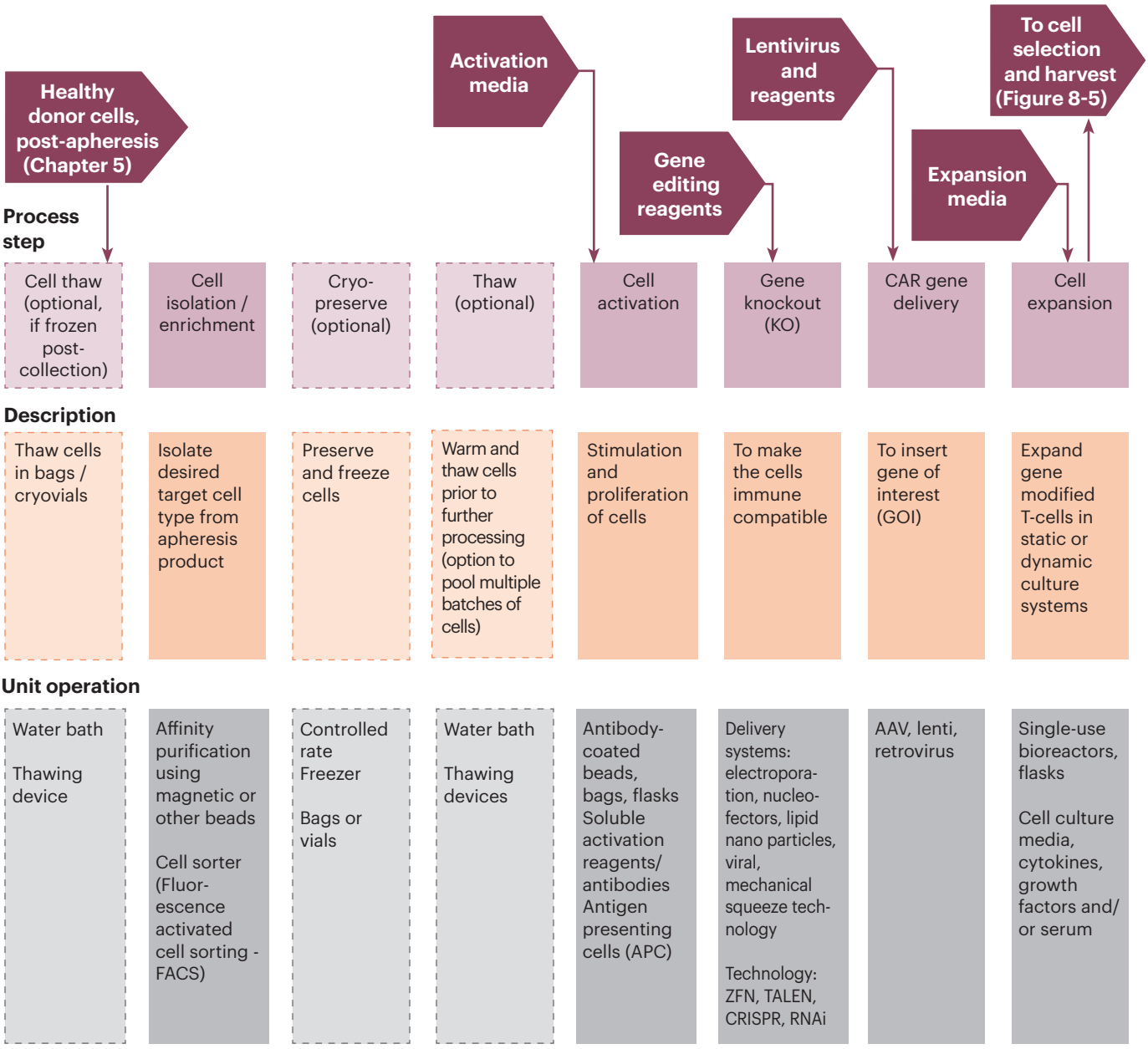
either a qualified/validated thawing time (e.g., a fixed thawing time approach) or via active sample temperature-monitoring probes capable of detecting an "end of thaw" condition (e.g., a fixed end-of-thaw temperature approach), with the former approach currently being the most common within the industry. In either case, the thawing time (typically ranging between 1-10 minutes) and "end-of-thaw" temperature are both container- and sample-specific, therefore requiring specific qualification/validation for each type of starting material/container combination, as well as consideration of the post-thaw GMP operations that should be designed to minimize the post-thaw exposure of cells to cryoprotectants such as dimethyl sulfoxide (DMSO).

CELL WASHING (VOLUME REDUCTION AND MEDIA EXCHANGE)

Upon completion of thaw, the starting cellular materials undergo a procedure—typically referred to as *cell washing*—aimed at removing the DMSO and other excipients that compose the cryomedia formulation that was utilized to freeze the starting apheresis. Long-term cell exposure to DMSO (>30-60 min) may result in negative cytotoxic effects,[13] thus driving the need to remove DMSO as rapidly as possible.

From a mechanistic perspective, cell washing refers to processes in which the original (input) media containing the cellular samples is replaced, or exchanged, by a target media, while targeting minimal nucleated cell loss or damage. A

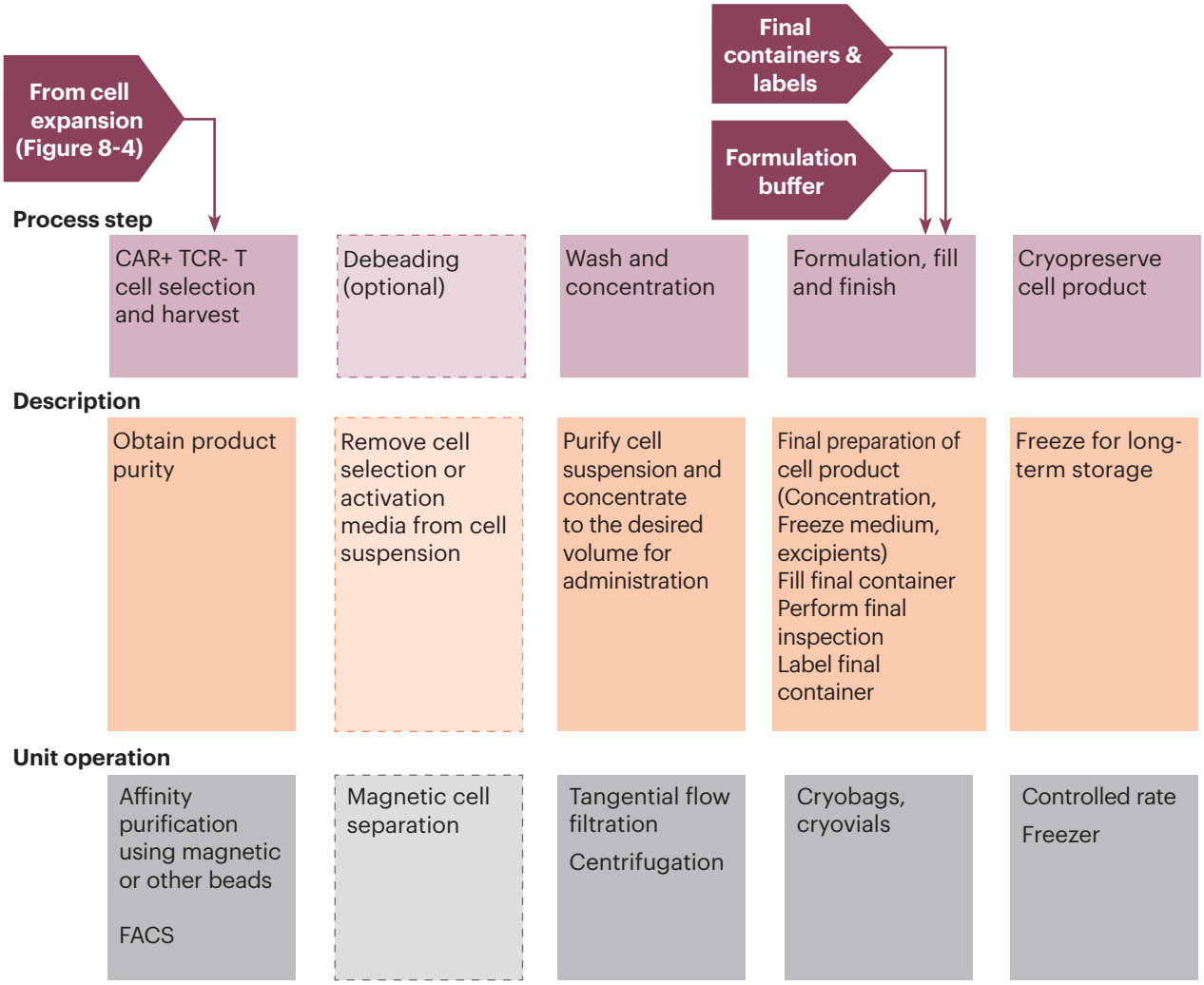
Figure 8-4: *Allogeneic cell therapy manufacturing process (cell preparation to expansion)*
(Adapted from BioPhorum Cell therapy manufacturing maps.) [11]



cell washing procedure in which there is no change in total volume between the input (pre-wash) and target (post-wash) samples is typically referred to as a *media exchange* operation (see Protocol 1 in Figure 8-6). Conversely, a cell washing procedure in which the post-wash volume is smaller than the pre-wash volume is referred to as a *volume reduction* procedure (Protocol 2 in Figure 8-6).

Media exchange and volume reduction functions are then often combined, as outlined in Protocol 3 in Figure 8-6, to provide a preparative workflow where the cells are not only transferred to a final target media but also concentrated into a lower volume, and therefore to a higher cell concentration. This combined media exchange and volume reduction protocol is particularly effective when handling large sample volumes, such as those typically associated with bioreactor harvests where sample volume might be up to several liters. By first performing a volume reduction, this sequence

Figure 8-5: *Allogeneic cell therapy manufacturing process (cell harvest to fill and finish)*
(Adapted from BioPhorum Cell therapy manufacturing maps.) [11]



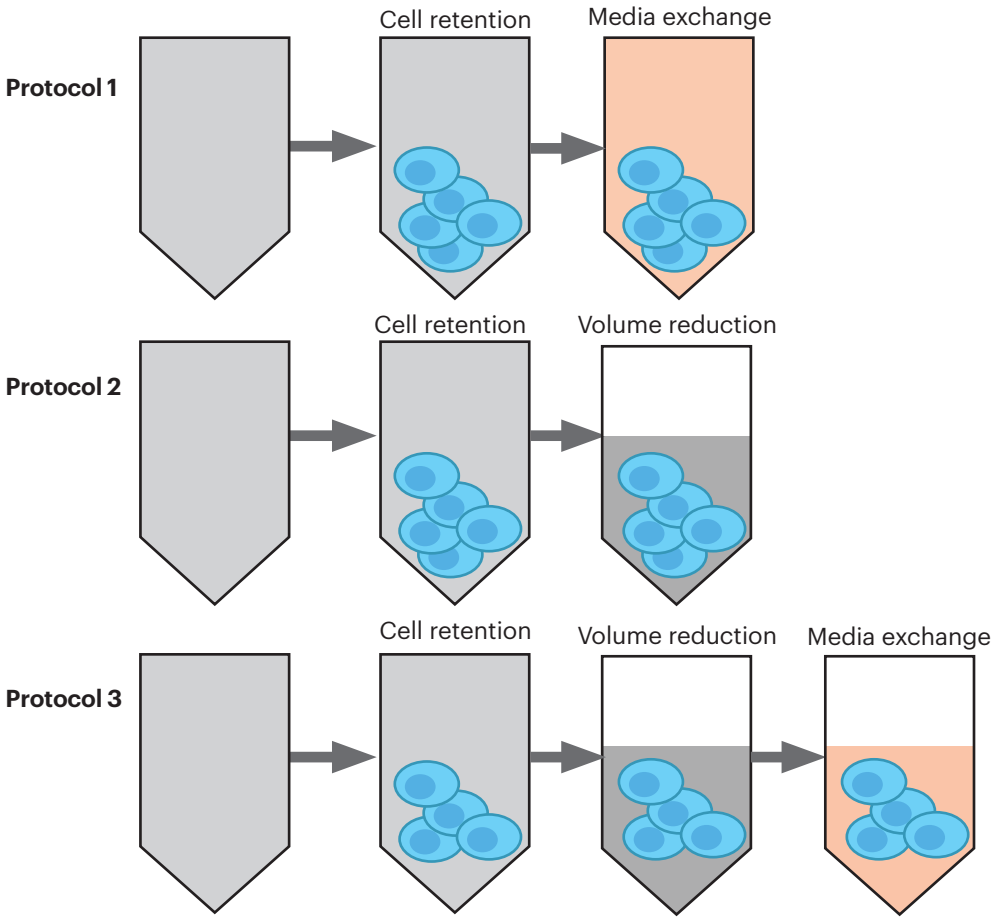
significantly reduces the amount of washing volume required to perform the subsequent media exchange.

Several cell washing methods/tools have been developed and are commercially available, each exploiting different physics:[14] these include centrifugation-based approaches (CellSaver by Haemonetics, Sepax by Cytiva), counterflow technologies (Rotea by Thermo Fisher, kSep by Sartorius), acoustic-based devices (Ekko by Millipore Sigma), and membrane-based technologies (Lovo by Fresenius Kabi). Despite the difference in underlying physics, selection of an appropriate washing tool is typically based on the following performance metrics:

Media Exchange Efficiency: The overall percentage of media components (including base media, additives, cytokines) exchanged or depleted within one washing cycle. The higher the media depletion (or exchange) percentage, the more efficient the washing procedure. For example, given a target depletion level (e.g., 99.9% or 2 log), it will take 2 cycles of a washing operation exhibiting 90% media exchange efficiency to reach the 2 log level, as opposed to 3 cycles for a system exhibiting 80% exchange efficiency.

Cell retention efficiency: The percentage of nucleated cells (or specific cell subsets) retained during the washing procedure. The higher the nucleated cell retention, the more efficient the technology. Conversely, washing operations do not aim to retain enucleated cells. Platelets whose size and morphology are significantly smaller than

Figure 8-6: *Example Cell Washing Protocols*



that of nucleated cells, are therefore largely depleted during a cell washing procedure. Red blood cells (RBC) are another cell subset that can be partly depleted via washing operations, although complete RBC depletion requires dedicated cell selection steps as outlined in the Cell Selection section below.

Throughput: The volumetric flow rate at which the system is capable of operating while providing the target levels of cell retention efficiency. The higher the throughput, the more efficient the tool. Generally speaking, there is a negative correlation between cell retention and throughput, with higher cell retention being associated to lower flow throughputs.

Minimal output volume: The smallest output volume in which the system can deliver the final washed cellular sample. A low output volume is preferrable, as it facilitates achieving a high cell concentration. This may be an important consideration in closed cell washing technology depending on the required downstream operations.

The performance metrics above should all be taken into consideration when selecting the best washing tool for a specific program or cell type. Additional aspects such as cost of goods and data integration should also be considered, particularly when mapping tool selection towards GMP and commercial applications.

CELL SELECTION

After completion of cell washing (DMSO removal), the washed apheresis material most often requires further processing to yield a more consistent starting material for the engineering and expansion steps. Variability of the starting material, particularly for patient-specific autologous therapies, increases the complexity of CAR-T cell production, potentially resulting in a failure to meet the dose requirements. As an example, it has been

demonstrated that specific cell populations can adversely impact subsequent processing steps such as activation (e.g., the attachment of CD14+ monocytes to activating Dynabeads®).[15] Furthermore, there is evidence that RBCs and monocytes interfere with the clinical efficacy of cell therapies.[16-19] The evidence of the deleterious impact of monocytes is further reinforced by the strategy to deplete these cells as a salvage operation when the patient starting material contains high levels of CD14+ cells and fails to pass requirements for further processing. Therefore, in most instances, pre-expansion cell processing is required to select and enrich the T-lymphocyte cell population.

To address such challenges, cell therapy providers have adopted different cell processing strategies, with some opting to expand the entire nucleated cell fraction, while others preferring to undertake cell enrichment steps prior to activation, transduction/transfection, and expansion. The exact nature of this preparative processing is dependent on the intended cellular fraction required, and is often a trade-off between purity and yield. As outlined in the previous section, the initial processing steps typically involve the removal of platelets and erythrocytes through a combination of washing and selection methods. Current approaches use legacy platforms adapted for lymphocyte enrichment, such as the Cell Saver 5+ (Haemonetics Corp.) and the COBE 2991 Cell Processor (Terumo BCT). Newer systems include the LOVO (Fresenius Kabi), Rotea (Thermo Fisher), and Sepax C-Pro (Cytiva).

The aforementioned technologies support the washing and selection of specific cellular components resulting in the enrichment of the T-lymphocyte population. Cell selection typically involves separation on the basis of physical characteristics, such as cell density or size, or on the basis of immunophenotypic properties. Ficoll-Paque is commonly used to deplete erythrocytes and granulocytes on the basis of density gradients (Ficoll-Paque has a lower density than erythrocytes and granulocytes, but a higher density than PBMC/MNC buffy coat). Typically, Ficoll gradient separations require open processing, however, adapted legacy platforms and newer systems support GMP-compliant, closed, automated Ficoll-gradient separation.

While density gradient separation supports effective erythrocyte and granulocyte depletion, other cell processing approaches are required for monocyte depletion and lymphocyte isolation. Two strategies are employed to achieve this: (1) elutriation and (2) antibody bead conjugates selection. Elutriation involves the use of counter-flow fluid and centrifugal force to separate cellular fractions on the basis of both density and size. The principal mechanism for elutriation involves introducing the heterogenous starting material into the system where the material is separated in a spinning chamber via centrifugal force. A buffer is introduced counter to the direction of the centrifugal force, and it is this counter flow buffer that separates the cells based on their size and density and supports cellular fractionations. These cellular fractions can then be sequentially removed, enabling the enrichment of the lymphocyte population. Elutriation is an effective method for discriminating between monocytes and lymphocytes. This technology has been effectively employed for other biotechnological applications, including dendritic cell vaccine production,[20] and is becoming increasingly common in CAR-T processes.[21] Systems that support monocyte depletion include the Elutra® (Terumo BCT), Rotea™ (Thermo Fisher) and Sepax C-Pro (Cytiva).

An alternative approach to lymphocyte enrichment is to use magnetic antibody bead conjugates, which can be used to support either positive or negative selection of target cells. This method has the additional advantage of enabling the enrichment of specific T cell subsets including CD4+, CD8+, CD25+, or CD62L+, and target cell isolation can be based on one or more markers. The antibody bead conjugates are introduced to a heterogeneous cell population where they attach to the target cell. A magnetic field is then applied to the cellular material. In the case of positive selection, the target cells are bound by the magnetic antibody bead conjugate and retained by the magnetic field, with the non-target cells passing through and separated as part of the waste stream. To obtain the target cell population, there is a washing step prior to removing the column from the magnetic field, after which the enriched population can be eluted from the column. In the case of negative selection, the target cell population is that which passes through the magnetic field in the initial flow stream, not bound by the magnetic antibody bead conjugates. GMP-grade, antibody-conjugated beads are readily available and are routinely

used with systems like the CliniMACS Plus and Prodigy platforms (Miltenyi Biotec). While this method provides additional cellular subset selection, it requires additional processing to remove the beads; however, the emergence of biodegradable MicroBeads (Miltenyi Biotec) may obviate the need for debeading as the target cells are free from antibody fragments and magnetic labels.

Other emerging approaches for cell separation include more traditional cell sorting using fluorescence activated cell sorting (FACS), buoyancy activated cell sorting (BACS), acoustic wave separation, and inertial microfluidics. Recent advances in cell sorting have increased the capability to sort labelled cells to achieve enrichment of rare populations of cells. New instruments by Miltenyi, Sony, Cellular Highways and others are able to increase sorting speeds and reduce processing times, making them more amenable to GMP processes. BACS achieves cell separation through the use of buoyant microbubbles that are composed of a gaseous core with a lipid, polymer, or protein shell. In the case of positive selection, microbubbles are coated with antibodies to capture the target cells. The microbubbles then float to the surface of the sample where they can be collected. Although primarily a research tool at present, commercially available, semi-automated platforms exist, including the X-BACS systems (Corning). Similarly, acoustic wave separation technology has proven effective at the research scale for cell separation. Although earlier in terms of its technology readiness level compared with more established approaches such as elutriation and antibody bead conjugates, acoustic cell processing is a promising approach involving the use of ultrasonic waves to enable a no-shear, solid-state approach to cell concentration, washing, and label-free selection. The Ekko system (MilliporeSigma) appears to be a promising acoustic cell processing platform purporting to support closed and automated, label-free cell processing. Inertial microfluidics have also been proven to effectively separate white blood cells from red blood cells at the research scale.[22] These systems utilize a curved fluid channel that causes a higher flow rate on the outside of the channel compared to the inside channel wall. Due to a variety of fluid flow forces, this can ultimately allow for the separation of red and white blood cells between the outer and inner walls of the channel. Therefore, this process is able to achieve effective, label-free separation. However, this technology is still early in development. While a fully-automated, inertial device has been demonstrated at the research level, no commercial products are currently available.

ACTIVATION

Selected or isolated starting T cells undergo an activation step to both enable the T cells to proliferate to facilitate gene transfer (e.g., lentiviral and retroviral transduction is more efficient in actively cycling cells) and expand the starting number of cells to achieve sufficient cells to meet patient dosing requirements. This activation is accomplished by engaging the cluster of differentiation 3 (CD3) receptor on the T-cell surface with activating monoclonal antibodies. The CD3 receptor provides the intracellular signaling capabilities, and is associated with the TCR protein complex on the T-cell surface capable of recognizing specific molecular signals as part of the immune response to pathogens, foreign particles, cells, etc., and reacting to them by inducing a proliferative response to deal with the immunological insult. An accessory signal, known as "signal 2," is required to provide co-stimulation and is thought to act as a confirming signal during the activation process. Signaling through the CD3 receptor without co-stimulation results in T cell dysfunction (anergy) or clonal deletion in an effort to avoid autoimmune events.[23-26]

This activation process is co-opted in CAR-T cell manufacturing and used to expand T-cell populations *ex vivo* to enable larger-scale cell production. One avenue for activating T cells through the CD3 receptor involves incubation with a monoclonal antibody, OKT3, to induce activation. This method is often employed when using bulk PBMCs in lieu of purified T cells. Interleukin-2 (IL-2) often accompanies OKT3 use in the culture to support T cell expansion, minimizing the need for external co-stimulation.[27] Another method uses two separate monoclonal antibodies targeting CD3 (signal 1) and CD28 (signal 2), co-stimulatory receptors mimicking a more physiologically relevant activation event where an antigen presenting cell (APC) would provide both signals to a T cell simultaneously. These two monoclonal

antibodies may be attached to a bead (e.g., Thermo CTS Dynabeads), included as part of a nanoparticle matrix (e.g., Miltenyi Transact), or utilized in other solid substrate formulations (e.g., adsorbed to plates or coated in bags). The prevailing outcome is similar in these instances in that T cells get activated and become more susceptible to transfer of the gene of interest via lentiviral or retroviral transduction. T cell activation with these various methods requires downstream considerations. CTS Dynabeads must be removed from the culture using a magnetic device to both stop the activation event and remove potential process-related impurities prior to administration to a patient. The Transact reagent can be diluted to dissociate the nanoparticle matrix and stop the activation event. Regardless of the activation mechanism, sponsors should be aware of the residual impurities, including murine antibodies, from that process step. Several strategies for clearance rely on dilution and additional cell washing steps through downstream processing operations, which will help with removal and minimize the presence of these reagents in the final product.

GENE-EDITING/TRANSDUCTION

Following activation, T-cells are genetically engineered to express the synthetic CAR. Significant advancements have been made in the gene-editing technology/transduction strategies to be applied to CAR-T therapies, and are outlined below.

Lentiviral transduction

A therapeutic product dose should be comprised of engineered cells that stably express synthetic antigens or targeting receptors. In the example of CAR-T cells expressing anti-CD19 targeting receptors, the transgene may be introduced with a lentiviral vector (LV), which has a safe integration character, to achieve persistent expression in the patient's cells. Lentivirus is an RNA virus where the transgene of interest (e.g., CAR) is integrated randomly into the genome of the host cell. Currently, third generation LVs are most widely used, typically produced using a 4-plasmid transient transfection system comprised of 3 packaging plasmids[28] and 1 self-inactivating gene transfer plasmid. Following transduction, the viral RNA is reverse transcribed to DNA, and, subsequently, the CD19 chimeric antigen receptor is expressed and transported to the host T cell surface.

T cells are sourced from a selection process done on an apheresis product from the donor or patient. It should be noted, given the clinical indication, such as pediatric acute lymphoblastic leukemia (ALL), adult B cell lymphoma, or solid tumors, that the donor cells may be exhausted (e.g., exposure to significant immune activation events leaving the cells with little proliferative capacity) or have been pre-treated via multiple rounds of chemotherapy to the extent that the cells may be hard to transduce.

Activated T cells are incubated with the lentivirus as the key component of the transduction process. Then, the virus is washed out before the expansion step. The expansion continues until a sufficient dose is reached, with the dividing cells continuing to express the transgene after infusion into the patient. The challenge in manufacturing autologous CAR-T therapies lies within the detail beyond this simplistic overview. In each unit process step, there are opportunities to improve and optimize each aspect of the process.

Much of the cost and complexity of CAR-T manufacturing and therapy can be attributed to the viral vector component of the process. Manufacturing viral vectors such as lentivirus is challenging in terms of standardization, stability, and yield. Improvements in the design of the lentivirus (including packaging efficiency), as well as scale (e.g., 10L to 200L) are current areas of industry focus. Increasing the transduction efficiency of the target T cell is a crucial challenge, and control of the culture conditions, including cell culture media, cell density, vector titer, temperature, and bioreactor design will help increase the process yield of engineered cells.

Additionally, there are ongoing efforts to find alternatives to viral transduction. One such effort is to achieve an effective, transient messenger RNA (mRNA) engineered CAR-T cell. The use of mRNA delivered to the cytosol avoids the challenges of viral manufacture and optimization. Such a therapy could be administered in a multi-dose regimen rather than a single-dose therapy. Though, to date, equivalent efficacy has been shown in a murine model[29] but not in the clinic, this route remains an attractive prospect. Another potential route to achieving stable expression of anti-CD19 receptor on T cells is

to edit-in the transgene at a site-specific location. Roth et al. demonstrated site specific knock-in in the literature,[30] but have not yet translated it to the clinic.

Gene editing

Developers have several options to enhance the performance of a CAR. Some of these options involve the transient or stable expression or inhibition of a factor. An excellent example of one such therapeutic option is the inhibition of PD-1. Immunologists differ on whether this inhibition should be transient or permanent. However, it is a frequent target for knock-out using a gene-editing tool. There are several clinically validated gene-editing platforms available to developers, including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9, Zinc finger nucleases, and megaTALs among others. Of these, CRISPR/Cas9 is ubiquitous due to its relative ease of use and flexibility.

The CRISPR/Cas9 system must be delivered without an integrating viral vector or more commonly by electroporation (see below) using an mRNA guide and a Cas9 RNP (ribonuclear protein). As the delivery is transient, the editing tool will perform its edit and then dissipate. However, the editing results will persist in the cell.

For a knock-in editing, this capability presents the possibility of an entirely non-viral, site-specific integration of a persistent transgene. Such a system has the potential to disrupt the current manufacturing process for advanced therapies.

Electroporation

Electroporation (EP) is the current leading non-viral delivery platform for cell engineering. This technology has been used in the production of clinical trial materials and relies on passing pulses of electric current through a solution containing cells. EP can be used to deliver a diverse range of cargos, including plasmids, mRNA, proteins, transposons, and gene-editing tools. Commercially available EP units include MaxCyte, Lonza Nucleofection, and Thermo Fisher Neon. Other systems have been developed and qualified for clinical use, including the Cellectis Pulse Agile. EP has been used in clinical trials to introduce transgenes into human primary cells for either transient or permanent expression. In transient expression, the therapeutic must be re-dosed. However, this also allows the clinician to monitor and escalate doses if needed. In the case of permanent expression, the EP system must deliver a transposon system such as Sleeping Beauty, PiggyBac,[31] or TcBuster™. The transposon system has the advantage of conferring stable expression; however, the insertion of the transgene is still at a random location. Site-specific integration into a safe harbor loci requires knowledge of the target sequences.

While EP is the most common non-viral delivery system, it is widely reported that it can cause cell damage.[32] Scalability and recovery challenges also exist. These issues notwithstanding, delivery efficiency and viability of functional cargos of commercial systems can be in the region of 90% at smaller scales[33] (for example at scales of 10^6-10^7 cells), but efficiency and viability generally trend lower as scale increases. It should also be noted that most commercial EP systems are fundamentally semi-continuous processing systems.

Lipid nanoparticles

Lipid nanoparticles (LNPs) are biodegradable polymeric structures that have been used *in vivo* and, more recently, *ex vivo* to deliver mRNA and RNPs to cells. They are currently being evaluated for the production of transient CAR-T therapies,[34] as well as for use in gene editing. Billingsley et al. demonstrated that LNPs have equivalent transfection efficiency to EP, but with lower cytotoxicity.[35] LNPs have a clear advantage in terms of their scalability, as there is no device component. Challenges include the need to establish and optimize the LNP chemistry and to improve process reproducibility.[36]

Mechanoporation

SQZ Biotech has applied its cell squeezing technology to clinically relevant cell types. Material is delivered to the cytosol by passing a population of cells through a microfluidic chip that compresses them, causing membrane disruptions that allow for the entry of macromolecules from the solution. The process is continuous, fast, and very controllable. However, scaling the technology requires significant parallelization in the context of T-cell therapy, where a target dose may be up to 10^9 cells. SQZ Biotech has shown data highlighting minimal genetic perturbation of the cells and excellent cell loading efficiency.[37,38] In addition, there are other emerging

technologies based on microfluidic chip formats that use forms of mechanical perturbation to deliver cargo into cells, such as those by CellFE and Kytopen.

Chemical transfection

Intracellular delivery by reversible permeabilization of the membrane has been demonstrated as a fast, simple, and scalable process.[39] Avectas has synthesized mRNA CD19-CAR cells at scale that are highly functional and potent. This chemical transfection technology can also deliver gene-editing tools with high-efficiency and viability and the technology can perform multiple, sequential edits. These qualities will be necessary for more sophisticated cell engineering processes.

EXPANSION

Expansion is a critical operation, epitomizing the statement "the process is the product" as it determines yield, has high impact on cost of goods (COGs), facility design, batch success, pipeline product platforms, and the ultimate quality and function of the final cell product. Expansion is particularly challenging, as it is the longest and, arguably, the most sensitive and unpredictable of the unit operations given the length of the operation and the variability of the donor and patient material feeding into it. Determination of the effective number of cells per dose from dose escalation studies will help identify the target process scale, the volume in which doses should be packaged, and the minimal lot sizes to be produced. Expansion is a constant battle between achieving desired yield while ensuring the cells are not too differentiated to be effective. The filling and cryopreservation unit operations are dependent on the expansion yield, which can be highly variable. Manufacturing operations scheduling can also be impacted as companies weigh accepting variable yield on a predetermined harvest day versus a variable harvest day based on the particular growth profile of an individual patient's cells.

The expansion unit operation offers a variety of choices regarding culture vessel and modality, culture length, media delivery and feed strategy, and media supplementation. It is also the unit operation that has seen the greatest amount of technological development. The selection of culture strategy and expansion unit operation are often based on the target cell number required for dosing, the target product profile (TPP), and may have facility fit considerations. Having as much of this information as possible before deciding the details of the expansion platform will help ensure the process delivers the correct cell product and can prevent being burdened with a system incapable of delivering the desired product in the most efficient and effective manner. For example, the TPP may elucidate dose requirements that dictate a particular expansion volume requirement. Most processes start at the bench using a manual method such as flasks or cell stacks. Moving from an open manual method to a closed and automated expansion method is likely required to produce the required number of cells and to meet regulatory requirements. Deciding how and when to automate a particular step or an entire process can have consequences. Utilizing a particular single-use disposable may increase costs or introduce particulates or leachables into the process. However, reducing the chances of microbial contamination and the ability to decentralize a process to use a less skilled work force may ameliorate such problems.

A major challenge for the expansion unit operation is the variability associated with the starting material (healthy donor material during research and development and patient material during clinical and commercial manufacturing). Because of this inherent variability, considerable care must be taken during development to account for potential performance differences between different healthy donors, individual patients, and patient populations. A well-designed process will contain process control strategies for critical process parameters, enabling effective responses to variable performance and mitigating batch failures during GMP production. The major expansion decision points discussed here can be divided into three major categories: process scale, culture duration, and development and manufacturing considerations.

An important consideration when deciding on an expansion platform and strategy is process scale. The final required cell number is a major driver of equipment and process decisions and varies greatly depending upon target, indication, and type of cell product (i.e., CAR vs. TCR, autologous vs. allogeneic). For example, an autologous product will likely be focused on scale-out

capability for manufacturing, while scale-up would be more imperative for an allogeneic product. Culture duration is another factor that needs to be considered before deciding on an expansion platform. Shorter cultures have more flexibility when it comes to culture vessel and modality, while longer processes likely need to plan for nutrient delivery, waste removal over extended periods of time, and impact on facility design. For T cells, shorter process retains the stem cell memory phenotype and central memory phenotype, while in a longer *ex vivo* expansion process, an effector/terminal effector and more short-lived *in vivo* population will emerge.[40] Specific choices regarding development and manufacturing considerations are key to designing the optimal process for a specific product. For example, a process that may seem simple to operate in a research environment may encounter significant challenges when the process is scaled-up (taken from benchtop and adapted to manufacturing scale) and run many times in a manufacturing facility. Knowing the specific needs, capabilities, and challenges of the intended manufacturing facility is key to streamlining the transfer of the process from development into GMP production.

Process scale

The choice of process scale for cell products is highly variable depending on the type of product and is largely driven by target cell numbers. Options for scaling include either scaling-up or scaling-out, the choice of which can be driven by autologous vs. allogeneic applications or by delivery time and cost drivers. The first major decision point when considering process scale is static vs. suspension culture (also referred to as 2-dimensional vs. 3-dimensional cultures). Each option offers particular benefits and challenges and must be considered carefully depending on the specific needs of each product.

Static culture options include traditional diffusion-driven gas permeable cell culture flasks (Corning), convection-driven gas permeable cell culture flasks (Wilson Wolf G-Rex), and gas permeable culture bags made from a variety of materials (Origen). These are basic technologies, making them simple to incorporate, with limited training required for operators that are skilled in sterile culture technique. Most are similar to technologies used in a research and development setting and are relatively inexpensive, making them frequent choices for start-ups and early-stage development organizations. While simple to operate, flask and bag cultures can be cumbersome when larger culture volumes are required. Flasks are inherently limited in size by the surface area required for proper gas and nutrient exchange, usually capped at 1 L for immune cell culture, making the scale-up option the addition of more flasks. More flasks also increase the number of open events and increase the possibility of contaminating all or part of a batch or dose. Gas permeable bags are available in a variety of sizes, but larger bags (1 L or greater) are challenging to handle from a mechanical perspective as they lack structure. Fitting large bags in incubators, moving them from an incubator to a BSC, and operations within the BSC can present handling difficulties. The inability to monitor the cells and culture conditions is a considerable disadvantage to static platforms. To determine how the culture is growing, the static cell layer must be disturbed, which may have negative effects on cell growth and culture environment. Manufacturers of such platforms generally advise against resuspending the culture often because of concerns related to cell growth. Recently, more complex automated and semi-automated static culture options, such as the Lonza Cocoon and Miltenyi CliniMACS Prodigy, have been developed that provide some of the process monitoring and control capabilities of suspension culture platforms (discussed below) within a closed system designed to be more standalone. However, these options come with constraints specific to static culture such as difficulty in cell counting and limited scale-up ability.

Diverse options exist for suspension culture as several technologies have been borrowed and adapted from traditional antibody cell culture. These options are generally more amenable to scale-up than static culture platforms. Foremost among these is the rocking motion bioreactor platform, which has been widely adopted in cell therapy manufacturing. Rocking motion cultures have been in use for decades as part of seed trains or production cultures for antibody products. A significant benefit of these systems is the scale-up ability this platform provides. Single-use bioreactor bags are available in a wide variety

of sizes (from working volumes of 300 mL to greater than 50 L). Allowing cells to grow in suspension removes the surface area constraints of static platforms, while active delivery of oxygen and other gasses creates an opportunity to achieve higher cell densities. In addition, the low shear stress produced by the wave-like mixing strategy allows the cells to remain fully in suspension, under low mechanical stress, while oxygen-containing air is constantly mixed into the culture.

Process analytical technology (PAT) is commonly used to continuously characterize the expansion process. Manufacturers of these rocking motion bioreactor systems have incorporated many process monitoring and controls capabilities. Bioreactor bag options include integrated process sensors for pH, dissolved oxygen, cell density, and perfusion membranes of various pore sizes for media exchange. Controls capabilities include control of rocking angle and speed, ability to deliver a specific mix of gasses (air, O_2, CO_2, N_2) via mass flow controllers at desired rates, and automated temperature, pH, and dissolved oxygen control. Increased process monitoring capabilities allow for intervention to respond to changing culture conditions. This is just the first step toward a big data approach where many aspects of the culture conditions are continuously measured.

Fed-batch feeding where a bolus of media is added to a bag or vessel when a particular pH, cell density, or metabolite concentration is reached is commonly used in cell expansion. While this approach is expedient and a holdover from antibody manufacturing, it can result in large swings in the concentrations of waste products and feed streams. In some cell types, high concentrations of lactate have been shown to inhibit growth. Most of these systems offer the ability to customize the expansion protocol. Process steps can be written into programs that an operator can manually execute or be automatically triggered by time interval or other input (i.e., pH, cell density). Because cells are already in suspension, it is not disruptive to the culture to count the cells as often as needed. Bioreactor bags are equipped with sampling ports to withdraw culture samples. It is important to ensure cells are in homogeneous suspension while a sample is pulled to get an accurate cell density reading. While these sampling ports are considered "closed," care must be taken when pulling samples, as the ports will be exposed to the open environment for a short time. Rocking motion bioreactor platforms can be easily scaled-out and scaled-up. Key factors for scale-out are the ability to run the same program on multiple units, monitor cultures for consistency, and employ single-use bags to enable simple clean up and reactor turn over.

Another option with similar benefits to the rocking motion bioreactor platform is the stirred-tank reactor. This is the traditional workhorse of antibody production processes but is just starting to be explored for immune cell culture. The impeller-driven mixing mechanism has the potential to produce significant shear stress to which immune cells are likely quite sensitive. However, because of their various impeller designs that exist to promote superior mixing and increase mass transfer under lower shear stress, stirred tank reactors can achieve very high cell densities and perfusion mechanisms in these systems are not limited in the same way rocking motion bag perfusion filters are, making them a potential option for programs requiring particularly high cell numbers. Another advantage of stirred-tank systems is the availability of high-throughput scale-down models capable of running larger design of experiment (DOE)-style and screening experiments. One disadvantage of stir tank bioreactors is the high cost of validation while scaling a process to produce the cells needed for advanced clinical trials and commercial manufacturing demands. Some newer cell expansion platform technologies that use bioreactor chambers that can be expanded during cell culture are becoming available and may be an option for ease of scale-up and reduced validation costs.[41]

Perfusion can be achieved by a number of mechanisms and varies with the expansion system. Rocking motion bioreactors (up to a certain, limited working volume of generally 10 L or less) can be equipped with perfusion membranes. These come in a variety of pore size options, depending on bag manufacturer and bag size. Stirred tank reactors and other systems can employ perfusion via hollow fiber tangential flow filtration (TFF) or alternating tangential flow filtration (ATF). Perfusion strategies can be tested and determined via development and "locked" via incorporation into an executable program for manufacturing. Some bioreactors such as the

Xuri Cell Expansion System are unique in offering both fed-batch and perfusion feeding capabilities.[42,43]

Hollow-fiber bioreactors adopted from kidney dialysis applications have been used to produce biologics for more than 20 years. Cells are cultured on one side of a semi-permeable membrane while metabolites, gas, and nutrients continuously perfuse through the membrane (Terumo, Quantum). The continuous addition of nutrients along with the constant removal of waste products produces a much different cell microenvironment than the fed-batch systems characteristic of most manual methods such as flasks, stacks, and bags. Automated systems like rocking bags and stir tank reactors offer perfusion-feeding strategies especially when high cell densities are required. A two-compartment culture system is advantageous in that it allows for an extra degree of control not possible with other configurations. Without perfusion-feeding strategies, the accumulation of unwanted and potentially inhibitory metabolites such as lactate and ammonia can cause growth arrest. Perfusion limits may become necessary to control the amount of media used and therefore the cost of the process. The small harvest volumes from hollow-fiber bioreactor cartridges are advantageous for downstream processing. Hollow-fiber bioreactors are available in several configurations, from a pump and single-use cartridge system to automated self-contained units. Different membranes with differing molecular weight cutoffs are available to accommodate a variety of applications. Scale is achieved by adding multiple units (scale-out) making them a better fit for autologous cell applications.

While single-use bioreactors offer many benefits, operation and maintenance of this equipment requires more training and investment in capital and process development efforts than most static culture options. Specific needs for individual programs should be carefully considered; pros and cons of various expansion systems should be investigated,[44] and costs and benefits properly analyzed before deciding on the optimal platform.

Culture duration

Determining the length of the expansion unit operation is variable, informed by the specific needs of a particular program. These decisions should be driven by the target product profile (TPP) and the quality TPP (QTPP), which define the required cell number and desired cell phenotype. Shorter cell culture duration has the advantage of fewer population doublings, shorter manufacturing facility times, and will likely require less nutrients. Longer cell cultures can produce more cells, but those cells are likely to have more variable phenotypes, take up more time in the manufacturing facility, and require more complex nutrients and waste management strategies. Upon sufficient characterization of cell growth in a given expansion platform, another approach is to have a standard culture duration regardless of cell number, which will improve operational efficiencies and make scaling operations easier.

Short expansion durations (a week or less) may be sufficient for many autologous CAR cell therapy programs. These processes have the advantage of being lower maintenance from the perspective of nutrient delivery and waste removal, and thus are likely more amenable to static culture options than longer processes. For processes requiring a week or more, nutrient delivery, waste removal, and media component stability must be carefully assessed and planned for. Perfusion is the most common technology available for nutrient delivery and waste removal, although this can be achieved in static cultures as well. In static flask cultures, spent media can be manually removed by careful pipetting, but adding new media invariably results in cell layer disruption. Alternatively, processes can be designed so that fresh media or concentrated feed is added without removing the spent media (fed-batch), reducing the number of manipulations and therefore simplifying the process.

For longer processes, it is necessary to also carefully determine the stability of media components. It is conceivable that for long processes, consideration may need to be taken for producing multiple, staged lots of media throughout production if particularly sensitive media components are used. For perfusion processes, room temperature stability must be characterized and proper timing determined for any media changes (removal of unused fresh media at room temperature and replacement with new fresh media from longer-term storage temperature). Newer technologies for refrigerated storage of media and reagents that are integrated

with the culture platform are on the horizon as more automation allows for longer periods of unattended processing.

Development and manufacturing considerations

A crucial aspect of expansion unit operation development is the ability and efficiency of transferring the process from the development lab to a GMP manufacturing facility. The two spaces are similar but there are distinct differences. Knowing what these differences are and how to plan for accommodating them is key to both smooth transfer and successful GMP production, regardless of the expansion platform chosen. There is, of course, greater flexibility when planning to manufacture internally as coordination between development and manufacturing should be more robust and development is likely to influence the equipment, workflow, and design of the manufacturing space. The early adoption of automated technologies is usually met with skepticism because of high equipment, disposables, and validation costs, but should lead to savings in fewer lost batches due to contamination, lower personnel requirements, and increased ease of transfer to a manufacturing environment. Robust, scalable, automated processes increase the facility throughput, therefore spreading the upfront capital expenditures over more products, resulting in reduction of COGs in the long run. If working with an outside vendor, such as a contract manufacturing organization (CMO), take care to align as early as possible on technology, process details, workflow, quality systems, etc. As in any professional relationship, clear and frequent communication, and technical and knowledge transfer are often key to success.

A "begin with the end in mind" approach to understand how the commercial and large-scale clinical trial GMP manufacturing processes will operate and the necessary steps to transfer the process from the development lab to the GMP manufacturing facility is highly advantageous for efficient and successful technology transfer. Early collaboration between the process development, manufacturing operations, and engineering functions can aid the establishment of a process development strategy that is aligned with scientific, operational, and facility needs. Development of the process for manufacturing is a key step for repeatable and reproducible production. Consultation with regulatory, Quality Assurance, and Quality Control functions is also beneficial at this time to ensure that the larger scale clinical and commercial processes can repeatably produce the same products with the required quality as the smaller scale early phase clinical products.

One important consideration for the expansion unit operation is closed vs. open processing. As mentioned earlier, traditional static culture flasks are simple from a technology and operations perspective. These processes are, however, considered to be open. Flasks must be opened in a BSC to manipulate the cells. This poses a contamination risk and is likely to prevent producing more than one product at a time in a single suite during GMP production. Static bags have the advantage of being equipped with syringe ports so samples can be removed and components added via a closed system. Bags can also be connected via weldable tubing for closed transfer between bags. Challenges arise, however, when larger volumes are in use (i.e., wash steps, large media additions) as it becomes more difficult to add and remove large volumes requiring multiple welds or additions via syringe. This imposes a contamination risk either by many repeated interactions with the port or by creating an open step where liquids are either poured in or drained out through an open connection (i.e., syringe with plunger removed, open drain port into waste vessel). The automated systems discussed earlier (primarily, rocking motion and stirred tank bioreactors, but also other systems such as the Lonza Cocoon, Terumo Quantum, and Miltenyi Prodigy) can largely be considered functionally closed. Most sampling in these systems is accomplished via some version of a syringe port and the systems are designed to make external connections via weldable tubing. The type and diameter of this weldable tubing can vary by manufacturer so it is important to plan carefully to ensure that all components required for the expansion unit operation (i.e., single-use reactors, media and waste bags, welders) are compatible with each other. Whether open or closed, simpler processes with fewer steps and manipulations have a lot of advantages, such as reduced probability of cross contamination and reduced resource requirements, leading to higher percentage batch success and lower COGs.

Pre-filled media bags save time and remove tedium from the process but can present connectivity challenges due to the wide variety of weldable tubing sizes and materials. Needless to say, connections to other unit operations should also be planned in advance to maintain closure, whenever possible, throughout the entire production process. Sterile connections between unit operations not only improve manufacturing efficiency and safety in a clean room but tubing welders can produce sterile welds in a lower-class clean space. Moving some or all of the unit operations out of the cleanroom would result in lower manufacturing costs.

Another important consideration for the development and manufacture of cell therapy products is the ability to monitor and control the process. Process monitoring and control are tenants of quality by design (QbD) and serve as the mechanism by which operators can react to process data in real time and use pre-determined "levers" to make planned process changes to keep the expansion process on-track. At the very least it is important to know what happened during the run. This process monitoring is particularly important when performing process changes during scale-up to ensure process parameters are properly controlled and the resulting product quality is maintained. Data collection through a laboratory information management system (LIMS) allows for electronic batch records, cell tracking throughout the process, lot-release test results, and source material management. When a mix of automated and manual processes are present, radio frequency identification (RFID) can be used to track a batch of cells. Additional consideration for electronic systems, data capture and documentation, and enterprise electronic resources are discussed towards the end of this chapter. When designing the expansion unit operation, there is often a trade-off between operational simplicity and process monitoring ability. Simpler systems, such as traditional cell culture flasks and static bags, often offer little opportunity to monitor and control process conditions while more technologically advanced systems, such as single-use bioreactors, offer a number of monitoring and control points. For example, a flask culture can monitor temperature and be set at a specific CO_2 percentage in an incubator while a bioreactor system offers monitoring and control of a number of parameters including both the setpoints and present values for each parameter. Examples include temperature, pH, gas flow, dissolved oxygen, pressure, rocking speed, and rocking angle. Parameters in these systems can often be monitored at user-specified intervals and recorded via operating software.

Probe technology is evolving and many systems now include single use or non-contact fiber optic probes for pH and dissolved oxygen, eliminating the need for calibrating, cleaning, and sterilization between runs. Different probe technologies based on capacitance are being developed to monitor cell growth and viability, which enable users to monitor cell growth without the need to physically remove samples.

Many process parameters can be controlled and monitored where the system will respond to changing inputs to maintain specific setpoints or run specified profiles. For example, gas mixture in these systems is often controlled by mass flow controllers that alter flow rates to maintain the specified percentages of certain gasses (i.e., CO_2, O_2). Proportional integral derivative (PID) controllers are also included in many cases that allow the system to maintain, for example, a specific pH setpoint, while allowing the user to determine the speed and intensity of the response by making adjustments to the PID controller settings. In development, the ability to monitor a number of parameters is imperative to identifying and characterizing critical process parameters. In manufacturing, it creates the opportunity to operate a more consistent process as more parameters are monitored and controlled during operation. Having this ability allows the cells in culture to experience a more consistent environment, which should lead to more predictable process outcomes.

A final consideration in the development of the expansion unit operation is which process analytics to include during development and manufacturing. Useful tools for development can include cell counting and viability analysis, metabolite analysis, and offline pH/gassing analysis via a blood gas analyzer. It is widely accepted that cell counting and viability analysis is imperative to include in both development and manufacturing of cell therapies as this is the most direct read

of cell health and expansion process success. Metabolite analysis can be a very useful tool, particularly during development when it can help to inform process decisions (e.g., particularly around feeds and perfusion strategy), and careful consideration should be made as to when and where this analysis should be included. Real-time metabolite monitoring will be the next step in automation, particularly in autologous cell processes where starting material variability requires precise process control. Several manufacturers provide different versions of these instruments and an array of assays is available. Use of these analyzers allows development scientists to understand more about the culture environment of the cells and how process changes or donor cell variability impact the culture environment. Blood gas analyzers provide an offline reading of parameters such as pH, dissolved oxygen (DO), and CO_2 and serve as an important tool for validating the accuracy of online measurements of these parameters. Online probes, while calibrated, often experience drift, whereas offline measurements, which employ multiple-point assessments during analysis, are widely considered to be more accurate.

Many technologically advanced expansion systems include the ability to correct online values to match those measured offline. While very useful for development, these types of analyses may or may not be necessary during manufacturing. These instruments, especially those used for metabolite analysis, are often expensive to purchase and costly to maintain from a reagent and operations perspective. Keeping the instruments functioning properly requires trained users and, most often, daily upkeep and maintenance. If an expansion process is robustly designed during development, metabolite analysis in the manufacturing facility may not be necessary. A blood gas analyzer, on the other hand, may still be required to maintain the accuracy of online probe readings. Considerations should be made when including such equipment in a manufacturing facility; DO changes rapidly once exposed to air so the instrument should be placed in relative proximity to the expansion system. Movement of this material is often via syringe and the transfer between expansion system and machine may be considered an open step (transfer to a closed tube is not advised if an accurate DO reading is required).

Expansion system summary

A wide variety of expansion system options exist for cell therapy products that vary greatly in degree of technology, complexity, scale, and capital investment. There is no ideal system that satisfies the goals and needs of every institution, so careful consideration should be made to the specific goals, opportunities, and constraints of each individual organization. The expansion system needs to support both the process and product goals as the two are inextricably linked (i.e., if a large number of cells are required for a product, the expansion system is likely to require the ability to perform media exchanges). This can be accomplished manually in a flask system or automatically via a number of mechanisms (perfusion via absolute filtration, perfusion via hollow fiber filtration, media reoxygenation via recirculation) available on more automated systems.

The major decision points around the expansion unit operation include process scale, culture duration, and development and manufacturing considerations. The determination of process scale weighs heavily on whether to choose a static or suspension culture, while culture duration informs decisions on nutrient delivery and raises questions on how duration may affect TPPs and QTPPs. Determination should be made on whether scale-up or scale-out is more relevant to a given program (based on process needs and business drivers such as time, cost and quality), as different expansion systems offer benefits and pose challenges to each. Development and manufacturing considerations will be specific to the capabilities and constraints of each organization. Will manufacturing be performed internally or externally? If internal, can systems be harmonized between development and manufacturing? If external, how can the process be designed in development while taking into account inherent differences between the internal development lab and the external manufacturing facility where customers have little to no impact on instrument and system choices? How much information should be collected during development and how much of that information must be monitored during manufacturing? Regardless of program goals and process details, all decisions should be guided by QbD principles so the resulting process is as robust as possible from a phase-appropriate perspective.

FINAL WASH AND VOLUME REDUCTION-DRUG SUBSTANCE

Upon completion of the cell culture phase, the expanded cells are harvested from the bioreactor or culture vessel to undergo a final cell washing operation. This last cell washing operation is performed to simultaneously fulfill three main purposes:

- Remove/reduce cellular impurities, additives, and non-target media components below acceptable release levels.
- Reduce the overall sample volume to increase the overall cell concentration to meet downstream formulation requirements.
- Perform a media exchange to transition the cells from the harvested culture pool to a basal formulation media.

Selection of an appropriate washing apparatus and protocol to fulfill the first requirement is particularly critical, as further impurity reduction typically is not possible after this step, with the exception of large particulates whose removal might be possible via coarse post-formulation filtration methods. In the "Cell washing" section, an overview of washing tools was presented, distinguishing them based on metrics such as underlying physics, efficiency, and throughput. In addition to such principles, the following factors should be considered towards appropriate selection of washing tools for DS preparation:

Impurity removal considerations: The final washing procedure should be designed to achieve sufficient depletion of contaminants and remaining media additives from upstream processes (e.g., BSA, cytokines) to meet the product-specific purity criteria necessary to satisfy the release specifications. As such, one of the critical steps towards the selection of a final washing tool is the definition of "target depletion levels" for media components, additives, and other impurities. These target depletion levels, and specifically the highest depletion level amongst all the components that need to be purified, will then determine the amount of volume washouts (or "volume equivalents," e.g., how many times the total original volume should be replaced with new media volume) that a washing operation will need to ensure the product is sufficiently purified.

As an example, let's take a harvest of a 1L cell culture whose culture conditions involved the use of two different cytokines with concentrations of 100 IU/mL IL-2 and 1 IU/mL IL-6. Let's also assume that the target wash output volume is 1L (e.g., no volume reduction), and that the final post-wash acceptable cytokine concentration levels are 0.1 IU/mL for both IL-2 and IL-6. Based on these parameters, the volume reduction must provide a 3 log (1000x) reduction in IL-2 concentration, as IL-2 represents the media additives that require the highest depletion level in this example (from 100 IU/mL to 0.1 IU/mL). Assuming the use of an automated washing system such as those discussed in the "Cell washing" section, and assuming a 90% volume exchange at each washing cycle, a minimum of three washing cycles (for a total of 2.7 volume equivalents, or 2.7 L total washing volume) will be required.

The optimal washing tool will provide the ability to perform the required amount of volume washout within the shortest operational window, while providing the highest cell retention.

Volume reduction considerations: Particularly for large harvest volumes, where the required media volume to perform the target volume washout might be significant (e.g., in the example above, the total washing volume would increase from 2.7 L to 27 L if the culture volume went from 1 L to 10 L), the ideal washing tool will provide the ability to concentrate the sample prior to performing the washing operation, as this will significantly decrease the total required volume to perform the required depletion of impurities. Several off-the-shelf tools exist that provide combined wash and volume reduction capabilities. The ideal final DS washing protocol will therefore be one in which the overall sample volume is first significantly reduced, followed by a washing procedure providing the required amount of volume washout based on the post-volume reduction volume. Applying this improved volume reduction and wash protocol to the example above, and assuming a 10x concentration step prior to washing, the overall culture volume would first be reduced from 1 L to 0.1 L (while the IL-2 cytokine concentration would still remain at 100 IU/mL) followed by a 2.7 volume equivalent wash, i.e., 0.27 L (as opposed to 2.7 L without volume reduction), to deplete the IL-2 level down to 0.01 IU/mL.

In addition to the media consumption considerations listed above, an even more critical design parameter for the volume reduction step is the "target DS concentration." The volume reduction step will indeed need to be capable of concentrating the cells to a concentration higher than that required to perform the final downstream DP formulation steps. For example, assuming a target 100×10^6 cells/mL DP concentration and a DP formulation protocol in which the DS is diluted 1:1 with cryomedia, the final DS wash will need to concentrate the cells to 200×10^6 cells/mL to enable the downstream dilution step.

Media exchange considerations: A key function of the final DS preparation wash is the ability to transfer the cellular product from the media in which the cells were cultured to a different buffer of choice that will constitute the "basal formulation buffer" in preparation for the successive formulation/DP manufacturing steps. Typically, basal formulation buffers are DMSO-free, but might contain excipients and other additives aimed at enhancing cellular function or recovery (e.g., dextrose). As some of these additives could potentially be limited in availability and high in price, it is sometimes favorable to consider a final DS washing approach where the main washing operation is performed using an excipient/additive-free buffer, followed by a final washing cycle of one volume equivalent that will place the cells in the final desired buffer composition.

Other considerations for selecting the appropriate wash/volume reduction tools include cost of goods, data integrity, and connectivity as described in the Physical Connectivity section.

FORMULATION

Formulation involves the mixing of the final cell product with a cryoprotectant and a diluent or freezing media to arrive at the desired concentration of cells.

Optimal formulation is critical for the success of a final cellular product that is stable, safe, efficacious, and meets regulatory requirements. Formulation is the process of combining cells, buffers, proteins, ancillary materials, and cryoprotectants, and is carried out immediately after the cells are harvested at the end of the manufacturing process. Formulation is a temperature-dependent and time-sensitive step since the harvested cells during this step are held in suboptimal environmental conditions and without nutrients. Appropriate formulation is needed to stabilize the cells so they can withstand stress factors such as temperature excursions, pH changes, and mechanical stress caused by handling, storage, shipment, and bedside preparation.[1]

Formulation strategies involve selection of the appropriate cryoprotectants and other excipients. The selection of excipients plays a key role in maintaining the CQAs of the final product. In addition to commercially available cryopreservation media, common formulation for mammalian cells historically includes 10-90% fetal bovine serum (FBS), 5-10% dimethyl sulfoxide (DMSO), and 1-20 million cells per milliliter that have been previously resuspended in an isotonic electrolyte solution, such as Ringer's lactate or Plasmalyte 148. While the use of FBS has been questioned, it is still permitted as an excipient. Serum-free cryoprotective agents are commercially available and, although proprietary, developers can reference a Drug Master File (DMF) for the regulatory agencies. Serum replacements include human serum albumin (HSA) and human platelet lysate (hPL). HSA is one of the most popular excipients in cell therapy because it is the most ubiquitous protein in blood and is known to create an optimal microenvironment for sustained cell viability. It acts as a scavenger of toxins and other reactive oxygen species, maintains pH, provides insulation, and maintains cell viability during cryopreservation without increasing osmolality.[45] Additional components of the final formulation can include dextran, which serves as an osmotically neutral volume expander and as parenteral nutrition, sodium chloride as a normal saline diluent, and stabilizers such as sodium caprylate and N-acetyltryptophanate that protect proteins such as HSA from oxidative stress.[1,46]

The cryoprotectant (DMSO) addition rate and the temperature at which this occurs must be carefully determined and rigorously controlled. If the cryoprotectant is added too quickly the cells may lyse; if it is added too slowly or at an elevated temperature toxicity becomes problematic.

There is no standard recipe for the final formulation and each cell type may perform differently following different manufacturing protocols. Additional factors such as cell concentration, sample volume, and even

sample container geometry may affect the final outcomes. Whatever system is implemented it is important to perform both short- and long-term follow up testing to ensure the viability of the product.

FILL AND FINISH OPTIONS

Fill-finish operations to load cell therapy products into appropriate containers are the endpoint of a product's journey through a GMP-compliant manufacturing process until the product is transported, thawed, and infused into the patient. After the formulation process is complete, the product is filled into the final product container (typically bags or vials), labelled, cryopreserved (if applicable), and stored in an appropriate environment (e.g., 2-8°C, -80°C freezers, or vapor phase liquid nitrogen) while batch release testing and certification is carried out. Following batch release, the product is packaged for distribution. The product is then transported to the clinical or commercial use site while carefully controlling the cold chain. At site, if supplied frozen, the product undergoes final thawing and potential cell wash or dilution steps prior to dosing the patient. The overall efficacy of the clinical product partly depends upon the efficacy of the bio-preservation steps within the process. A thorough understanding of the impact of cryopreservation, thawing, and preparation of the product for administration to patients is essential to ensure its efficacy as the product is of most value at this point. These include formulation, aliquoting into bags or vials, cryopreservation, short- or long-term banking, maintaining the cold chain during transportation to the clinic, and the final thawing and potential cell wash or dilution steps prior to dosing the patient.

In conventional practice, cell therapy products may be presented in either vials or bags. Both of these options have advantages and disadvantages, and developers must choose according to closure integrity (the container must be hermetically sealed and provide adequate physical protection to ensure specimen integrity is maintained throughout processing, storage, and distribution), sample stability (the container must ensure that the product remains stable over long periods of time), and ease of access to the product. When deciding, developers should consider all safety and quality standards and patient needs. Selection of the final container should therefore take into consideration particulate levels, leachable and extractable profiles, and material integrity at a range of temperatures achieved during cryopreservation, storage, shipment, and the thawing process.

Traditionally, cells have been cryopreserved in vials. Vials are especially valued for durability under extreme temperature conditions and amenability to storage under a variety of conditions. Additionally, they can be more easily combined to meet a variety of doses required in early phase clinical dose escalation studies. As cell therapy processes are developed, washing cells, resuspending cells in freezing media, and finally aliquoting into cryovials is a manual process mostly carried out in a BSC. Automated solutions for vial filling exist, having been developed for pharmaceuticals and more recently biologics. Vials are suitable for seed banks and large-scale allogeneic cell therapies, but they are not as convenient for autologous cell therapies due to the requirement to be processed in a BSC and thus unsuitable for administering cells at a clinical site by the end user. Other drawbacks include the size limitation (i.e., cell doses that may exceed billions of cells may not fit in a vial) and the inability to hermetically seal the system. Vial closure systems, whether screw caps or rubber stoppers, do not allow complete closure and therefore pose contamination risks to the product. More recently, automated vial filling platforms have been developed that use more sophisticated vials with filling ports and various retrieval options that facilitate sterile weldable connections.

Bags are commonly used as the final container for cell therapy products due to the existing abundance of infrastructure for processing, freezing, and storing bag container systems from the long history of bag usage in blood banking systems. Cryopreservation of cells in bags facilitates administering large numbers of cells at the clinical site with a sterile weldable connection, a luer lock, or a spike connection. From the manufacturing perspective, the ability to aseptically weld and hermetically seal cryobags is extremely beneficial and eliminates the need for a BSC or similarly controlled environment. However, the fill process can be uniquely challenging. While commonly used for larger volumes, new bag formats are available in several sizes between 5 and 10 milliliter volumes.

Even given the advantages bags offer, several drawbacks do exist. There are cases where samples must be stored in

smaller volumes than is appropriate for a bag format; due to the relatively brittle nature of the material out of which most bags are made, sample stability at typical storage temperature may be compromised, especially during transport and manipulation of the sample during thaw. Perhaps most significantly, bags carry a risk of compromised container integrity. A study by Khuu et al.[47] investigated a series of catastrophic bag failures first noticed in 2001 that were associated with four specific bag lots made from poly (polyethylene-co-vinyl acetate). While no serious adverse patient effects occurred, extensive bag failures led to cell product contamination, increased product preparation time, increased antibiotic use, and increased costs to replace defective products. In another study[48] investigating cases from 2000 to 2006, integrity loss of 3.5% of bags was observed during bag thawing at 37°C.

Sealed or semi-closed vials, such as the AT-Closed Vial® from Aseptic Technologies, offer both the scalability of traditional screw top cryovials and the closed system advantages of bags. The ability to fill and seal AT-vials without removing the septum minimizes the contamination risk. Both bags and vials are available with traceable bar coding and RFID tags to identify and record batch information, testing results, and other quality documentation. Many of these containers are supported by the FDA DMF, with extensive data provided in their validation packages.

Many fill and finish protocols start out as manual operations in a BSC. As lot sizes increase, because the overall manufacturing process is scaled out/up, manual operations quickly become a bottleneck. Several devices have been launched into the marketplace that are capable of rapidly filling bags or vials with formulated cell products. While capable of quickly filling multiple bags or vials and greatly reducing contact time in DMSO, some devices lack the capability to cool formulated cell products and some must be used in an isolator (Aseptic Technologies M1 and L1, Sexton Biotech AF500). Other manufacturers have automated the entire unit operation including mixing and cooling capabilities (Miltenyi Prodigy, Terumo Finia, Sexton Biotech Signata) and the ability to fill both bags and vials (in some cases). The current automated offerings are somewhat limited when large-scale is needed and may require multiple devices to accomplish the fill and finish without increasing the DMSO exposure time to levels that impact product quality.

After products are filled, they must be appropriately inspected and labelled. The type of label and printing ink used needs to be suitable for adhesion to the primary container, cryopreservation, and thawing of the product so that the label remains attached to the primary container and legible. Incorporation of all the information required on the label can pose a challenge, especially when using vials as the label area is often quite small. Labels that extend the printable area (e.g., "Flag" and "leaflet" labels) are available and development studies on thawing times and selection of appropriate thawing equipment should be carried out with the chosen label to ensure establishment of robust end-user protocols and product quality prior to patient administration. Labeling for licensed CAR T cells must conform to the requirements in 21 CFR Part 201 and 21 CFR Part 610 Subpart G. Autologous CAR T products must be labeled "FOR AUTOLOGOUS USE ONLY", and the label should also include at least two unique identifiers to confirm patient identification prior to administration.[49] For allogeneic products where batch sizes are much larger than autologous product, the time taken to visually inspect and label the product is much longer so it is important to design the fill-finish process to ensure that all critical process parameters such as hold times and temperature are still met.

Ultimately, the choice of final container and label must be suitable to the developed manufacturing process, be sufficiently scalable for commercial success, and must accommodate the end-user. With the current available options that can offer simplicity, flexibility, and scalability, one of these containers can likely suit cell therapy developers' product needs.

CRYOPRESERVATION

For the clinical or commercial distribution of cell therapies, the standard convention at this time is to perform cryopreservation prior to distribution either to the patient or for temporary storage until required use, where the cells will be thawed and administered. This is principally due to the fact that cryopreservation provides significant flexibility in manufacturing and patient scheduling, facilitates the quality control release process for a DP, and allows for transportation of the therapy to the patient. Working with fresh, non-cryopreserved cell therapy products increases the risk to patients because all subsequent operational activities (labelling, packaging, batch record review, QP release, and

shipping) must be rushed so that infusion to the patient can occur within the product stability time window. As such, the time allotted for release testing and quality assurance review is minimal, often resulting in patient infusions with limited data available (e.g., interim sterility reads). Moreover, the lack of cryopreservation significantly increases the risk of a cell therapy product shortage as only limited inventory can be built, especially in the allogeneic space.

The current established best practice for T cell therapies is formulation in a DMSO-based cryoprotectant and processing into either blood bags (typical for autologous therapies) or cryovials (common for allogeneic therapies), followed by cell cryopreservation utilizing a highly reproducible, controlled-rate freezing system suitable for the type of primary packaging (bags or vials) that can support a cooling rate of −1°C min^{-1} or slower to preserve high levels of cell viability. This is because the rate at which a cell solution is brought from a physiological temperature to a cryogenic temperature has been shown to be a key factor in cell survival. It has been demonstrated that rapid cooling rates (i.e., greater than −10°C min^{-1} have led to a notable reduction in viable cell count if the subsequent warming process for administration is slower than 6.2°C min^{-1}.[50] The loss in cell viability is believed to be due to the creation of amorphous ice structures in the cells, which when subsequently thawed at slow rates lead to ice recrystallization causing the mechanical disruption of cells. Implementation of a controlled cooling rate also decreases variability in the outcome of the cryopreservation process.

There is no one-size-fits-all cryopreservation approach for cell therapy processes. One needs to consider the type of cell, cryoprotectants, cell concentration, and the primary packaging in the optimization process for cell cryopreservation. Each of these aspects could affect the final product quality.

- **Cryoprotectant.** The cryoprotectant should be selected such that the final product is stable, safe, efficacious, and meets regulatory requirements. Typically, to avoid osmotic shock and temperature-related toxicity, cells are cooled down to 4°C before the same temperature DMSO-containing cryoprotectant is added. Maximum contact time should also be assessed to determine the operational time window before cell viability and functionality drops.
- **Cell concentration.** The cryoprotectant's power is best leveraged within specific cell concentration ranges.
- **Product container (bag or vials).** The material and the geometry of the final container will have an impact on heat transfer and homogeneous cooling/warming rate of the product.

Recommendations would be to utilize QbD approaches to identify the CQAs that provide consistency in meeting the TPP of the final product. This process is particularly critical for cell therapies where, unlike many other bioprocesses, the properties of the final product (viability, recovery, and cell function) post-thaw represent the cumulative effect of all of the processing steps and reagents used in the process, where each step may influence the total number and viability of cells after completion of the manufacturing and cryopreservation processes. The current recommendation for a minimum viability standard for cell therapies is at least 70% (based on FDA guidance,[51] and often higher) post-thaw after both formulation and cryopreservation. In addition, assessing post-thaw vitality or apoptosis as predictive signals of cell viability drop, and post-thaw functionality, ensures a more holistic readout of the quality of the cryopreservation process, therefore generating a stronger dataset to support decision-making when locking down the process.

For cell therapies, best practices require that cells be stored at temperatures below -150°C. Storage in temperature-controlled environments and systems to prevent temperature excursions are commonly used. The stability of cells in storage is most strongly influenced by two factors: transient warming and background ionizing radiation. Proper training and product handling protocols can help reduce transient warming of samples during retrieval of samples from a biostorage location.

In conclusion, the success of CAR-T manufacturing relies on a series of critical processes, from the processing of cellular starting material (apheresis) to cryopreservation, to ensure product yield and quality. Each step involved in the manufacturing process has its own technical considerations and challenges, and continued work is being performed in the cell therapy field to address these challenges and optimize each process for various therapeutic applications. While

this chapter covers the technical considerations of each unit operation, manufacturers must also ensure the safe delivery of the cell therapy product to end users at clinical administration sites; therefore, cold chain storage, distribution, and logistics must also be considered.

Electronic Systems Considerations

Enterprise control is the ability to combine control, intelligence, and process management to enable business optimization that is inclusive of digital information (IT) and production operations (OT). It is the deliberate act of synchronizing business strategy with operational execution in real-time to enable closed-loop business control across an enterprise. Additionally, it is the start of a data collection capability that allows for data feedback loops into process optimization. In the context of cell-based therapy manufacturing, these entail considerations for electronic systems for manufacturing execution (MES), quality management (QMS), process information management (PIMS), release testing and laboratory information management (LIMS), document management (DMS), warehouse management (WMS), computerized maintenance management systems (CMMS), and other systems designed to ensure track and trace abilities, enhanced documentation and robustness of the manufacturing and testing processes.

ENTERPRISE SYSTEMS ARCHITECTURE OVERVIEW

Enterprise IT/OT Systems commonly follow the ISA 95 standard which is based upon the Purdue Enterprise Reference Architecture (PERA). ISA 95 focuses on segregation of the IT and OT domains with strict level-based control and dataflows intended to prevent unauthorized access or changes to the OT systems. With the introduction of Cloud and IIOT (Industrial Internet of Things) within the last 10 years, many institutions are implementing a modified hybrid-based model to enable Cloud/Edge technology at each level of the architecture.

While the hierarchy of ISA 95 details a logical separation of systems utilized for designing digital solution architectures, the model can be simplified into Business Systems, Data Systems, and Operational Systems.

ENTERPRISE SYSTEMS IN DETAIL

Business systems

Business Systems are those traditionally used across the enterprise and encompasses commercial functions for sales, business operations for finance, accounting, and HR, and corporate business planning. One common business system is the enterprise resource planning (ERP) system. Other examples include product life cycle management (PLM), supply chain orchestration (SCO), customer relationship management (CRM), and human resource management (HRM). Reaching further into business operations we find document management systems (DMS), warehouse management systems (WMS), computerized maintenance management systems (CMMS), quality management systems (QMS), track and trace (T&T) systems, learning management systems (LMS), and finite scheduling (FS) systems. These are typically of enterprise-scale, and often require a significant investment in human resources, dollars, and time.

ERP

An enterprise resource planning (ERP) system is typically a suite of integrated applications that an organization can use to plan their main business processes. ERP systems track the status of business commitments such as orders, purchase orders, payroll, warehouse management, financial operations, and planning. An ERP system facilitates information flow between these business functions and manages connections to outside stakeholders.

In cell-based therapy commercial manufacturing, ERP systems are critical as the pharmaceutical manufacturer needs to keep track of all patient orders along with critical must-have inventory. For manufacturers with allogeneic processes, ERP systems are also important as one donor gives life to a number of recipients and the ERP system keeps tracks of all this information.

The most common ERP systems used in the life sciences space are SAP, Oracle, and Microsoft Dynamics, all of which are also available in the cloud.

SCO

Supply chain orchestration (SCO) platforms have recently evolved for advanced therapy medicinal products (ATMPs) or more commonly cell and gene therapy manufacturers. SCOs control the chain of identity (COI),

which is a unique patient ID, and the chain of custody (COC), which ensures the name of anyone who has touched the product and where the product has traveled are recorded. To facilitate ATMP sector growth, the supply chain infrastructure is critical in supporting the industry from clinical trials to commercial distribution of life-saving treatments to more patients in a safer and more secure manner. The Health Insurance Portability and Accountability Act (HIPAA) in the United States and the General Data Protection Regulation (GDPR) in the European Union are the foremost regulations all SCO platforms must adhere to. The patient journey, as some vendors call it, is tracked within this platform. Similar to both autologous and allogeneic processes, the first steps include creating a unique identifier or COI for the donor / patient, drawing blood from the donor / patient (apheresis process), centrifuging the product, securing the blood bag by printing barcoded labels, integrating with shipping and logistic vendors to ship the blood bag to manufacturing facility, preparing the final product for shipment back to the hospital or clinic after manufacturing is complete, and then infusing the final product into the patient.

Notable companies that use cloud-based solutions to track needle-to-needle patient monitoring include TrakCel, Vineti, Hypertrust, FarmaTrust, and Danaher (previously GE).

HUB Services

New to the industry and specifically valuable to the cell and gene therapy industries is the entry of HUB Services. HUB services provide connections between a patient and their health care providers, pharmacies, distributors, and insurance companies, while at the same time ensuring privacy as provided by HIPAA regulations.

HUB Service providers include large health providers such as AssistRx, CareMetx (Walgreens), Omnicare Specialty Care Group (CVS), OptumRx (United Health) and Sonexus Health (Cardinal).

FS

Finite scheduling (FS) systems in the cell and gene industry are gaining prominence as more cell therapy manufacturers reach clinical trials and patient population grows from a mere 100 at start to more than 10,000 patients when the cell-based therapy product is fully commercialized. FS systems support resource scheduling with finite capacity, production sequences optimization, materials management, multi-scenery analysis, dynamic constraints management, laboratory room scheduling, and patient scheduling with direct integration to SCO to allow nurses to book a slot for patients. An FS system allows cell therapy manufacturers to do global and local production planning (patient orders) and dynamically allocate production orders. The following benefits are obtained: improvement of finite capacity work plan and patient order tracking, production lead time reduction, inter-operational and line buffer reduction, prompt material calling from suppliers, inventory-level cost reduction, and improvement of transportation efficiency.

The most notable FSs are Körber Pharma PAS-X, sedApta, and Bio-G (purchased by Emerson in 2019).

DMS

A document management system (DMS) is used to receive, track, manage, and store documents and eliminate paper. They keep a record of the various versions created and modified by different users (version tracking). It is often viewed as a component of enterprise content management (ECM) systems and related to digital asset management, document imaging, workflow systems, and records management systems required by the company's QMS. Documents stored in a document management system, such as procedures, work instructions, and job aides, provide evidence of documents under control. Failing to comply can cause fines, loss of business, or damage to a business's reputation. Important aspects of document control include reviewing and approving documents prior to release, reviews and approvals, ensuring changes and revisions are clearly identified, ensuring that relevant versions of applicable documents are available at their points of use, ensuring that documents remain legible and identifiable, ensuring that external documents (such as customer-supplied documents or supplier manuals) are identified and controlled, and preventing unintended use of obsolete documents.

Paper documents have long been used in storing information. However, paper can be costly and, if used

excessively, wasteful. Document management software is not simply a tool, but it helps manage access, and track and edit stored information. A DMS is an electronic cabinet that can be used to organize all paper and digital files. It helps businesses convert paper documents to digital files and store them in a single hub after they are scanned and digital formats get imported. An important benefit of digital document management is the "fail-safe" environment it provides to safeguard documents and data.

The most common DMSs include Veeva's Vault DMS, Qumas, and Microsoft SharePoint.

CMMS

A computerized maintenance management system (CMMS) is a computer database of information about an organization's equipment maintenance operations. This information is intended to help facility maintenance workers do their jobs more effectively by helping to determine when equipment will require maintenance and which equipment is predicted to fail. This helps management make informed decisions to calculate the cost of equipment breakdown repair versus preventive maintenance for each piece of equipment, possibly leading to better allocation of resources. Cell-based therapy manufacturers spend a considerable sum of money on equipment, as the equipment used in this space is very intricate and complicated, and the CMMS helps ensure the equipment runs as expected. CMMS data may also be used to verify regulatory compliance. To properly control the maintenance of a facility, information is required to analyze what is occurring. Manually, this requires a tremendous amount of effort and time. A CMMS also allows for record keeping to track completed and assigned work orders in a timely and cost-effective manner. Cell-based therapy manufacturers have started using CMMSs extensively to better control and organize maintenance management.

The different components of a CMMS include, but are not limited to, equipment data management, predictive and preventive maintenance, labor, work order system, scheduling and planning, vendor management, inventory control, purchasing, budgeting, and asset tracking.

The most common CMMSs are Blue Mountain, SAP PM, and IBM Maximo.

QMS

A quality management system (QMS) is a collection of business processes focused on consistently meeting customer requirements and enhancing their satisfaction. It aligns with an organization's purpose and strategic direction. It is expressed as the organizational goals and aspirations, policies, processes, documented information, and resources needed to implement and maintain it. The ISO 9000 family of standards is probably the most widely implemented QMS worldwide–the ISO 19011 audit guidelines apply to both pharmaceutical and medical devices—and deals with auditing management systems, including principles of auditing, managing an audit program, and conducting management system audits, as well as guidance on evaluating the competence of the individuals involved in the audit process.

The two primary guidelines for life science manufacturer QMSs and related services today are the ISO 13485 standards and the US FDA 21 CFR 820 regulations.

Quality system requirements for the life science industry are internationally recognized as a way to assure product safety and efficacy to customers. The FDA constituted the rule in Code of Federal Regulations 21 CFR 820, which all medical device manufacturers must follow. A typical quality system includes management controls; design controls; production and process controls; corrective and preventative actions; material controls; records, documents, and change controls; and facilities and equipment controls. Pharmaceutical drug manufacturers are regulated under a different section of the Code of Federal Regulations.

The QMS covers processes such as calibrations, internal audits, corrective actions, preventive actions, identification, labeling, and control of non-conforming products to prevent its inadvertent use, delivery or processing, and purchasing and related processes such as supplier selection and monitoring.

Common IT systems to manage a QMS include Veeva's Vault QMS, TrackWise from Sparta (purchased by Honeywell in 2020), SmartSolve from IQVIA, and MasterControl QMS.

LMS

A learning management system (LMS) is used for the administration, documentation, tracking, reporting, automation, and delivery of educational courses, training programs, or learning and development programs.

Learning management systems are designed to identify training and learning gaps, utilizing analytical data and reporting. LMSs are focused on online learning delivery but support a range of uses, acting as a platform for online content, including courses, both asynchronous- and synchronous-based. An LMS offers classroom management for instructor-led training or a flipped classroom, used in higher education, but not in the corporate space. Modern LMSs include intelligent algorithms to make automated recommendations for courses based on a user's skill profile, and to extract metadata from learning materials to make such recommendations more accurate.

The most notable LMS in this space is ComplianceWire.

Data systems

Data systems are key to the collection, storage, and sharing of manufacturing data. Newer data systems additionally add functions for the analysis of data as well as sharing and collaboration of both data and insights garnered from it. For this simplified grouping, data systems would include manufacturing execution systems (MES), laboratory information management systems (LIMS), process information management systems (PIMS), and data historians.

MES/EBR

A Manufacturing Execution System (MES) is a computerized system used in manufacturing to instruct and document the transformation of raw materials to finished goods. An MES provides information that helps manufacturing decision makers understand how current conditions on the plant floor can be optimized to improve production output. These systems work in real-time to enable the control of multiple elements of the production process (e.g., inputs, personnel, machines, and support services). In cell-based therapies, the MES covers all steps in the manufacturing process and typically it helps in reducing cycle time of the process along with reviewing only critical deviations ensuring compliance and data integrity to batch records. An MES includes electronic batch records (EBR), albeit MES is much more, and operates across multiple function areas such as management of product definitions across the product life cycle, resource scheduling, order execution and dispatch, production analysis and downtime management for overall equipment effectiveness (OEE), product quality, or materials track and trace. The MES creates the EBR by capturing the data, processes, and outcomes of the manufacturing process, and often can result in elimination of paper batch records.

MES systems in the cloud can integrate with almost any system and can be used as a source of truth for shop floor manufacturing. MES or digital EBR systems in the cloud are advantageous for cell-based therapy manufacturers as they control the chain of identity (COI), chain of custody (COC), chain of condition, and time-to-delivery, which are critical aspects for cell-based therapy manufacturers in the cell and gene space. MES applications seamlessly integrate with supply chain orchestration (SCO) platforms in absence of a formal ERP system. An MES system also keeps track of CQAs, critical process parameters (CPP), QTPP, and integrates with a PIMS for cross-product analysis of these parameters.

The most notable MESs in the life sciences space include Körber Pharma (formerly Werum IT Solutions) PAS-X, POMSnet Aquila from POMS, Emerson Syncade, and Rockwell FactoryTalk Pharma Suite.

LIMS

A laboratory information management system (LIMS) is designed to improve laboratory productivity and efficiency by keeping track of data associated with samples, experiments, laboratory workflows, and instruments. A LIMS can feature specialized capabilities for research and development laboratories, process development and manufacturing laboratories, or bioanalytical laboratories. The LIMS can also act as one source when using the four-eyes principle, automating workflows and tracking all the important sample information, data, workflows, and QA/QC results a lab generates each day.

The most common LIMSs in the life sciences space include LabVantage, Thermo-Fisher LIMS, StarLIMS by Abbott, and Labware.

PIMS

A process information management system (PIMS) is employed for process monitoring from the collection of unique raw materials during apheresis through batch production to clinical endpoint data. A PIMS supports adherence to regulatory authority requirements like continued process verification (CPV) by providing analytics for monitoring and reporting capabilities as well as supporting investigations. These systems are unique as one of the few systems designed to curate data from paper, spreadsheets, and other data sources existing at all levels of the organization. Common examples include curation of data from paper batch records with data from a LIMS, ERP, Historian, and QMS.

One cell-based therapy usage of a PIMS system is for management of V2V (vein-to-vein). The PIMS allows linking blood characteristic information collected from blood donations, the resulting manufactured materials quality information, and non-patient identifying efficacy data from hospital medical records to allow for visibility into how the therapies are performing along with any possible correlations of adverse effects due to manufacturing quality problems or raw materials.

Two popular PIMS providers include Skyland PIMS and BIOVIA Discoverant.

Data historian

A data historian (also known as a process historian or operational historian) records and retrieves production and process data by time; it stores the information in a time-series database that can efficiently store data with minimal disk space and fast retrieval. Time-series information is often displayed in a trend or as tabular data over a time range. As cell-based therapy sponsors accumulate large amounts of data from complex equipment, a data historian comes in handy where a cloud-based system can integrate with any equipment and store the data in real time (microseconds). Commercial off-the-shelf data historians have several advantages over home grown systems and off-the-shelf relational databases such as off-the-shelf data acquisition interfaces with control systems.

The most notable data historian systems include OSIsoft PI (purchased by Aveva) and Rockwell FactoryTalk Historian.

Operational systems

Operational systems include automation and typically follows ANSI S88 standards set by the industry. The most common systems in this group are the supervisory control and data acquisition (SCADA) system, distributed control system (DCS), data historian system, environmental monitoring system (EMS), building automation system (BAS), and equipment monitoring systems. These systems use sensors placed across the entire plant to collect data and allow users to make informed decisions on the floor. Commonly used in traditional pharmaceutical and biotech industries, these systems have gained prominence in the cell and gene therapy space as well. They are highly complex systems that require an automation engineer and high capital to support the high operating cost.

For cell-based therapy manufacturers, although each of these is important, we cover the EMS and BAS as these are prevalent at every cell-based therapy manufacturer who has reached Phase 1 clinical trials. DCS and SCADA systems are often needed for much larger scale processes such as large and small molecule manufacturing.

EMS/BAS

Cell-based therapy manufacturers use an environmental monitoring system (EMS) for managing controlled environments as it is designed to evaluate, manage, and reduce the risks associated with everyday environmental monitoring. An EMS controls the whole process, not just scheduling for individual sampling points; it also captures all critical quality parameters for environmental controls. These systems integrate with LIMS, MES, instrumentation, and mobile sample acquisition in a paperless environment.

Some key features include utilities monitoring, viable and non-viable sampling, comprehensive reporting capabilities, automated investigation management and root cause analysis, facility map for visualization of positive counts, integration to particle counters and air samplers, and integration to process monitoring and QC lab instrumentation.

Several notable EMSs include Novatek EM, ThermoFisher EM, and Siemens Life Science Laboratory.

Building automation systems (BAS) connect and automate functions inside a building including lighting,

HVAC, fire, and security systems. They can additionally monitor facility assets that may directly impact environmental monitoring, such as doors that separate environmentally sensitive spaces.

BAS vendors include Honeywell, Johnson Controls, Schneider Electric, United Technologies, Emerson Electric, and Siemens among other providers.

ENTERPRISE SYSTEMS RECOMMENDATIONS

For a commercial manufacturer annually handling 30,000+ patients, most of these enterprise systems are critical as the volume of patients is high and it is crucial that the entire business works in coherence to remove silos and manage all data electronically in the cloud.

For a preclinical or clinical trials manufacturer, the initial patient volume is small; therefore, only an MES or EBR, PIMS, LIMS, DMS, and environmental monitoring system are needed to minimize reliance on paper and spreadsheets. From experience, these are the only systems early-stage manufacturers use and have significantly progressed in their clinical trials while capturing the relevant regulatory data at each stage.

IT CONSIDERATIONS

Regardless of differences in specific technologies utilized for the manufacturing process, a consistent and enduring challenge for every cell and gene therapy company is the collection, storage, retrieval, analysis, and reporting of the data. When selecting technologies to employ, considerations into cost, resource investment, single- or multi-vendor, off-the-shelf (OTS) versus custom solutions, schedules, and spreadsheets come into play.

Firms rarely go all in from the beginning with respect to the IT environment. Instead, firms often purchase piecemeal to solve "problems at hand," incurring tremendous burdens on the organization as they take evolutionary steps forward. The best practice to minimize costs and burdens is to envision the idealized final state for data management and reporting, and then design the steps necessary to reach that final state. Creating an "IT Maturity Model" is critical for planning and guiding this IT investment roadmap.

A common problem to consider while developing this roadmap is deciding to take the lowest cost, simplest, and seemingly easiest path forward for managing data (e.g., Microsoft Excel). Putting 21 CFR Part 11 and validation concerns aside, this decision also puts a burden on gathering, entering, contextualizing, tracking, and creating the analytics for all that gathered data. Invariably, Tech Ops leadership realizes that a large percentage of time and resources are spent on managing spreadsheets. At this point, it is time to consider new systems. An obvious problem is the legacy data in the spreadsheets that will later need to be available for analysis in the new tools. While Excel is often the easy decision at start-up, as companies climb up their maturity model to early-stage product development, they should consider the critical timing for cutover to automated systems to keep resources on revenue generating activities, rather than spending months trying to unravel spreadsheets, or worse—paper, with historical data.

As with every company, management of human capital (captive or outsourced) and human resources are IT costs best optimized. It is recommended that companies consider technology decisions that lower costs involving human capital to allow focusing funds on core IP development and manufacturing. Due to advancements in security architectures, cloud technologies are rapidly being adopted in the life sciences arena, allowing firms to redirect precious resources from large and expensive IT data centers to more important IP challenges that bring value to the business.

Whether a company should incorporate a single-vendor or multi-vendors with integration is important for the future of the business. Since the life sciences industry tends to swing back-and-forth on which vendor approach is ideal, one should consider the risks and benefits of both paths forward. If partnering with a single vendor, the therapeutic developer should take into account whether the vendor can prioritize their needs given much of the control has been placed in the provider's hands. Selecting a multi-vendor approach mitigates this risk and provides flexibility to the therapeutic developer, however integration among vendors could be an issue and therefore needs to be evaluated thoroughly.

Deciding whether to use OTS or custom solutions can be difficult. An OTS solution comes at a fraction of the cost of a custom solution but gives everyone the same product, therefore capabilities rarely provide 100%

of a therapeutic developer's desired needs. However, OTS solutions can usually be installed and be ready to run much faster, allowing a company to extract value from these solutions sooner (often referred to as time-to-value). Additionally, the product vendor provides new functionality and system maintenance. As a best practice, therapeutic developers should perform a gap analysis of critical business requirements against current and planned features. If gaps are determined to be critical, they should assess whether there are other ways to fill the gaps at lower cost. If a custom solution is deemed the desired approach, they need to determine what budgetary sacrifices are needed to justify this development and the respective ongoing support.

Schedules must also be considered when selecting technologies to employ, i.e., how early a good practice (GxP) system needs to be up and running for each area of the business, and whether validation is needed. If validation is required, one needs to understand the impacts of deciding to validate at initial deployment or later by working with the QA organization. Additionally, risk-based approaches to validation that further accelerate the software's time-to-value should be explored.

Conclusions

This chapter presents the principles of cell-based therapy manufacturing, which involves numerous steps and unit operations, each associated with its own complexity. In particular, considerations for manufacturing of an example CAR-T product are described. The complexity of this process warrants the implementation of diverse technologies throughout its manufacture to ensure an appropriate product yield is achieved to meet the clinical dose requirements, while the product quality, safety, and efficacy is maintained. Continuous advancements in production technologies are being made in the cell therapy field to allow for increased yield, while maintaining product quality. In addition to the platform innovations (e.g., new gene editing techniques, new expansion hardware, automated fill and finish platforms), advancements are also being made to support process improvements through increased process control, incorporation of data analytics, and automation. These are facilitated by innovations in hardware such as new non-invasive sensor development, imaging capabilities, and software improvements to complement these innovative technologies.

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Chapter 9

Analytical Technologies for Cell Therapies

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Background

Cell-based therapies play a vital role in transforming human health as they offer solutions for many unmet medical needs. The commercial launch of CAR-T products Kymriah™, Yescarta™, Tecartus™, Breyanzi™, Abecma™, and most recently Carvykti™ generated tremendous excitement and have resulted in more therapies advancing through clinical trials towards commercial approval. These advancements have fueled the need to develop robust and well-characterized analytical methods that are critical for demonstrating safety, identity, purity/impurity, quantity/strength, and potency of the final products. Effective analytical technologies also play a vital role in choosing raw materials, controlling the manufacturing process, determining the quality of intermediates, and ensuring a safe and effective final product.

Cell-based therapies are unique and are often described as "the process is the product" where the final product is comprised of living cells. The complexities associated with cell therapy manufacturing require a broad set of analytical tools. It is critical to develop and employ analytical assays early in the process development stage since they provide insights into the attributes of the cells throughout the manufacturing process, and help enable process changes and control of the final product.[1]

This chapter focuses on the approach to analytical assay development, progress of analytical technologies, and challenges related to process and product characterization for cell-based therapies. While this chapter uses a CAR-T product as an example to illustrate the various analytical technologies used during manufacturing and final product testing, many of the principles outlined here are applicable and beneficial to a broader profile of cell therapy products.

Critical Quality Attributes (CQA) and Quality by Design (QbD)

According to ICH Q8, critical quality attributes (CQAs) can be defined as the physical, chemical, biological, or microbiological properties of the cell product that should be within an appropriate limit, range, or distribution to ensure the desired product quality. These properties are identified early in the product development process. Their criticality is determined using quality by design (QbD) principles, as outlined in Chapter 4, and are continuously refined throughout the development by characterization studies. Successful characterization of these attributes through implementation of analytical technologies depends on product understanding, including:

- Interlink between CQAs of the product (including potential CQAs from early product development) and analytical methods
- Performance attributes of the process
- Performance attributes of the methods
- Key characteristics of the various starting raw and in-process materials

In the case of cell therapies, it is vital to develop analytical assays that build upon the relationships between donors' or patients' starting materials, raw material variables (media, supplements, cytokines/growth factors, extracellular matrix substrates), product variables (expected mechanism of action, cell phenotype, marker expression, proliferation profiles, metabolic profile, apoptosis, etc.), and process variables (medium perfusion or exchange rate, feeding regime, pH, dissolved oxygen, etc.). This is to ensure that product quality is maximized and CQAs are maintained within the expected ranges throughout the product life cycle. Such strategies involve designing several specific assays during the early process development stage and subsequently refining and streamlining select assays for good manufacturing practice (GMP) as the product's CQAs and characteristics, and the manufacturing process are better defined. This ensures standardization and reproducibility, reduces deviations, increases quality, and consistently meets release criteria for the final product. This also ensures a smooth transition from clinical to commercial manufacturing.

Some unique challenges associated with cell therapies include inherent variability in the starting material, lack of reference materials, limited quantity of manufactured lots, multiple and diverse mechanism of actions, paucity of cells available for quality control (QC) and stability testing, and inability to sterilize the final product. Despite several advancements, cellular therapeutics often experience challenges and delays in clinical trials due to a lack of suitable analytical methods that are reproducible and meet the regulatory standards.

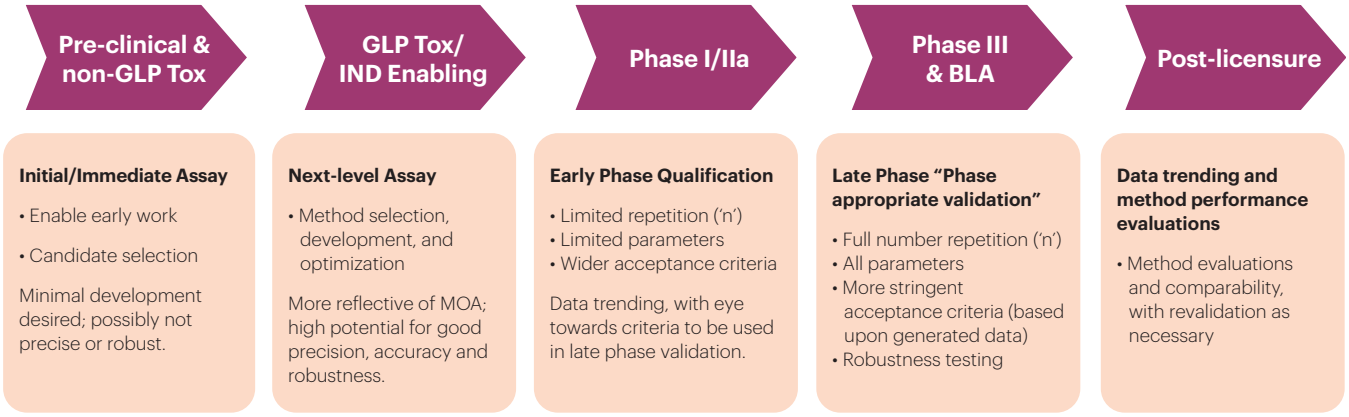
Approaches to Analytical Assay Development

PHASE APPROPRIATE, FIT-FOR-PURPOSE ASSAY DEVELOPMENT APPROACH

Fit-for-purpose is a commonly used phrase when discussing method validation. The concept maintains that the level of validation should be tailored to an assay's intended use. For example, qualification is acceptable for early development, but full validation is required for the Biologics License Application (BLA) or Marketing Authorization Application (MAA) stage. The decision to develop fit-for-purpose assays should be made in the early stages of product development. Fit-for-purpose assays in cell therapy are designed for their usability by the QC and development labs. Figure 9-1 shows an example of an assay development pathway. It is important to note that the exact activities for each phase can vary based on the therapeutic program, for example Phase II might be performed separately prior to the pivotal Phase III.

Bioassays are a good example to consider in a discussion of fit-for-purpose assays as they have inherent variability, require extended culture periods, use multiple (variable) cell types, and generate more than one readout. While at times it may be necessary to validate a complex bioassay, it is worth considering in early product development whether there are simpler alternative assays or surrogate assays that are easier to develop in a shorter time. Using several assays during early development may help to identify orthogonal methods that may be more straightforward to validate.[3] This approach could also provide several options for an assay that adequately controls potency, instead of "starting from scratch" after

Figure 9-1: *Traditional assay life cycle development roadmap*[2]



an assay is deemed inadequate late in development. These could then be bridged to or complemented by more elaborate potency methods, with subsequent validation prior to Phase III.

Assay or method qualification typically happens during early clinical phases. During qualification, understanding an assay's key performance parameters (specificity, sensitivity, accuracy, precision, etc.) is vital, however, there is no pre-set acceptance criteria and robustness is not studied in detail. Complete validation is performed after the manufacturing process is "locked down" and the product is in its final matrix because the validation process is matrix dependent. This typically happens prior to initiation of clinical Phase III studies or before the BLA/MAA stage. Analytical technologies demand a strategic approach for assay qualification and validation due to intra- and inter-assay variabilities and intrinsic differences between distinct cell types. Performing a risk assessment exercise early in the development helps identify the CQAs (this topic is addressed in Chapter 4), and ultimately helps inform the timing of qualification and validation for the corresponding release assays. For "for information only" (FIO) assays (discussed in later sections), such risk assessments can inform whether validation is necessary.

COMPENDIAL ASSAYS

Only limited qualification is required for compendial assays as they are driven by strict pharmacopeial (USP/EP) requirements. For assays such as appearance, pH, and osmolarity, only a single qualification run with the appropriate reference material is required before conducting a GMP sample analysis.

PATHWAY FOR ASSAY/METHOD VALIDATION

The pathway to assay validation is illustrated in Figure 9-1, and is typically achieved through the following steps:

- Protocol-driven exercise with predefined acceptance criteria
- Non-compendial method validation
- Validation according to ICH Q2 guidelines

For validation, the following parameters are generally evaluated:

- Specificity
- Linearity
- Accuracy
- Precision (repeatability, reproducibility, and intermediate precision)
- Range
- Limit of quantitation (LoQ)
- Limit of detection (LoD)
- Compendial method validation (verification of product with the method)

It should be noted that, depending on the purpose of the assay, not all parameters are required for validation. For example, limit of detection is often not required for potency and purity.

It is often challenging to follow the roadmap presented in Figure 9-1 for analytical assay development in cell therapies. Some of the challenges include starting material (e.g., patient sample) variability, limited

numbers of lots and small lot sizes for autologous products, inherent variability of the production process, variability in potency, and product stability concerns. This is especially true for autologous therapies as the final product is administered in small lot sizes to one patient. Typically, only a small amount of in-house reference material is available for conducting multiple tests and the distinctive nature of cell therapy makes it challenging to develop commercial reference standards for long-term use.

GENERAL GUIDANCE ON ASSAY DEVELOPMENT, QUALIFICATION, AND VALIDATION

After an analytical method is selected for product testing, it evolves through development and optimization to become qualified for use in clinical trials.[4]

1. Qualify and validate high-risk assays that measure safety parameters (e.g., mycoplasma and endotoxin tests) for use in humans.
2. During process development, qualified assays should be continuously subjected to re-optimization or requalification if procedural adjustments are necessary to maintain suitable performance.
3. Before method validation, performance history should be established to predetermine acceptance criteria for indicated validation parameters.
4. A complete GMP validation of assays used for release, stability, and in-process testing is required for submitting a BLA or MAA. Ideally, assays should be validated prior to initiation of pivotal clinical studies.

When changing an assay, a risk assessment should be performed to determine how the assay change impacts evaluation of the cell product. The requalification strategy of assays upon change is dependent on the degree of the change.[5]

Categories of Analytical Assays

Analytical assays can be grouped into distinct categories:

- Use of assays at specific stages during the manufacturing process. These include analytical assays for:
  - Starting material (e.g., apheresis product, cord blood, bone marrow, cell lines)
  - Intermediate material (in-process analytics)
  - Final product (identity, purity, impurities, potency, and safety)—these tests are also used to determine product stability

- Types of assays needed to characterize various properties of the cells as a final product. These include:
  - Cellular properties (cell number, cell size, cell morphology, phenotype, genomic stability)
  - Biochemical properties (cell viability, metabolism, gene and protein expression)
  - Cellular responses and functions (cell activation, cell proliferation/expansion, cell differentiation, cell function such as cytokine secretion, antigen recognition, and cytotoxicity)

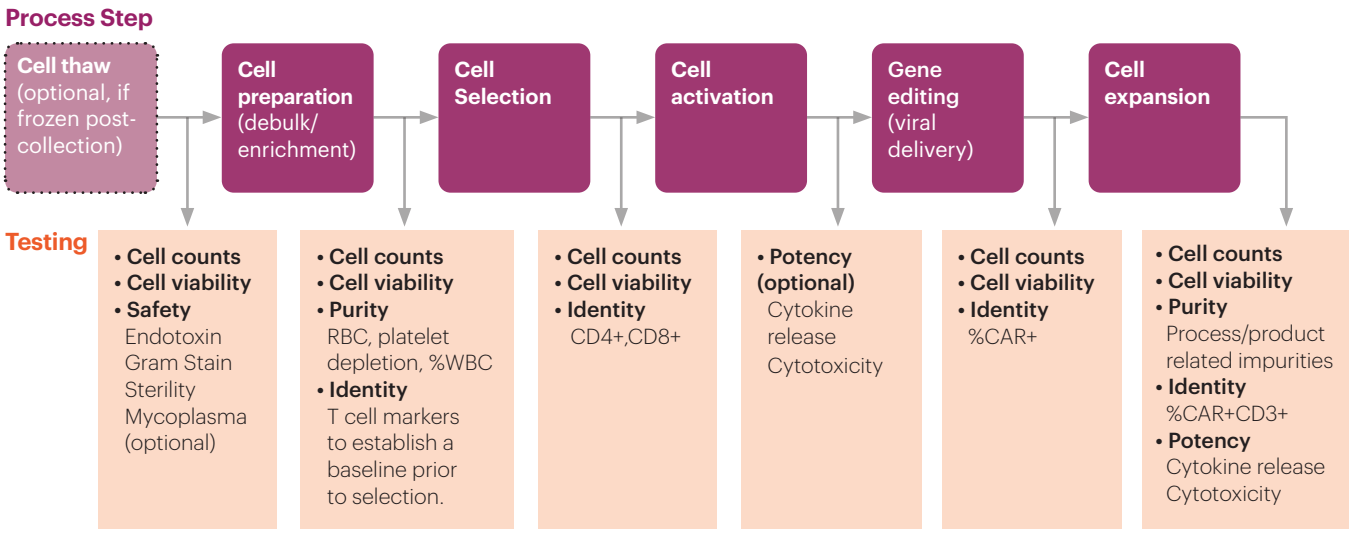
For CAR-T manufacturing, the FDA recommends appropriate in-process testing to be conducted at relevant process steps to achieve and maintain control of the manufacturing process.[5] Results from these tests can be used to guide manufacturing decisions at critical steps, such as when to change media or harvest the cells. In-process testing regimens for CAR T cells typically assess multiple parameters (e.g., viability, cell number, cell phenotype, CAR expression). Figures 9.2-9.3 depict an example testing paradigm for an autologous CAR-T product throughout the manufacturing process. Details on each analytical assay are described in the subsequent section.

It is important to note that the exact testing strategy for every product is different depending on the indication of interest and modality, therefore, risk assessment is critical during process development to determine which tests are necessary at which time points. If certain impurities at a certain process step are considered high risk, assays to identify or quantify them need to be done at that specific point during the process.

FOR INFORMATION ONLY (FIO) ASSAYS

Oftentimes a sponsor or manufacturer may decide to use assays or tests that are under development to evaluate product characteristics or method performance capabilities. These tests are designated as "for information only" (FIO). FIO assays, while not used for final product lot release, are vital for understanding additional quality attributes of

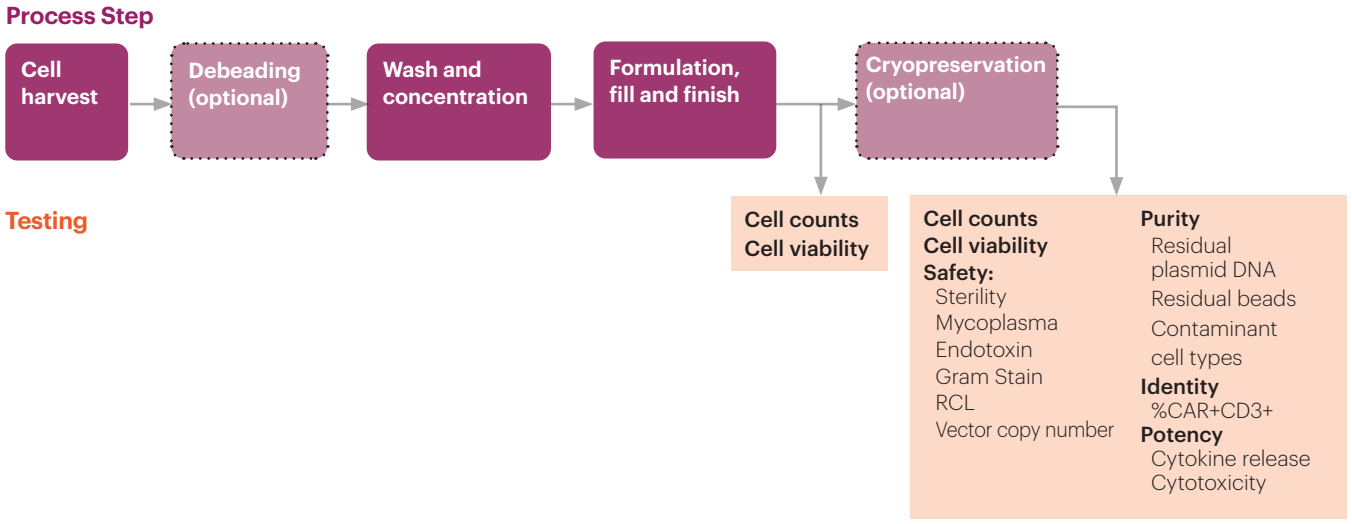
Figure 9-2: *Autologous cell therapy testing paradigm (cell preparation to expansion)*



the cellular product and the mechanism of action (MOA). They are also helpful in gathering more information about the key steps of the manufacturing process or final product. FIO assays are indispensable as backup assays if there are problems validating existing release assays (e.g., the assay is too inherently variable, supply shortage, or discontinuation of critical materials). FIO assays can be tracked throughout process changes to reinforce comparability or help identify noncrucial characteristics (e.g., determining time-points in culture when cells stop producing a specific cytokine even if that does not affect its target function in patients). After sufficient information is gathered, specification and release criteria can be redefined and qualified in later stages of clinical trials.

A phase-appropriate strategy may be considered for the FIO assays. The purpose of Phase I is primarily to evaluate drug safety. If the FIO assay attributes could potentially impact patient safety, they should be well-characterized to ensure no adverse impacts on patient safety. Health authorities may require the qualification of FIO assays for such safety related attributes and implementation of the FIO safety assays on the certificate of analysis. As part of the development, FIO assays could enable a deeper understanding of MOA and contribute to the implementation of MOA as they may be reflective of potency before the pivotal trials.

Figure 9-3: *Autologous cell therapy testing paradigm (cell harvest to fill & finish)*



A cautionary approach in FIO assay development and disclosure may prove beneficial because if the FIO assays are deemed to be strong from a safety and potency perspective, the regulatory agencies may require the sponsor to convert such FIO assays into final lot release assays. If sponsors are not prepared for such a demand, it will delay the regulatory submission timeline.

Analytical Assay for Cell Characterization

The Code of Federal Regulations (CFR) is the codification of the general and permanent rules published in the Federal Register by the departments and agencies of the Federal Government. Title 21 of the CFR (CFR 21) is reserved for rules of the Food and Drug Administration. 21 CFR Part 610 is reserved for General Biological Products Standards that also applies to cell therapy products.

The analytical assay-related regulations in 21 CFR Part 610 can be subdivided into the following categories:

- **Safety (610.12, 610.13, 610.40)**
  - Sterility
  - Pyrogen testing
  - Mycoplasma
  - Opportunistic viruses (adventitious agents)
  - Vector copy numbers
  - Replication-competent lentivirus (RCL)
- **Identity (610.14)**
  - Specific test to distinguish it from other products manufactured/tested at the same site
- **Purity (610.13)**
  - Free of extraneous materials
- **Potency (610.10)**
  - Measurement of biological function/activity
- **Constituent Materials (610.15)**
  - Ingredients, Preservatives, Diluents, Adjuvants, Excipients, Ancillary materials

SAFETY (MICROBIOLOGICAL SAFETY, 610.12, 610.13, 610.40)

Safety testing is conducted to confirm that the product is not contaminated with bacteria, mycoplasma, fungi, adventitious viruses, endotoxins, or replication-competent viruses derived from viral vectors. Safety is critical at every step of the manufacturing process and can be assured by testing of raw materials, testing of intermediate samples, minimizing contamination risks during manufacturing, and testing the final product prior to release. Safety testing is further categorized into sterility, pyrogen testing, mycoplasma, gram stain, replication competent lentivirus, and vector copy number.

Sterility

Sterility testing is required to demonstrate the absence of viable micro-organisms. The sterility test is a conventional test that is based on the growth of microorganisms (bacteria, yeast, and fungi) in two culture media incubated at two temperatures with a final visual reading after 14 days. The methodology of this test is harmonized between the different pharmacopoeias (USP, Ph. Eur, and JP) since 2010. As stated in Ph. Eur 5.1.1, sterility testing cannot guarantee that the product is sterile but it does help to ensure that the validated and controlled production process provides assurance of the sterility of the finished product.

This chapter presents some differences to consider between cell therapy products to that of typical pharmaceutical drug products. Cell therapy products have one or more of the following characteristics:

- Short life span (hours to days)
- Aseptic manufacturing with several steps, often manual, in different locations (hospital, clinic, therapy center)
- Limited or very limited quantity of product
- Each batch is unique (one patient, one batch) for autologous products
- Used in the context of treatment for life-threatened patients (cancer, rare genetic diseases)
- Very high manufacturing cost
- High or very high cell concentration
- Thermosensitive
- May not contain preservative agents or protection against micro-organisms
- The manufacturing process (temperature, media, raw material, presence of oxygen) is very favorable to microbial contamination

Most of these characteristics make the application of the traditional sterility test technically or economically impossible.

Since 2010 and the evolution of alternative microbiology methods, several rapid sterility methods have been validated for the release of pharmaceuticals. These methods are still based on growth but the result could be accelerated by changing the reading method. The best known are the Milliflex® rapid technique based on ATP detection or the BACT/ALERT® or BD BACTEC™ that is based on CO_2 emitted during growth. With the emergence of these rapid microbiology methods, several guidance documents were published on the implementation of such an approach, e.g., the European Pharmacopoeia Chapter 5.1.6, the Parenteral Drug Association (PDA) Technical Report No. 33, and USP <1071>.

The ATP-based methods can interact with the cell products and the CO_2-based methods take more than one week to produce a result. However, these methods have paved the way for the development of new methods with guidelines on validation methodologies. It is important to point out that validating alternative methods remains complex and time consuming, and requires expertise in several areas including statistics.

Alternative microbiology methods can solve the time issue but do not address the challenges presented by the availability of a limited number of samples. The European Pharmacopoeia has started to address this issue of sample quantity with a dedicated chapter, 2.6.27: Microbiological Control of Cellular Products. In 2012, the FDA removed the notion of minimum quantities to be tested from 21 CFR 610.12 and replaced it by a justification of the quantity to be tested. This point relating to quantities is still unclear and is a crucial issue for the industry.

Based on experience and in accordance with the evolution of regulations, the sterility test must be based on the growth of microorganisms since non-growth techniques such as qPCR are not able to demonstrate the viability of a microorganism and its ability to grow. The second important feature of the ideal method is the ability to investigate quickly in case of a positive test. Investigation is key to understanding the source of the contamination and to take appropriate action if the product has already been delivered to the patient. The third important feature of the ideal method is automation of a data analysis process that is specific to the microorganisms.

In this context, developing a hybrid method with prior enrichment and measurement of the evolution of the quantity of microorganisms by sensitive and specific methods could be one solution, increasing sensitivity, reducing time-to-result, and limiting the amount of sample needed for the analysis.

Pyrogen testing

For cell therapy products, the FDA Guidance for Industry "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)," issued in January 2020, requires testing for endotoxin to verify the absence of pyrogenic contaminations. The EMA guidelines have a similar requirement. The endotoxin assay is based on an enzymatic reaction of limulus amebocyte lysate (LAL) to endotoxin, which can be detected by clotting or quantified using kinetic methods based on chromogenic substrates. The composition of the products generally allows testing according to USP <85>, EP 2.6.14, and JP 4.01 after simple dilution of the cell suspension to remove interference with the assay. Control of in-process intermediates can be achieved with cartridge-based systems, allowing generation of test results within minutes, while testing for batch release is generally performed using validated chromogen kinetic methods.

Testing for endotoxin as described in USP <85>, EP 2.6.14, and JP 4.01 is the primary assay used for measuring the presence of pyrogens, but there are other assay formats emerging that provide relevant information on the pyrogen content of a sample. The endotoxin test is specific for endotoxins but pyrogens are a diverse group of contaminants whose common denominator is their ability to induce a fever reaction after contact with human monocytes/macrophages, so testing for endotoxin alone might not be sufficient to verify the absence of pyrogens. Among those, microbial contaminations by gram-positive bacteria cause pyrogenicity that is not detectable with the endotoxin assay. EP 5.1.10 requires verifying the absence of non-endotoxin pyrogens during the production process and in the final product as a prerequisite for using the endotoxin assay for batch release, while 21 CFR 610.13 requires demonstrating the equivalence of the chosen test method to the rabbit pyrogen test, which effectively also means that the absence of non-endotoxin pyrogens must be demonstrated. For this demonstration, the rabbit pyrogen test is universally accepted, but it is tedious and

prone to misinterpretation due to individual differences in the biological reaction of the test animals. A full *in vitro* alternative is available with the monocyte activation test, where pyrogenicity is measured based on the cytokine expression of human monocytes/macrophages after contact with the product. This method is also useful for evaluating the potential impact of bioburden on the pyrogenicity of the cellular product.

Mycoplasma

Mycoplasma testing is performed to ensure that the product is free from mycoplasma contamination. The compendial methods for mycoplasma detection, as described in USP <63> and EP 2.6.7, are increasingly being replaced by nucleic acid-based techniques (NAT) as they provide faster results and, depending on the mycoplasma strain, higher assay sensitivity.

Validation design guidelines for NAT are described in EP 2.6.7, JP XVII "Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products," and general chapters EP 2.6.21 "Nucleic Acid Amplification Techniques," and USP <1223>.

Real-time polymerase chain reaction (PCR), also called quantitative PCR (qPCR), is the preferred method for cellular samples because this technique allows detecting almost all Mollicutes species that can contaminate cell cultures, even fastidious strains that cannot be detected with the compendial culture method. Moreover, fast results (few hours to 1 day) can be obtained in comparison to the culture method (28 days).

Despite its effectiveness for cell-based therapies products, some analytical points must be considered:

- Kit or PCR reagents must detect a broad range of mycoplasma (Mollicutes) species. More than 100 species is recommended.
- As mycoplasma can adhere to the cell surface, cells must be tested. Testing of supernatant only might lead to false-negative results.
- Due to the possible co-purification of cellular DNA and potential mycoplasma DNA, PCR chemistry must be carefully considered. It is highly recommended to use hydrolysis probes such as TaqMan® probes. Use of unspecific DNA stains should be avoided to prevent unspecific amplification and false-positive results.
- Cell concentration must be known for each test and properly controlled. Depending on the chemistry and the primers/probe design, concentration above 1-5 million cells per input volume may lead to PCR inhibition and sometimes to false (low)-positive results. A matrix-specific validation by testing a defined cell concentration or a "worst-case" (highest) concentration is a good analytical approach to prevent unexpected results.
- As qPCR does not guarantee a 100% results reliability rate, using a back-up detection kit or identification by DNA sequencing of the qPCR amplicon are appropriate options.
- To detect as low as 10 CFU/mL as recommended by Pharmacopeia, input volume tested should be at least 1 mL (using mycoplasma standards with GC/CFU ratios <10). If this volume is not available for testing, use a "hybrid" mycoplasma method, combining enrichment steps by culture with subsequent PCR detection.

Gram stain

Gram stain is a fast method to confirm the absence of bacteria, either gram-positive or gram-negative bacteria. The staining procedure reveals peptidoglycans present in the bacterial cell walls and a positive stain indicates microbiological contamination.

Replication competent lentivirus

Replication-competent lentivirus (RCL) could theoretically occur, but has not been observed with third-generation replication-incompetent self-inactivating (SIN) vectors. Detection of the vesicular stomatitis virus G (VSV-G) gene in a drug product (DP) can be used as the RCL surrogate. RCL can also be tested by the co-culture method as part of the vector release specification.

The design and optimization of any PCR methods involves the selection of the primers, probes, PCR reaction parameters, and PCR reaction reagents. Due to the nature of the qPCR reaction, it could be subject to potential PCR inhibition from the matrix of the sample or preanalytical processes. Alternatively, droplet digital PCR can be used instead of qPCR to partially alleviate the effects of matrix interference and remove the need for standard calibration.

Vector copy number

Vector copy number (VCN) is a surrogate measure of lentiviral transduction, which detects vector transgene integration into the host cell genome. It is intrinsically required for the CAR protein expression and efficacy. Lentiviral transduction presents a theoretical safety risk of insertional oncogenesis, thus VCN is also considered a safety assay.[6] In the VCN method, genomic DNA (gDNA) extracted from the DP is used as a template in a multiplexed, qPCR reaction to monitor vector integration.

IDENTITY (610.14)

The identity of the final product is established using unique distinguishing characteristics to confirm that the product contains the intended cellular and noncellular components. The manufacturing process/workflow in cell therapies can range from days to weeks with various cell manipulations including but not limited to selection, activation, expansion, and cryopreservation. Cells are subjected to various media, cytokines, and culture conditions (static, dynamic, hypoxia, normoxia, etc.). Such complex workflows can introduce both intended and unintended changes. Identity testing is essential to address the concern that the unintended changes do not change the biological nature and function of the cells or lead to phenotypic drift in the final cell product.

Identity testing of cell therapy products includes the use of flow cytometry as it is well established in the research community and has translated well to GMPs. Flow cytometry entails the staining of live (or fixed) cells with fluorescently labeled antibodies that recognize cell surface or, in some cases, intracellular expressed proteins.[1] For example, all hematopoietic stem cells express CD34 and all T-cells CD3, Mesenchymal stem cells (MSCs), a very common cell type in clinical trials, must be positive for CD73, CD105, and CD90 and negative for CD45 and CD34. Other markers may also be tested for absence (e.g., CD14/CD11b, CD79alpha/CD19, and HLA-DR).

PURITY (610.13)

Purity testing is carried out to evaluate and confirm that the final product contains minimal undesired components, such as contaminating cell types, dead cells or debris from dead cells, and residual process reagents that may affect the purity of the cells.

In cell-based therapies, it is impossible to ensure that the final cell population will be 100% homogenous, since the starting material is human material and the manufacturing process involves multiple steps that affect cells differently. A cut-off for minimum purity must be decided based on the mode of action of the cell product and potential impacts to safety. Likewise, there should be a cut-off for cell viability, post-production, or post-thawing, and for all other cellular components.

Flow cytometry is often used in identity and purity methods with potentially different instrument platforms. The phenotypes of the CAR-T cells are important since the cells are the therapeutic agents, which could impact the efficacy, PK, and safety. Phenotypes currently considered critical for product release are viability, %CD3+, and %CAR+. The staining and gating of the different cells could be method dependent. The fluorescence minus one (FMO) gating, the selection of dye and antibody conjugation, and the expression levels of surface markers are considered to ensure the balanced responses and accuracy of the method. The representative samples are important as it is not unusual to observe potential impurities inferences.

POTENCY (610.10)

Potency is defined as the quantitative measure of biological activity and is necessary to confirm that the product possesses the inherent or induced biological functions relevant for the treatment of the intended clinical indication. Ideally, potency assays measure all aspects of the therapeutic and biological ability of a product.

For CAR-T cells, important aspects include transfer of the CAR gene into T cells and ability of the transferred genetic material to elicit the desired biological effect in impacted T cells. If the product includes more than one active ingredient (cell type or cell line), the potency assays should be able to measure the potency of all active ingredients.

Potency assays should be quantitative and can include animal-based bioassays, cell-based bioassays, or analytical assays (which are designed to measure the immunochemical, molecular, or biochemical characteristics of the product). In general, health authorities prefer a cell-based bioassay, but are open to inclusion of analytical assays to enable early clinical development. In addition, animal-based bioassays are often

discouraged to limit animal use where possible. Use of an analytical assay for a marketed product typically requires data to demonstrate that the assay provides acceptable control over relevant product attributes and that a cell-based bioassay is not needed. Some of the challenges encountered in establishing potency tests for cell-based therapies include the inherent variability of starting material, availability of limited material for testing, heterogenous product composition, sub-optimal assay precision, lack of established reference standards, incomplete understanding of MOA, multiple active ingredients, limited stability, and the potentially complex fate of a product *in vivo*.

Potency tests, along with a number of other tests, are performed as part of product conformance testing,[7] comparability studies,[2] and stability testing.[3] These tests are used to measure the product attributes associated with product quality and manufacturing controls, and assure the identity, purity, strength (potency), and stability of products. Similarly, potency measurements are used to demonstrate that only product lots that meet defined specifications or acceptance criteria are administered during clinical investigation and following market approval.[8]

During early-stage development, extensive characterization should be performed to generate knowledge on MOA and support the appropriate potency. For CAR-T products, potency characteristics commonly tested include the number of transduced T cells and the functionality of antigen-specific T cells such as cell killing and cytokine release (e.g., interferon-gamma [IFN-γ] release in response to CD19-expressing target cells).[9,10] Potency characteristics of other cell therapy products have to be defined based on the putative MOA. Often a combination of several (often cell-based) assays is required to generate a more holistic picture of Drug Product potency.

While there are functional assays in place for certain cell therapies like CAR-T products, the complex nature of the MOA of most cell therapies presents a limitation to potency assay development. In the case of CAR-T, the current IFN-γ production and cytotoxicity assays still cannot accurately predict the product's clinical outcome. Different technology platforms to monitor the cytokines include AlphaLISA, Meso Scale Discovery (MSD), and Luminex.

It is therefore necessary to monitor clinical outcomes and use statistical methods and correlative analysis to determine relationships between the product CQAs and the clinical outcomes. This exercise allows for a better understanding of the manufacturing variables that impact product quality and clinical outcomes, which ultimately dictate the product control strategy (topic covered in Chapter 10).

Potency assays reflecting the MOA might require combinations of extended cell cultures combined with methods to determine target gene expression, morphological cell characterization, or determination of cellular function. As projects mature through different clinical phases, a thorough evaluation should be performed to implement the potency on the most reliable and QC friendly platform.

Ideally, potency assays should be executed using full-dilution curve analyses, allowing constrained four- or five-parameter fit logistics to be used. To date, this has been difficult to achieve when assessing cell-based therapeutics (autologous or allogeneic CAR-T). As a result, there may be a tendency to use minimal (or even single) dilution of therapeutic (i.e., a single E:T ratio) and determine an absolute amount of cytokine generated at that E:T ratio in the case of CAR-T cells. This may be, unfortunately, misleading as different CAR-T lots/donors may provide dose/response curves (e.g., holding target cells constant, and titration of the CAR-T cells) that are not equivalent, resulting in different rank-order of cytokine production by the different lots/donors depending upon the specific E:T ratio used.[11] Thus, using the current approach of one fixed E:T ratio, it is possible to derive a misleading interpretation of DP potency. This could be especially important with the use of allogeneic CAR-T cells, where understanding the "off-the-shelf" potency of the DP might be used to determine consistency.

Molecular Assays to Confirm Gene Editing

With the increasing understanding of cell biology and genetic editing capability, gene knock-in and knock-out are being introduced in new cell therapies to both confer the antigen specificity and modulate the immune

interactions such as PD1 checkpoint pathways. For example, CRISPR technology has been used to produce the T cell receptor (TCR) cell therapy that has both PD1 knock-out and transgene knock-in into the TRAC loci. To monitor the genetic modification, qPCR and droplet digital PCR (ddPCR) were developed.

Additionally, as a nuclease (e.g., CRISPR) is used to prepare the T cell genome to receive and insert the CAR gene, the potential for an improper insertion (off-target) must also be assessed, generally via droplet digital PCR (ddPCR).

Additional need for molecular control arises from cell therapies generated from pluripotent stem cells. Clone selection and generation of Master and Working Cell Banks carry the risk of introducing point mutation and chromosomal aberrations into the DP, which need to be tightly controlled to prevent interference with or potentiation of oncogenic properties.

Genomic Characterization

Allogeneic therapies may require genomic characterization as long-term culture of cells can alter the genomic composition and lead to chromosomal abnormalities. Such abnormalities may further affect protein expression, cell function, safety, and purity, therefore the genomic stability/normality of the material should be assessed at multiple timepoints.[12]

The timepoints where genomic characterization is most important are:

1. The source cells during establishment of master and working cell banks.
2. Manufacturing processes that including genetic modification of a cellular product.
3. Manufacturing processes that rely on extended culture and cell expansion to meet the final dose.

Currently, several methods are used to assess these changes, including g-banded karyotype, chromosomal microarray, fluorescence in situ hybridization (FISH), or sequencing.[13] It is important to note that all methods have limitations or trade-offs in resolution and sensitivity. Therefore, genomic characterization may need to be tailored to the product and processes being utilized in manufacturing.

Impurities

Impurities can be categorized as product- or process-related impurities. In CAR-T therapies, product-related impurities can originate from leukapheresis products and are comprised of non-T cells, residual tumor cells, non-viable T cells, and non-transduced T cells, all of which could be monitored by the flow cytometry methods.

In other certain cell therapy products, product-related impurities can refer to its pluripotent origin, where residual pluripotent stem cells need to be tightly controlled. The lower limit of quantification (LLOQ) in flow cytometry methods is typically not sufficient to support the acceptable impurity levels (e.g., <0.01% residual pluripotent cells in the DP). DNA-based methods such as qPCR and ddPCR are currently being evaluated for their potential to be integrated in respective DP release panels.

Process-related impurities include materials employed or added during product manufacturing such as residual beads, antibiotic, or cytokines. As we introduce new reagents such as activation and gene editing reagents, we also need to demonstrate process control and develop new analytical detection methods. A stage-appropriate approach should be taken for control of process-related impurities. The approach should be based on a risk assessment for each impurity, and strategies to control each impurity should be prioritized according to risk. Full process and method validation for clearance of process-related impurities is typically not required until late-stage or before BLA filing. If activation beads are used during manufacturing, a key impurity assay in the release specification could be a residual beads assay (e.g., imaging technology). Overall, the detection and process validation of impurities clearance must be demonstrated or the high-risk impurities could be monitored as part of the release specification.

Cell Line Identity

Confirmation of cell line identity (distinct from cell type identity) is strongly encouraged for cell-based therapies that utilize cell lines as starting materials. A profile of cell lines used as starting/source material should be established at the earliest possible timepoint followed by regular testing at specific pre-determined

time points to ensure confidence throughout the manufacturing process.

Cell line identity testing can be accomplished via short tandem repeat (STR) testing or a similar method. STR can discriminate cell-based products from one another as well as identify contaminating cells.

Stability

In addition to release testings, some of the assays discussed so far are used to determine the stability of the cell therapy products. The FDA recommends that product stability studies for CAR-T cells should include testing to determine product shelf life, as well as in-use stability,[5] which covers a much shorter time frame (e.g., hours to days) to establish the duration cells can be held under specific conditions.

IN-USE STABILITY

The purpose of in-use stability testing is to establish a period of time during which a product can be used while retaining quality within an accepted specification once the container is opened. In-use stability is critical for delivering reproducible results for cell products (freshly applied or frozen), significantly influencing product quality. Example studies that can be done to determine in-use stability include determination of maximum hold time between product thaw and administration. For CAR T cells that are administered fresh, stability information for the intended hold time between final formulation and administration should be provided.[5] According to the EMA Guidance on In-use Stability Testing of Human Medicinal Products (CPMP/QWP/2934/99), the test should be designed to simulate the use of the product in practice as closely as possible, taking into consideration the fill volume of the container and any dilution/reconstitution before use. Throughout the duration of the in-use stability test period, the product should be stored under conditions as recommended in the product literature.

The appropriate attributes of the product susceptible to change during storage should be determined over the period of the proposed in-use shelf life. These can include, but might not require, all analytical assays for cell characterization listed in the earlier section.

STABILITY TESTING TO SUPPORT SHELF LIFE

Product stability testing is required to ensure that the cell therapy product (DP) is, at a minimum, stable for the duration of the clinical trial.[14] To determine the product shelf life, stability indicating assays should be identified through stressed stability samples studies. The stability-indicating assays should be related to the functional, phenotypic, and physicochemical properties of the DP. Accelerated stability should be evaluated to support the product handling and short excursion of storage conditions. According to the existing product inserts for marketed therapies, the formulation and storage conditions are often based on dimethylsulfoxide (DMSO) and cryopreservation. A detailed study of cryopreservation of autologous CAR-T products has been conducted that revealed changes in mitochondrial dysfunction, apoptosis signaling, and cell cycle damage pathways.[15] The study also confirmed that the function and phenotypes in the release panel are not impacted in the target storage condition. At such low temperatures and using an adequate formulation, the stability of the CAR-T drugs was demonstrated in the study.

An illustrative example of a stability testing plan is provided in Table 9-1. Note that not all attributes need to be tested at all time points. The frequency of testing could vary based on the product-specific applications. The shelf life and storage conditions could be justified by the stability data with the representative cellular products in the right container. Under optimized cryopreserved storage conditions, the DP can be stable to support the clinical and commercial applications.

Process Analytical Technologies (PAT)

In-process testing or process analytical technology (PAT) is defined as a system for analyzing and controlling the manufacturing process through timely (in-process) measurements of:

- CQAs
- Overall performance attributes of raw materials and (cellular) byproducts
- Overall final product quality
- Real-time evaluation of the manufacturing process

Table 9-1: *Example stability testing plan for an autologous CAR-T product to determine shelf life.*

Attribute category	Test	Time points following lot release (months)							
		0	3	6	9	12	18	24	36
Viable cell count	Cell counting	✓	✓	✓	✓	✓	✓	✓	✓
Cell viability	Cell counting	✓	✓	✓	✓	✓	✓	✓	✓
Safety	Sterility (USP <71> / EUR Ph 2.6.27)	✓				✓		✓	✓
Identity	% CAR+ CD3+	✓	✓	✓	✓	✓	✓	✓	✓
Potency	Cytokine release (ELISA)	✓	✓	✓	✓	✓	✓	✓	✓

Due to the inherent variability of starting cellular material and changes in the characteristics of cells as they go through the manufacturing process, a significant level of in-process testing is required to ensure product quality and consistency. Table 9-2 lists the most common PAT technologies that could be used for cell therapy development.

Many challenges exist for offline analysis used in cell therapy. The analysis often occurs outside the GMP manufacturing facility in a QC lab or a contract testing organization. In some cases, the results are available at a later time, after the process is complete. Sample handling is another challenge as the samples need to be drawn several times, which may contribute to contamination risk. The information cannot be used for real-time process modulating.

Online PAT provides an opportunity to gather the information in a timely manner, and potentially provide this information to improve the process in a timely and efficient manner.

Table 9-2: *Example in-line and at-line PAT technologies for cell therapy process monitoring*[16]

Technology	Measurement
NIR spectroscopy	Cell culture metabolites (glucose, glutamine, lactate, ammonia), viable and total cell density, osmolality
Raman spectroscopy	Cell culture metabolites (glucose, glutamine, lactate, ammonia), viable and total cell density, osmolality
Fluorescent sensors	pH and dissolved oxygen
Refractive index	Compositional changes
Multi-wavelength fluorimetry	Amino acids
Holographic imaging	Cell shape/size, cell viability
Impedance	Biomass/cell viability
Turbidimetry	Biomass
HPLC	Media components (amino acids, sugars, proteins, metabolites)
LC-MS	Media components (amino acids, sugars, proteins, metabolites)
Coulter counter	Biomass/cell viability
Imaging	Cell size/shape, cell viability
Photometric analyzers	Cell culture metabolites (glucose, glutamine, lactate, ammonia)

NON-INVASIVE AND NON-DESTRUCTIVE IN-PROCESS TESTING FOR CELLULAR ANALYSIS

Real-time inline monitoring of cells as they undergo a variety of manipulations in a closed system during GMP manufacturing is important to evaluate the process parameters and maintain the CQAs of the final product. Such sample monitoring efforts at multiple time points within the workflow, while essential, also pose significant risks. Sampling of cells is invasive and can introduce contaminants into the production line. It can also terminally remove cells that constitute the final product and therefore is destructive.

Non-invasive and non-destructive inline monitoring solutions that directly interface with closed automated systems for cell analysis offer real-time assessment in a standardized and reproducible manner.[17] They enable direct assessment of phenotype, morphology, viability, proliferation, and functionality of cells throughout the workflow. Such technologies can also monitor cell health, pH, oxygen levels, and metabolites and help in determination of optimal process parameters.

Currently there are a few technologies that offer such options. One example is the Ovizio inline, automated microscope that generates reliable viable cell counts based on their patented double differential digital holographic microscopy (D3HM) technology. Sampling of cell populations can be remotely carried out in real-time, without any labelling or cell removal. Its versatility makes it compatible with off-the-shelf stirred tanks, rocking motion bags, or any static and dynamic culture vessels.

Other examples are PreSens (Precision Sensing) Optical sensors, Sonotec ultrasonic sensors, and BugLab sensors that can measure some cell attributes without direct contact. Single use sensors can play a vital role if they can be redesigned as non-invasive or no-contact sensors.

Cell viability can be measured based on radiofrequency impedance technology. This can be achieved by single-use probes integrated into rocking bags or bags placed in incubators (dynamic/static culturing of cells) that allow for continuous monitoring of cell expansion in bags vs. daily monitoring or monitoring on specific days/time points. This technology is particularly helpful as sample availability and access to the culture system is a limitation in cell-based therapies.

The technologies for monitoring the phenotypes and functions of cell therapy are largely offline, as in the QC methods. Online technologies are challenging to develop because of cell complexities. However, with the increasing development of cell therapies, further advances for online or inline analysis are expected.

PROCESS ANALYTICAL TECHNOLOGIES FOR MEDIA NUTRIENTS AND METABOLITES

For CAR-T cell therapy, the media is designed specifically to promote T cell expansion. The media contains amino acids, growth factors, cytokines, other nutrients, and metabolites, where PATs are available for each of these analytes. Various analytical tools have been utilized to monitor the media, metabolites, and secreted proteins in the cellular therapeutic process. For example, chip-based capillary electrophoresis-mass spectrometry (CE-MS) has been utilized to provide all the essential amino acids concentration information in less than 10 minutes.[18]

Cytokines are important components of the T cell culture and have been monitored by immune assays with platforms such as MSD or AlphaLISA. The immunoassays provide the specificity and sensitivity for these analytes. Other technology could potentially be developed to monitor the cytokines. For example, an online device based on monolith was designed to enrich low concentration of proteins and coupled with MS detection. The integrated setup can analyze the protein concentration down to pM range with cytochrome C as the model protein. Such sample enrichment may enable the analysis of low concentration proteins by liquid chromatography–mass spectrometry (LC-MS).[19]

Non-invasive methods (Raman or NIR detectors) have been developed for monitoring cell attributes using sensors where the sensors are not in physical contact with the cells or media. For example, optical sensors such as near infrared and Raman spectroscopy are non-invasive and can provide continual in-line monitoring of parameters associated with cell metabolism such as glucose and glutamine consumption and production of lactate and ammonia. Raman Spectroscopy can be used for cell cycle state and chemometric modelling to monitor nutrients and metabolites in real-time without the need for sampling the cultures, thus reducing the chances of contamination and human errors. However, analytical data from

online optical systems is typically not amenable to direct interpretation. Further advancement in process analytical technologies will enable process development scientists to modulate the process based on the real time inputs.

Conclusions

Diverse analytical technologies have been implemented to characterize cell therapy products to ensure the quality, safety, and efficacy of this promising new modality. Continuous advancements in analytical tools are being made in the cell therapy field to allow for timely process monitoring and for proper implementation of controls. These include new molecular assays to shorten the release time, multi-parametric platforms integrated throughout the process, multivariate data analysis, smart analytics, and data integration. They are facilitated by innovations in hardware such as new non-invasive sensor development, increased resolution in imaging capabilities, and software improvements to complement these innovative technologies.

Advancements in cell-based therapies are critically tied to enhancements of analytical assays. The application of artificial intelligence (AI) techniques and machine learning is beginning to be recognized in the advanced therapy field to build predictive models for personalized treatments with engineered stem cells, immune cells, and regenerated tissues. AI could enable data learning and prediction of clinical outcomes, thus optimizing cell therapy clinical trials through precise planning, simplified patient recruitment and retention, resulting in reduced complexity and costs.[20] Complementing current analytical tools with machine intelligence could have an exponentially high impact on the cell therapy field to dramatically reduce product release time and accelerate commercialization.

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CHAPTER 10

Control Strategy

CHAPTER LEAD

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Chapter 10 | Contents

Due to similarities in control strategy development across various types of cell therapy products, some of the overall content and flow of this chapter was based on, but adapted from when applicable, Technical Report No. 81: Cell-Based Therapy Control Strategy, a document published by the Parenteral Drug Association (PDA). The authors of this A-Cell chapter acknowledge the work of the authors and editors in constructing the PDA document.

For further details on the PDA document, please refer to:

Parenteral Drug Association. Technical Report No. 81: Cell-Based Therapy Control Strategy. https://www.pda.org/bookstore/product-detail/4638-tr-81-control-strategy-for-cell-therapy. February 2019.

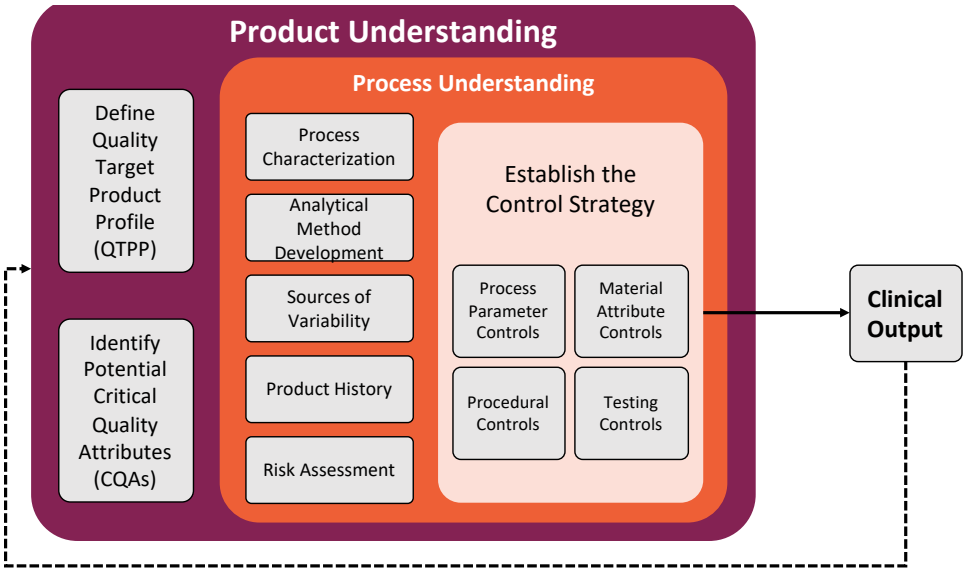
Introduction

Manufacturing control strategy represents a comprehensive, risk-based approach to ensuring that a generated drug product is of the highest quality. It seeks to minimize variability in all inputs and processes that impact the ultimate quality and clinical performance of the product. According to the International Council for Harmonisation (ICH) Quality Guideline Q10 definition,[1] control strategy is:

> A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.

Underlying the definition of control strategy is the recognition that both product and process understanding evolve throughout the life cycle of drug product development. The robustness of the initial control strategy depends on the extent of scientific knowledge available at idea inception. Development should start with a basic understanding of the quality target product profile (QTPP, Chapter 3), built on a general idea of what the

Figure 10-1: Overall control strategy development.



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product must eventually accomplish. Early on, critical quality attributes (CQAs, Chapter 4) described in the QTPP may be informed by preclinical data, processing runs, and rudimentary understanding of the various sources of risk to the product or the patient. As process and analytical methods are more fully characterized, an increased understanding of how critical reagents, critical process parameters (CPPs), and in-process and release controls shape the control strategy begins to inform how the manufacturing process can be manipulated to optimize favorable profiles of CQAs. Valuable information comes in later-phase development, as clinical efficacy and safety data begin to emerge, and in post-approval settings where real-world patient outcomes are reported. As such, the control strategy must evolve over time as more is learned about the product and about how various process inputs affect its quality or performance.

A holistic integrated control strategy is created from smaller-scale control strategies that address all essential parts of the manufacturing system: process parameter controls, material attribute controls, procedural controls, and testing controls (Figure 10-1). Clinical data encircles and influences further characterization and refinement of all elements of the control strategy as new data become available, long-term safety profiles are established, and as increased understanding of patient heterogeneity and variability are obtained. Patient-centric data not only confirms the true criticality of various quality attributes, but it may also help to identify aspects of the manufacturing process where control of variability is less essential. Thus, the control strategy not only addresses the life cycle of the cell therapy product, but also the life cycle of its own, characterized by continuous improvements as new knowledge is gained. In its refined state, it incorporates a thorough understanding of all risks to the product and, most importantly, to the patient.

As new product knowledge is gained, sharing and managing that knowledge across all involved parties (e.g., cell therapy developer, vector and cellular drug product production facility, contract development and manufacturing organizations (CDMOs), and clinical trial management organizations) helps to assure the ongoing effectiveness of the control strategy. Breakdown of knowledge sharing at any point could compromise the integrated control strategy and threaten quality and commercialization of the cell therapy product.

RISK-BASED APPROACH TO CONTROL STRATEGY

Quality-by-design (QbD) principles of criticality analysis and risk management underlie the formation of a control strategy that can guide and grow with a cell therapy product through various stages of development

(Chapter 4). This risk-based approach is supported by the European Medicine Agency's (EMA's) Committee for Advanced Therapies and is discussed in two recently published guidelines.[2,3] Additionally, the U.S. Food and Drug Administration (FDA) has published several guidance documents that outline a risk- and science-based approach to ensure product quality and patient safety.[4-8]

Risk analysis should be employed early in development and be continued through all validation steps and commercial scale-up. Knowledge of various quality attributes can be used to score each attribute according to the severity of harm it poses to the patient, the likelihood of its occurrence, and the ability of analytical methods to detect its occurrence.[9] As the goal is to minimize overall risk to patients, stringency of controls can often be increased for CQAs that pose medium-to-high risk to patients. Though the initial risk assessment is often based on limited product knowledge, as more is learned about CQAs and CPPs, the risks that various inputs pose to overall product quality become clearer. Enhanced understanding of these risks helps to define appropriate control points and testing stringency and is essential for the development of a robust integrated control strategy.

CONTROL STRATEGY AND REGULATORY EXPECTATIONS

A validated control strategy that allows consistent production of a safe, pure, and potent drug product with defined stability is central to regulatory approval of any cell therapy product. Demonstration of the control of all processes (e.g., laboratory controls of analytical methods, control of incoming raw materials) through their initial conception, improvement, and optimization is essential. Expectations for demonstrating control of cell therapy processes have evolved over the last decade, both within the United States and European Union (EU) regulatory communities. Chapter 2 addresses regulatory expectations as product development moves through key timepoints.

Touchpoints with regulatory agencies occur at all stages of drug development, from the pre-Investigational New Drug (IND) meeting through all phases of clinical study. The integrated control strategy, along with data from process characterization and validation studies, is presented to regulatory agencies within filing documents; any changes to product specifications or alterations of the control strategy post-approval must be submitted and approved by regulatory bodies. If the manufacturing process is incompletely characterized or insufficiently validated, the manufacturing process may lack robustness to support commercialization and the strategy may not be accepted, further delaying the commercialization of the cell therapy drug product. Thus, taking the time to establish, monitor, and improve the holistic control strategy is the most efficient approach for any company producing a cell-based therapy. Regulatory responses to filings regarding all aspects of the integrated control strategy such as raw material controls, procedural controls, CPPs, in-process controls (IPCs), and intermediate/release specifications will also help inform overall strategy across programs. In addition to facilitating regulatory approval, a solid holistic control strategy framework will allow the cell therapy developer to remain competitive in this rapidly expanding field as key clinical questions surrounding cell therapy begin to be answered.

CHAPTER AIM

This chapter seeks to guide the reader through the development of a robust integrated control strategy for a hypothetical chimeric antigen receptor (CAR)-T cell therapy product in the setting of current regulatory framework and guidelines. The content will illustrate steps in the risk-based approach to control strategy generation. Differences between allogeneic and autologous approaches to CAR-T cell therapy production will be highlighted where applicable.

Process Parameter Controls

After establishing a list of CQAs (e.g., cell concentration, vector copy number, % CAR+ CD 3+) or potential CQAs (e.g., viability, residual plasmid DNA) essential for the CAR-T cell product efficacy and safety, the manufacturing process must be developed and rigorously evaluated to ensure consistent delivery of a quality product. Initially, a broad map of the production process should be created utilizing tools such as a simple flow diagram, deployment flow chart, SIPOC (supplier, input, process, output, customer) diagram, or IPO (input, process, and output) diagram. The product obtained after each step of the production process should be described in detail and the specific parameters that govern the overall process must be

Table 10-1: Example IPO diagram for CAR-T expansion unit operation (assuming a 3-D suspension culture in a rocking motion/stirred tank bioreactor)

Controllable parameters		
• Number of single-use bioreactor bags	• Culture duration	• Rocker/agitation speed
• Cell seeding density	• Incubation temperature	• Feed volume
• Media volume for each bag	• $\%CO_2$	• Feed timing
	• Dissolved oxygen	

Unit Operation Inputs	Cell expansion unit operation steps	Unit Operation Outputs
Starting materials	1. Seed cells and media into bioreactor bag	**Performance attributes**
• Cells from previous step (activation / gene editing)		• Cell counts • Cell viability
Raw materials	2. Incubate for 5 days with a set agitation speed / profile	**Quality attributes**
• Culture medium • Serum • Culture feed • Antibiotics	3. Add media feed	• Safety: sterility, endotoxin, mycoplasma • Purity: dead cells, contaminating B cells, process-related impurities (e.g., remaining beads) • Identity: % CAR+ CD3+ • Potency: cytotoxicity, cytokine release
Consumables	4. Incubate for 5 additional days	
• Single-use bioreactor bags • Transfer bottles / bags • Serological pipettes • Pipette tips • Filters • Sterile centrifuge tubes • Welder blades	5. Obtain cell count / viability	
Equipment	6. Harvest; transfer cells to another bag for further processing	
• Incubator • Biological Safety Cabinet (BSC) • Tissue culture pipettor • Rocking motion bioreactor/ stirred tank bioreactor • Aseptic welder • Grade B / Grade C room		

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identified. An example of a flow diagram for an autologous CAR-T manufacturing process, including the process step and associated unit operations, is presented in Chapter 8. An example IPO diagram for the expansion step of CAR-T manufacturing is shown in Table 10-1. Such process mapping exercises should be performed for all steps/unit operations involved in the overall manufacturing process.

Risk to the quality of the product can be introduced during any step of the manufacturing process making selection of appropriate process parameters essential. The criticality of various process parameters is ultimately determined by evaluating their risk to CQAs, which are monitored throughout the manufacturing process to ensure that the QTPP is met. Not all process parameters will be deemed to contribute risk to the overall quality of the product; those that are truly critical go on to become essential elements of the manufacturing control strategy.

Due to the inherent nature of cell therapy, the influence of patient heterogeneity on process parameter controls should be well-understood. This may require performing additional characterization studies using numerous healthy donor materials so that a clear understanding of patient contribution versus process contribution to overall process variability can be determined. That being

said, patient-derived starting material may have intrinsic properties that affect CAR T manufacturing due to disease state and prior treatment; therefore, when using patient-derived starting material, additional manufacturing process development may be recommended for autologous CAR T cells.[10] A variance component analysis approach, which estimates the contribution of each random effect to the overall variability within a process, may be used to decouple various sources of variability (e.g., patient, process, analytical) to help inform the most appropriate process control strategy during development and commercialization.

PROCESS CHARACTERIZATION

To be completed efficiently and allow manufacturers to move quickly from late-stage clinical trials to global commercial filings, process characterization should take in a comprehensive, stepwise, organized approach. Oversimplification or shortcuts in process characterization studies to achieve quicker development timelines could lead to less-than-robust processes that may result in delayed regulatory approvals or difficulties with commercial manufacturing if processes are insufficiently understood. Process characterization is typically performed within a well-developed and qualified scale-down model, allowing the large number of experiments required to characterize a process and to select the best-performing ancillary material (e.g., media, viral vector, transfection reagent) to be executed on a small scale. Attempting to fully characterize a process at a manufacturing scale may be both cost- and resource-prohibitive.

During initial process characterization, one-factor-at-a-time (OFAT) studies may serve as a starting point to better understand how single inputs affect process outcomes. However, design of experiments (DoE) approaches are preferred over OFAT studies because they provide information on how multiple inputs interact with each other. Knowledge of CPPs generated from DoE studies are used to define the operating ranges for various process parameters that ensure consistent performance of the process. One such range is the Proven Acceptable Range (PAR). As defined by ICH Q8(R2),[11] a PAR is:

> A characterized range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria.

In 2017, the EMA issued a Question and Answer document[12] to facilitate a better understanding of parameter ranges and how they should be presented in regulatory filings. The document further defines a PAR as allowing deliberate change in one parameter without changing the other parameters outside their normal operating range (NOR) or target. The PAR should be adequately justified for all process parameters, regardless of whether they are critical or noncritical. The document goes on to define a NOR as a region around the target operating conditions that contains common operational variability (variability that cannot always be controlled to a precise value). The NOR does not represent deliberate adaptation of the process and does not cover a parameter range that affects the quality of the process output. If a NOR were to affect the quality of the process output, then a PAR for that parameter should be established. The disclosure of NORs within regulatory filings is intended to better quantify the actual uncontrollable operational variability of process parameters and should be presented as what is practically achievable by available equipment.

Data obtained from comprehensive process characterization studies are used to confirm CPPs and to develop meaningful IPCs, which together serve as the controlling documentation for the actual manufacturing process. The IPCs establish guidelines and define limits for process parameters and operations, deviation from which could result in an adverse impact to product quality. IPCs should be performed at key intermediate stages of the manufacturing process and may cover a variety of quality aspects:[13]

- Molecular (genomic integrity, identity; vector copy number [VCN]; transduction efficiency; on- and off-target modifications)
- Cellular (target cell identity/purity; cell growth kinetics; cell viability; hold-time stability)
- Process-related (temperature, pH, media consumption, dissolved oxygen and/or carbon dioxide, metabolite concentration, agitation, acid/base addition, perfusion rate)
- Microbiological (presence of infectious particles, absence of adventitious contaminants, sterility, mycoplasma)

To have a comprehensive and effective process characterization program, it is beneficial to have the most representative process conditions and equipment to facilitate meaningful small-scale, high-throughput process screening and development. The entire system must be considered, including

the equipment and software required to generate, evaluate, and analyze all process-related data. In the case of a CAR-T process characterization, this complete system should include flow cytometry for the cell phenotype characterization during selection/activation, the automated small-scale bioreactor for expansion, metabolite analyzer, representative process analytical technologies for in-process testing and characterization (including visualization of cell killing assays, as required), as well as the software to generate DoE study results based on the data generated. The higher the degree of automation at each of these steps, the faster the process characterization will be. It is important to note that analytics and software capabilities need to be synergistic to avoid a bottleneck where more data is generated than the software can analyze. Figure 10-2 shows a potential solution for a CAR-T manufacturing process that can be used to efficiently generate process-related data (potential CPP), evaluate the CQA for each condition tested in your DoE plan, and eventually analyze all these data to confirm the CPP. This example is not meant to serve as a recommendation for any particular equipment.

Failure Mode and Effects Analysis (FMEA)

As a part of the risk-based approach to establishing a meaningful integrated control strategy, risk assessment tools such as the Failure Mode and Effects Analysis (FMEA) can be employed. Other risk assessment tools to determine criticality of a product's quality attributes are discussed in Chapter 4. The FMEA provides a method to evaluate potential failure modes for processes and their likely effect on outcomes and/or product performance,[15] and is calculated as Severity (the impact of failure) × Occurrence (the probability of failure) × Detection (the detectability of failure). Each of the three factors are assigned a score, and when multiplied will result in the Risk Priority Number (RPN), which ranks the failure mode, prioritizes risks, and evaluates risk mitigation.[16] Since it

Figure 10-2: *Potential solution for characterization of a CAR-T manufacturing process.*

Synergy between manufacturing unit operations and data analytics ensures a comprehensive process characterization.[14]

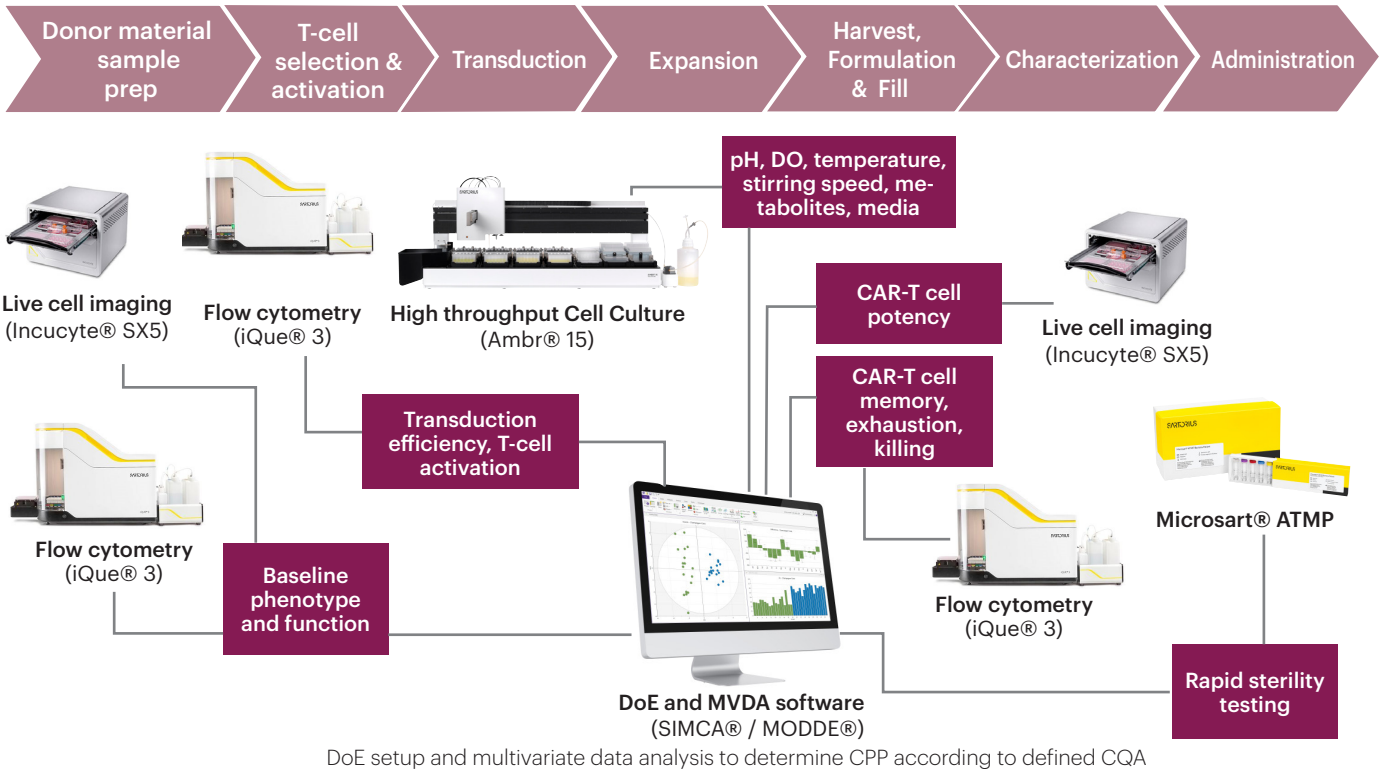

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Table 10-2: *Example FMEA for CAR-T expansion process.*

Failure Mode and Effects Analysis for process steps and inputs

process step or input	**Potential Failure Mode(s)** In what ways can the Process Step or Input fail?	**Potential Failure Effect(s)** What is the impact on the Key Output Variables once it fails?	**Potential Cause(s)** What causes the Key Input to go wrong?	**Severity**	**Occurrence**	**Detection**	**RPN (Risk Priority Number)** SxOxD	**Current Controls** What are the existing controls and procedures that prevent the Cause or the Failure Mode?	**Improvement Actions Recommended** What are the actions for reducing the occurrence of the cause, or improving detection?
Media preparation									
	Incorrect concentration of serum / growth factors added to media	Insufficient cell growth	Operator error	1	1	3	**3**	Training, SOP / batch record	Verify operator training
	Improper transfer of cell culture media into bioreactor bag	Contamination	Operator error, defect in single-use disposables	5	1	3	**15**	Training, SOP / batch record	Verify operator training; prepare extra media and bioreactor disposables
Cell seeding									
	Incorrect number of cells seeded	Insufficient cell growth to fulfill dose requirements	Cell counter failure; operator error	4	1	3	**12**	Training, SOP / batch record	Equipment requalification / calibration; verify operator training
Cell expansion									
	Incorrect incubation temperature	Cell death; insufficient cell growth to fulfill dose requirements	Incubator failure	4	1	1	**4**	Extra incubators available	Equipment requalification / calibration; additional incubator temperature monitor
	Incorrect pH control	Cell death; insufficient cell growth to fulfill dose requirements	pH controller failure; operator error	2	1	3	**6**	Training, SOP / batch record	Equipment requalification / calibration; verify operator training
	Incorrect DO control	Cell death; insufficient cell growth to fulfill dose requirements	DO controller failure; operator error	4	1	3	**12**	Training, SOP / batch record	Equipment requalification / calibration; verify operator training
	Incorrect bioreactor agitation speed	Cell death; insufficient cell growth to fulfill dose requirements	Bioreactor failure; operator error	3	1	2	**6**	Training, SOP / batch record	Equipment requalification / calibration; verify operator training
	Incorrect feed concentration / feed timing	Insufficient cell growth to fulfill dose requirements	Operator error	2	1	1	**2**	Training, SOP / batch record	Equipment requalification / calibration; verify operator training
Harvest									
	Insufficient cell recovery from harvest	Reduced cell yield	Incomplete transfer due to operator error	4	2	1	**8**	Training, SOP / batch record	Verify operator training
	Improper connection for cell transfer out of the bioreactor bag	Contamination	Defective connector, weld failure, operator error	5	1	3	**15**	Training, SOP / batch record	Verify operator training

requires scoring for occurrence and detectability, FMEA relies on product and process understanding and is typically performed in later stages of development (e.g., during the control strategy development stage), where sufficient data is available to predict occurrence and detectability. FMEA principles can be applied to all aspects of the manufacturing process, including equipment and facilities, and might be used to analyze a manufacturing unit operation and its effect on CQAs. It identifies elements/operations within the system that render it vulnerable. The output/results of FMEA can be used as a basis for identification of improvement actions in the control strategy, particularly for process inputs associated with a high RPN.

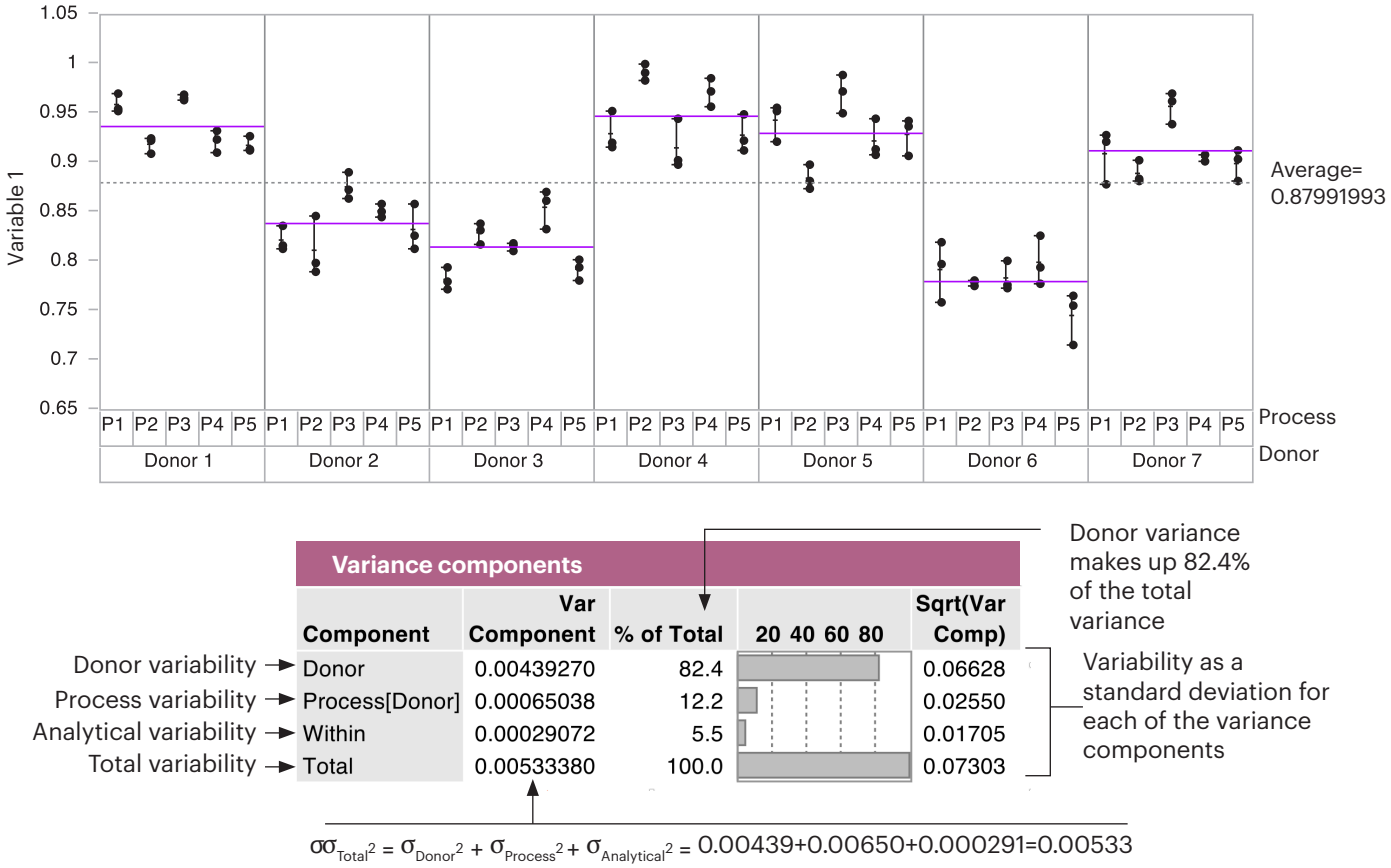
An example of FMEA applied to a CAR-T expansion process is presented in Table 10-2. In this case, each factor is assigned a severity, occurrence, and detection score between 1 and 5; the highest overall risks are conferred by process inputs that present the greatest impact of failure (severity) from possible contamination or reduced cell yield that could result in insufficient dose for the patient. Further characterization of these high-risk process inputs (i.e., how these parameters affect the final product CQAs) should be performed, which will then inform the improvement actions for future operations.

Design of Experiments approaches

A key tool used during process development and characterization is DoE, which utilizes statistical and analytical methods to determine the impact of multiple inputs (e.g., process parameters) on product CQAs.[17] The DoE approach can help to identify process parameters that have the greatest impact on CQAs (the CPPs) and to identify

Figure 10-3: *Example DoE including multiple donors, highlighting inherent patient heterogeneity.*

Material from 7 donors are each processed 5 times and results are analyzed in triplicate, revealing that donor variance comprises 82.4% of the total variance in the data.[18]



Source: Ashton R, et al. Presentation at: CASSS Cell and Gene Therapy Conference. June 10, 2019. Reprinted with permission.

secondary relationships that may influence CPPs orthogonally. These CPPs proceed to more in-depth characterization, whereas parameters that impart the lowest risk to CQAs may be excluded from further characterization. Thus, DoE approaches increase the understanding of truly critical elements of the overall process as well as identify secondary relationships of materials or process parameters that may exert influence on the critical parameters, while also improving the efficiency of process characterization. DoE helps to minimize the number of experiments required to obtain the necessary process information without compromising the output, by ensuring a minimum interaction between all experiments. A case study of a DoE involving one donor is provided in the text that follows. Importantly, due to potential patient heterogeneity, use of multiple donors in the DoE may be necessary (Figure 10-3).

Case Study

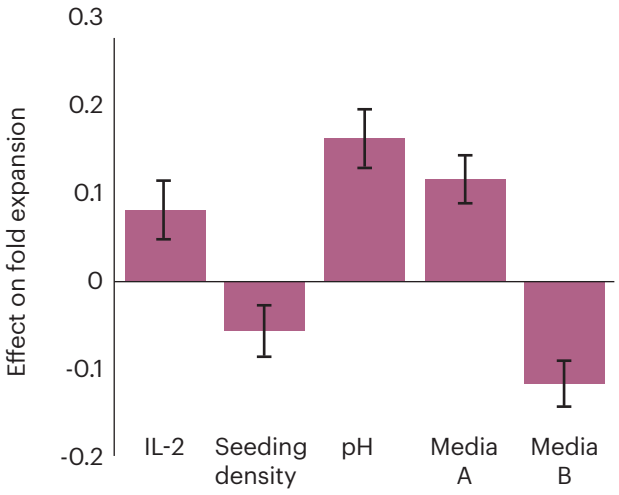
An example of DoE used to determine the optimal culture conditions for primary T cells in a small-scale stirred tank bioreactor (Ambr® 15) is presented below (Table 10-3). The goal of this DoE is to screen different parameters (factors) and evaluate the effect of these factors on T-cell expansion, as measured by two responses: cell growth (fold expansion) and cell viability. Other parameters, such as temperature (37°C) and culture volume (10 mL), are kept constant. A linear, reduced combinatorial design DoE approach was taken, with replicates of 4 experiments.

The result of the DoE is analyzed and presented in a coefficient plot (Figure 10-4), which represents the size and direction of the relationship between each factor in the model and the response variable (fold expansion) while other terms are kept constant. Based on these results, higher fold expansion can be achieved through higher IL-2 concentration, lower seeding density, higher pH, and the use of Media A.

With the knowledge gained from this initial DoE, a second round of experiments was performed with a reduced number of factors: pH, IL-2 concentration, and dissolved oxygen (DO) (Table 10-4), while keeping seeding density at 5×10^5 cells/mL and using Media A. The results

Figure 10-4: *Example coefficient plot for T-cell expansion study in small-scale bioreactors.*

The size and direction of the relationship between each factor and fold expansion was investigated while other factors were held constant.[19]



Source: Hupfeld J, et al. Optimization of T-Cell Expansion Using the Sartorius T-Cell Exploration and Characterization Solution. Sartorius application note: https://www.sartorius.com/en/applications/cell-and-gene-therapy/cell-therapy/cell-therapy-guide/optimization-of-t-cell-expansion-app-note. Reprinted with permission.

Table 10-3: *Factors screened in DoE for optimization of T-cell expansion in small-scale bioreactors.*[14]

Factors	Optimization Range
IL-2 concentration (U/mL)	50; 125; 200
Seeding density (cells/mL)	5×10^5; 1×10^6
pH	7.0; 7.3; 7.5
Medium	Media A, Media B

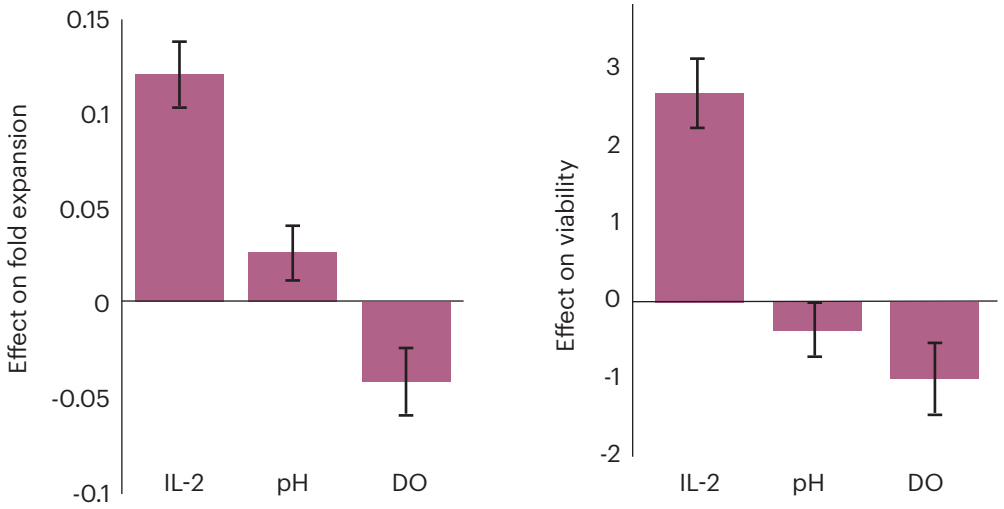
Table 10-4: *Factors screened in second DoE for optimization of T-cell expansion.*[14]

Factors	Optimization Range
IL-2 concentration (U/mL)	50; 125; 200
pH	7.2; 7.4
DO (%)	50; 70; 90

Source: Adapted with permission from Sartorius webinar "Accelerating CAR-T process development: The power of process insight." https://www.sartorius.com/en/applications/cell-and-gene-therapy/cell-therapy/cell-therapy-guide/accelerating-car-t-process-development-process-insight-webinar.

Figure 10-5: *Coefficient plots for T-cell expansion and viability from the second optimization study.*

Second optimization for fold expansion and viability identifies IL-2 concentration as a CPP with strong positive impact.[19]



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show IL-2 concentration as the most important factor for both fold expansion and viability; IL-2 concentration is therefore identified as a CPP. This DoE also indicates that higher DO might have a negative effect, and pH is shown to have a small effect on the responses (Figure 10-5).

To better visualize the outcome of this study, a sweet spot plot can be generated from the DoE results (Figure 10-6), which shows the optimal process range that can be applied in future scale-up. In this case, to achieve the predetermined CQA of ≥8-fold expansion and ≥90% viability, the T-cell expansion process should be operated with IL-2 concentration above 160 U/mL and DO below 85%.

Further characterization should then be performed to confirm that the optimal process conditions obtained through the DoE fulfill the CQA requirements. In the case of CAR-T manufacturing, characterization of potency can be performed following expansion and harvest by quantifying CAR-T killing of target tumor cells. In the example below, CD19+ CAR T cells (effector cells) were co-cultured with Ramos cells (antigen-positive target cells) at different ratios, and selective cell killing was monitored over time using live cell imaging (Figure 10-7). Effective killing of antigen-positive cells was observed. More extensive potency characterization can be done *in vivo* or in humanized mouse models.

Figure 10-6: *Sweet spot plot to inform optimal ranges of process parameters to achieve certain CQAs.*

Optimal process range was identified to achieve a minimum fold expansion of 8 and viability of 90% while pH, stirring speed, seeding density, and medium type were kept constant.[19]

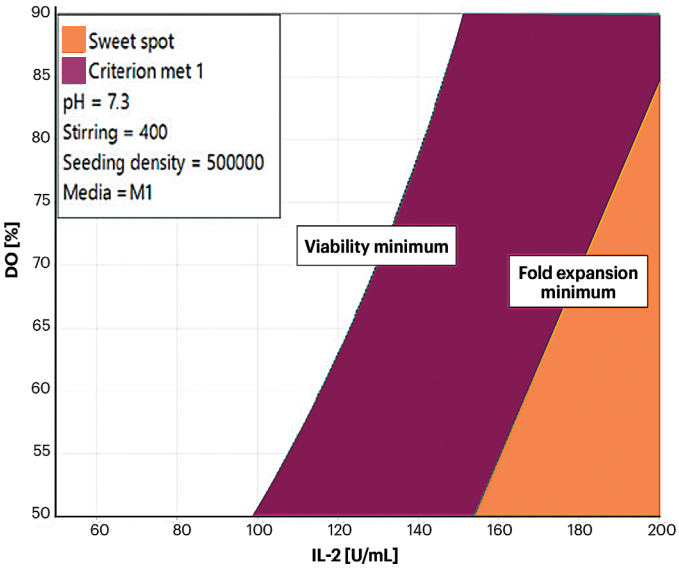

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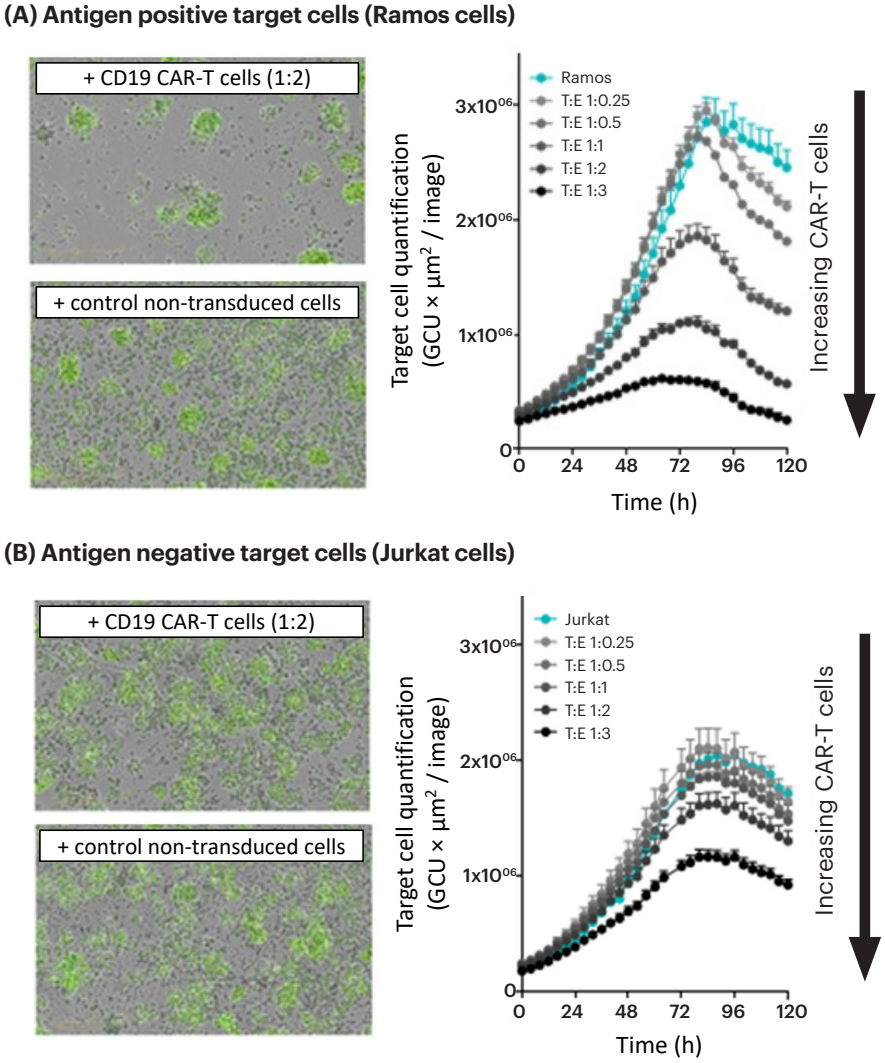
Figure 10-7: *Characterization of CAR-T potency through quantification of cell killing assay.*

CD19+ CAR T cells were co-cultured with antigen-positive target cells (A) or antigen-negative target cells (B) at different target: effector (T:E) ratios. Target cells were labeled in green and images were taken at 72 hours following co-culture.[14]



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PROCESS VALIDATION

Following development of the IPCs, process performance qualification (PPQ) runs are executed at commercial manufacturing scale to validate the process and confirm that it provides adequate control of product quality. The number of PPQ runs required will depend on adequacy of starting materials, level of risk assessed, and complexity of both the molecule and the process.

Guidance regarding the overall approach to process validation has been provided by the FDA.[20] This guidance aligns process validation activities with the product life cycle concept, incorporating three key stages of process validation:

- Process design, during which the commercial manufacturing process is defined based on knowledge gained during clinical development and scale-up activities;
- Process qualification, which seeks to evaluate whether the process is capable of reproducible commercial manufacturing (via the execution of PPQ runs); and

- Continued process verification, which provides ongoing assurance that the process remains in a state of control during routine production.

PROCESS COMPARABILITY

Changes to the manufacturing process (vector or drug product) are inevitable throughout the development, commercialization, and post-approval life cycle management of a cell therapy product. These changes may occur when new knowledge is gained about CQAs and CPPs, when processes are scaled-up and/or transferred between manufacturing sites, or when process improvements or technological advances such as automation occur. Manufacturers must demonstrate that resulting vector or drug product are comparable before and after the change. According to ICH Q5E,[21] the goal of comparability exercises is to ascertain that pre- and post-change drug product is comparable in terms of quality, safety, and efficacy. As such, manufacturers must provide relevant technical information (based on a combination of analytical testing, biological assays, and clinical/nonclinical data in some cases) showing that process changes will not have an adverse impact on the quality, safety, and efficacy of the drug product. Similarity of quality profiles must be demonstrated across validation batches and the methods used to detect differences must be appropriately selected and justified. In many cases, a paired-run approach for both vector and drug product manufacturing changes is utilized to ensure that the true comparison of process changes can be evaluated while minimizing the potential impact of donor variability in the cell therapy space that may confound a more conventional comparability strategy used for biologics. More details on comparability expectations throughout the product life cycle are provided in Chapter 2.

Material Attribute Controls

Materials used during manufacturing processes can be sources of significant variation across all types of drug products.[53] Materials of poor quality can introduce contaminants and impurities that impact the viability, purity, potency, and safety of the final product. According to EU guidelines on good manufacturing practices (GMP) for biologically active substances and medicinal products for human use, control of material quality is especially important during the manufacturing of cell therapy products, where final sterilization is generally not possible and the ability to eliminate microbial by-products is limited.[22] Material controls can be established once critical material attributes (CMAs) and potential risks generated from materials are identified. Control strategies must be defined for each CMA so that variations can be detected, managed, and minimized.

RAW AND STARTING MATERIALS

The selection of high-quality raw and starting materials is paramount to ensuring the quality of a final CAR-T cell product (Chapter 5, Chapter 7). Risks associated with raw materials can range from viral contamination of processing reagents to heavy-metal impurities present within excipients used for final product formulation. Raw material quality can also vary between lots and suppliers and can be impacted by changes to supply-chain logistics. For example, purified enzymes obtained from different sources may vary in their specific activity, whereas hydrolysates may vary in their composition. Materials of biologic origin are often poorly characterized, but avoidance of their use is rarely feasible in the manufacturing of cell therapy products. Where biological raw materials must be used, a key to controlling overall risk to the final drug product lies in demonstrating the ability to eliminate risks from raw material variability.[53]

Starting materials for CAR-T cell products include primary cells of human origin (Chapter 5) and vectors (Chapter 7). Several safety risks are inherent to cells sourced from humans:[53]

- Materials can be contaminated with adventitious agents (e.g., bacteria, viruses, fungi, parasites)
- Materials can have tumorigenic potential
- Materials can elicit an immunogenic reaction

For autologous CAR-T cell products, primary cells are extracted from individual patients with cancer and one batch represents one patient. Hence, variation and unpredictability between patients is the major cause of batch-to-batch variability in drug product production processes. Conversely, for allogeneic CAR-T cell products, a lot or batch derived from a single healthy donor's cells

can be used to treat multiple patients (or an individual patient with multiple doses). With either approach, viable cell count estimates can be misled by cellular impurities within apheresis materials, such as myeloid cells or circulating tumor cells. Complicating the process further, primary cells begin to change soon after removal from the body and cell viability can quickly be compromised in the absence of adequate controls. Changes to morphology, cell growth potential, and cell-surface markers may impact the ultimate performance of the cell construct. As such, developing strategies to ensure the uniformity and consistency of apheresis processes is of utmost importance. Strategies to control the quality of collected cells include auditing of the apheresis center to better understand and standardize collection protocols, equipment selection to standardize processing conditions, equipment maintenance, personnel training, storage of the apheresis product, and shipping conditions prior to drug product manufacturing.[53] Additionally, a thorough understanding of donor screening and testing requirements implemented by apheresis providers is warranted, including their collection of donor history (e.g., previous treatment exposure, prior illness, complete blood count with differential, and absolute lymphocyte count), which could impact the quality of incoming material that may impact the probability of manufacturing success during downstream processing. All components of the transportation process must be well-characterized, including the impact of transport time and temperature on cell viability and growth potential.

Inherently, the cell sourcing strategy for allogeneic CAR-T cell products diverges from that of an autologous product. The aim of an allogeneic CAR-T cell product is to be off-the-shelf ready for infusion into a recipient. Allogeneic CAR-T cell s are derived from healthy donor cells that have been gene-edited to allow administration to patients unmatched for human leukocyte antigen (HLA). Donor screening criteria for allogeneic products are extremely robust, seeking to eliminate the introduction of any adventitious agents that could cause disease or any immunogenic material that could precipitate graft-versus-host disease (GVHD). In addition, phenotypic markers (e.g., PD1, CD27, and CD8) on donor cells can be important predictors of CAR-T cell expansion and response in patients. As such, screening and selecting healthy donors whose T-cells express a high frequency of these markers may improve response to allogeneic CAR-T cell products.[23]

Vectors are effectively treated as starting materials when used to manufacture genetically modified cells for cell therapy products, even when that modification occurs *ex vivo*, as with CAR-T cell products. According to the EMA, "principles of GMP shall apply from the bank system used to produce the vector onwards."[13] Whereas EU regulatory agencies very clearly view vector (and all components used to make the vector) as starting material,[13] language from the FDA is less clear, referring to vector as a "critical material."[24] Nevertheless, expectations regarding the vector are similar across regulatory bodies, as vector attributes influence the final drug product. For example, vector attributes have a direct impact on the cell therapy product VCN post-transduction. Further discussion on control strategy, as it relates to vector, is included in Chapter 7 of *Project A-Gene*.[25]

ANCILLARY MATERIALS

Primary cells, as well as the intermediate and final CAR-T cell product, come into direct contact with multiple ancillary materials during production. These materials can vary widely in quality depending on the source and vendor, and therefore should be characterized to better understand the risk they impart to the CAR-T cell manufacturing process, final product, and patient. The FDA recommends that human- or animal-derived components are not sourced from geographical areas of concern for potential viral and/or transmissible spongiform encephalopathy agent contamination and that components be tested appropriately for adventitious agents.[10] In addition, sponsors should qualify ancillary materials for quality, safety, and potency through vendor qualification programs and incoming material qualification programs, as appropriate. Principles of ancillary materials risk management, qualification program, and reagents consideration for cell-based therapies are covered in Chapter 6.

Procedural Controls

Having procedural controls in support of current GMP manufacturing operations allows both the vector and the CAR-T cell therapy manufacturer to mitigate risks

imposed by personnel, facility design, equipment, and environmental conditions. Procedural controls should be clear, concise, and leave no room for interpretation by manufacturing staff. This drives consistency into processes, making them more robust and reducing overall variability as much as possible. Manufacturers should continually assess whether improvements should be made based on periodic reviews for trend deviations/investigations and as a routine part of their internal quality review or annual product quality reviews as required by health authorities.

ASEPTIC PROCESSING

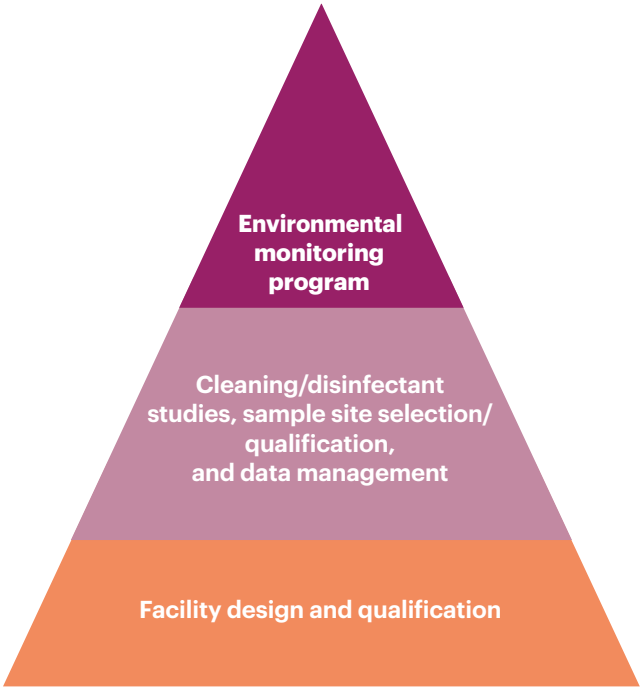
Manual processing of CAR-T cell products is becoming less common due to advances in automation and closed-system manufacturing processes. Where manual processing continues to be used, minimizing introduction of contaminants and impurities relies heavily on the individual operator's understanding of microbiology and aseptic technique.[53] In semi-automated and closed-loop systems, manipulation by individual personnel is more limited, making overall product quality less dependent on the individual operator. In such systems, aseptic processing relies on ensuring that equipment and its associated materials (e.g., tubing, vials) are properly sterilized.

The need to describe aseptic controls in the overall control strategy is discussed in both EMA and FDA documents.[2,26] Sufficient control must be demonstrated for individual operator training, qualification of manufacturing procedures, aseptic manufacturing, and microbiology. Process simulations can be used to validate manual and semi-automated/closed-loop aseptic processes, as described by both the FDA[26] and the Parenteral Drug Association (PDA).[27,28]

PERSONNEL

A complex, experienced workforce poised to adapt to a rapidly evolving field is required to develop and manufacture CAR-T cell products. Safe, effective, and consistent manufacturing of CAR-T cell products requires establishment of a quality culture at the organizational level as well as a staff with in-depth product knowledge and thorough training in both aseptic manufacturing and product-specific processes. Significant emphasis must be placed on education, practical experience, and demonstrated competency of the personnel.[53] The European Commission recommends that manufacturing personnel "possess the appropriate qualifications, adequate practical experience relevant to intended operations, and [have a] clear understanding of their responsibilities, including knowledge of the product appropriate to the assigned task."[2] It also recommends that, prior to participating in routine manufacturing operations, personnel should participate in successful aseptic process simulation tests.[2] Similarly, the World Health Organization provides policies for appropriate personnel hygiene levels and health conditions, as well as procedures for preventing the transmission of communicable diseases from raw and starting biological materials.[29,53] Personnel must be trained, and retrained as necessary, to perform their assigned responsibilities adequately.[30] This extends to personnel involved in the cleaning and maintenance of areas used for aseptic processing.[31] In addition, manufacturing facilities must have appropriate redundancy in both personnel and organizational structure to ensure thorough review of all manufacturing and testing activities.

Figure 10-8: *Foundation for the environmental monitoring program.*



ENVIRONMENTAL MONITORING AND CONTROL OF FACILITIES AND EQUIPMENT

Environmental monitoring is an important control element when manufacturing CAR-T cell products. Facility design (Chapter 11) provides the foundation of a successful environmental monitoring program (Figure 10-8); design and qualification staff must consider elements such as surface finishes and cleanability; air filtration, flow, and pressurization; flow of waste from personnel, material, and equipment; gowning procedures and certification; and the number of personnel to be accommodated by the facility.[53] For autologous CAR-T cell products, the facility may need to accommodate the processing of multiple individual patient batches in parallel. Design considerations are different for allogeneic CAR-T cell products, where one large batch destined for multiple patients is produced at a time. Regardless of approach, the environmental monitoring program should be supported by cleaning and disinfectant studies[32] as well as sample site selection/qualification and data management. Several resources exist for the development of a successful environmental monitoring program.[2,26,33]

Facility controls should consider the segregation of materials, product, and personnel such that mix-ups and cross-contamination are minimized. The type of product being manufactured must also be considered (i.e., whether autologous or allogeneic processing will be performed). Equipment must be "fit for the purpose" of CAR-T cell manufacturing and controls should be established for cleaning and sanitizing all equipment.[34]

Testing Controls

Quality control tests for CAR-T cell drug products center on demonstrating a favorable CQA profile by direct or orthogonal controls using validated methods throughout the entire production process. At a minimum, testing strategies must show that all drug product release specifications are met prior to release and infusion into patients. According to the ICH Q6B, a specification "establishes the set of criteria to which a drug substance, drug product, or materials at other stages of its manufacture should conform to be considered acceptable for its intended use."[35] As such, quality control tests must consider the mechanism of action of the final drug product in the overall control strategy. Specification limits and the precision required by the analytical methodologies used for testing must consider all product-related knowledge and should reflect accumulating consensus and advances within the rapidly evolving CAR-T cell therapy field. Specification limits may also be tied to clinical outcomes through correlative analysis techniques to evaluate the potential impact of CQA profiles on clinical endpoints such as safety, efficacy, and pharmacokinetic profiles.

Testing can be divided into routine testing (i.e., release testing, as required by regulatory authorities and done on every lot prior to disposition) and periodic testing (i.e., testing performed on sufficient batches to demonstrate that a process is in a continuous state of control via validation, comparability, stability, or periodic monitoring studies). Quality tests are a key part of any manufacturing control strategy. Phase-appropriate testing controls can be employed to support early development where limited knowledge about the process and product is available. The analytical methods used to support characterization and release should be constantly evaluated during development, and improvements made based on increased product and process understanding or where technological improvements are available to improve reliability, robustness, and reduce variability. As such, the tests themselves must be developed and improved throughout clinical development, with final commercial validation performed to support commercial licensure. The final control strategy should define when testing should be performed, which assays will be used, and the acceptable range for each CQA that ensures a product of acceptable quality. Additional information on analytical method strategies is found in Chapter 9.

ROUTINE TESTING

In-process testing monitors the quality of cells, clearance of process-related impurities, or safety attributes by testing CQAs at critical production steps or intermediate product stages. The understanding of appropriate in-process testing for any CAR-T cell product grows throughout the development life cycle of the product. Early on, assays that monitor product quality and patient safety should be performed after most process steps to determine which steps are most critical and which assays are best able to pick up on process parameters

that impact quality attributes of the intermediate or final drug product. As the product moves into later phases of development, an evolution of the control strategy continues as analytical methods are optimized, new methods are potentially added, and appropriate acceptance criteria for in-process and release testing are established based on process characterization and clinical development studies. Final specifications of a commercial drug product should also encompass relevant real-world experience obtained through clinical studies, where correlative analysis can be performed to better inform the practical relevance of the observed range of CQAs and the potential impact on clinical efficacy, safety, or pharmacokinetic parameters. The benefit of correlating to clinical studies lies in the ability to justify potentially wider specification acceptance criteria that have been demonstrated safe and efficacious across the broad range of clinical experience. Due to the greater inherent variability of patient material within the cell therapy space, the ability to correlate CQA profiles to clinical outcomes to justify wider specification acceptance criteria will allow greater flexibility for patient dosing across the broader range of manufacturing experience.

Routine release testing is performed on every lot to confirm alignment with established specifications. When combined with in-process testing, control of the manufacturing process and final drug product from batch-to-batch can be demonstrated.[36] Release tests center around characterizing products according to their identity, potency, purity, safety, and dose/viable cell number (see Table 10-5 and Chapter 9).

Table 10-5: *Examples of release/characterization testing for CAR-T cell products.*

Goal/target of assay	Attribute being tested
Viable cell number	**Dose**
Sterility	**Safety**
Mycoplasma	
Replication-competent lentivirus	
Vector copy number	
CAR+ cells	**Identity**
T-cell markers (e.g., CD3+, CD4:CD8 ratio)	
Count/concentration of transgene-expressing cells	
Markers of cell viability	
Residual LV particles or plasmid DNA	**Purity**
Undesired cell types (host tumor cells, WBCs, B cells)	
T-cell activation reagents/beads	
Cytotoxic potential	**Potency**
Interferon-γ secretion	

CAR+: chimeric antigen receptor-positive; LV: lentiviral vector; WBCs: white blood cells

PERIODIC TESTING

Full characterization of manufacturing processes requires more than in-process and release testing of intermediate and/or final drug product. Even extensive release testing only confirms that a particular process was successful; however, it does not fully demonstrate control of the process itself. PPQ runs and other periodic testing (e.g., comparability, stability, periodic monitoring) are required to ensure that the process remains in a state of control.

According to the FDA's Guidance for Industry on Process Validation, the PPQ is part of process qualification and seeks to "confirm the process design and demonstrate that the commercial manufacturing process performs as expected."[20] In general, PPQ runs will feature a higher level of sampling and scrutiny of process performance than what is common during actual commercial production. In some instances, PPQ runs may be performed at the extremes of normal operating ranges (NORs) to confirm that process control remains intact at these extremes.

The PPQ protocol must specify the manufacturing conditions, controls, testing, sampling plan, analytical methods, acceptance criteria, and expected outcomes. PPQ lots should be manufactured using the intended commercial process by the personnel who will routinely be in charge of each production step, and should meet the standards described within the specification. Once a sufficient number of PPQ runs have been performed, a full PPQ summary report documenting results, deviations (along with product impact statements), and an assessment of adherence to the written PPQ protocol should be prepared.

Incorporation of stability testing into the control strategy provides a high degree of confidence that the drug product is stable within its expected shelf life and under all transport, storage, and conditions of use during patient administration. According to the United States Pharmacopeia (USP) chapter 1049, "protocols to establish stability of a patient-specific therapy should use materials from multiple donors and at least three lots."[37] Stability during process holds at the manufacturer or at the medical center(s) should be assessed, as should stability at key in-process points if drug substance is stored before final processing and filling (as drug product). Assays used in stability testing must be capable of detecting changes in the relevant CQAs being measured. Robust stability-indicating assays are required to test for CQAs that may be expected to degrade over time in storage; these assays must verify that storage conditions maintain the purity and potency of the drug product within predetermined specification ranges and within a certain time frame, which will then dictate the product shelf life. Notably, aberrant potency assay results can be indicators of changes in stability. With CAR-T cell therapies, cryopreservation is the key to a stable platform, as it enables transportation of both the leukapheresis material and the final drug product back-and-forth between the manufacturing site and medical center. Cryopreservation principles are discussed in detail in Chapter 8.

Significant guidance on stability data requirements can be found in ICH Q5C and ICH Q1A(R2).[38,39] In general, for either drug substance or drug product, data from at least three batches representative of the manufacturing scale of production should be provided, with "representative" applying to manufacturing process, storage conditions, and containers. If claimed shelf-life is greater than 6 months, a minimum of 6 months of data should be provided at the time of submission to regulatory agencies (for shelf lives of <6 months, data requirements should be considered on a case-by-case basis). Where material volume is sufficient as an output of the drug product manufacturing process during pre-approval and pre-licensing, the recommended intervals for stability testing frequency are as follows:

- ≤1 year shelf-life: stability studies monthly for the first 3 months, then at 3-month intervals thereafter
- >1 year shelf-life: stability studies every 3 months during the first year of storage, every 6 months during the second year, and annually thereafter (note: this may be applicable to allogeneic CAR-T cell approaches)

Though stability testing ideally follows ICH guidance, cell therapies are often constrained by small lot size and limited starting materials. As such, acceptable creative approaches to stability testing exist, including bracketing and matrix designs where multiple lots are utilized to holistically build an "ICH-like" picture of the stability profile.[40] In these cases, more than three batches may be required to build the holistic stability framework. Additionally, reduced stability testing may be appropriate after approval or licensure where data are available that demonstrate adequate stability.[39]

An additional stability testing consideration relates to the need to maintain potency during the in-use (post-thaw) phase, prior to recipient infusion, which also may be extended in allogeneic approaches. Regulatory guidance on in-use testing is sparse; the EMA[41] advises that:

"The appropriate physical, chemical, and microbial properties of the product susceptible to change during storage should be determined over the period of the proposed in-use shelf life. If possible, testing should be performed at intermediate time points and at the end of the proposed in-use shelf life on the final remaining amount of the product in the container."

Data obtained from in-use stability testing helps support information contained in the label regarding the preparation, storage conditions, and period during which the drug product can be used. Typical in-use studies are measured in hours, so care should be taken to ensure studies carried out can be tested immediately to verify potential impact of storage post-thaw prior to patient administration. For further discussion on product and in-use stability, including an example stability matrix for a CAR-T product, refer to Chapter 9.

Whenever a change is made to manufacturing materials or processes, a risk of altering one or more critical quality attributes of the drug product exists. As such, comparability studies on pre- and post-change drug products must be conducted to show that the resultant products are similar in terms of efficacy and safety. Much of the existing guidance on comparability studies stems

from ICH Q5E,[21] with insights on comparability incorporated into recent guidance from both the FDA[24] and the EMA.[42] Comparability claims should not rely solely on routine release specifications; rather, a prospective comparability study design should accompany all filings and may include characterization studies, forced degradation studies, and long-term stability studies. Additionally, retrospective analyses comparing efficacy, safety, and pharmacokinetics of pre- and post-change product can be utilized to show comparability.[43] Due to the inherent variability of patient starting material, split-run approaches should be utilized to ensure that within a specific donor, the process change can be assessed pre-change vs post-change. Having sufficient paired runs will allow statistical analysis of the process differences to be well understood beyond the inherent material variability impact on such studies. Including a comparability testing protocol within the control strategy to support any future changes could demonstrate a commitment to upholding the utmost efficacy and safety of a CAR-T cell product.

Table 10-6: *Clinical factors examined in correlative analyses.*[44,45]

Factor	Potential correlative relationship
Patient characteristics	Age Prior treatment exposure Tumor characteristics T-cell attributes
Clinical efficacy	Overall response rate Complete response rate Best overall response Duration of response Progression-free survival
Clinical safety	Cytokine release syndrome Cytopenia Neurotoxicity Oncogenesis (or lack thereof)
Pharmacokinetics/ pharmacodynamics	Area under the curve Maximum concentration Time to maximum concentration

Control Strategy as a Function of Clinical Relevance

CORRELATIVE ANALYSIS TO CONNECT DRUG PRODUCT CQAS TO CLINICAL OUTCOMES

Correlative analysis employs statistical methods, subject-matter review, and advanced analytics infrastructures to explore potential associations between drug product quality attributes (particularly those related to purity, strength, and potency) and clinical outcomes (i.e., efficacy, safety, and pharmacokinetic profiles).[44] These analyses can inform the true criticality of quality attributes as well as the tolerance levels for their specifications. Correlative analyses also facilitate a better understanding of how variation within quality attributes impact both product performance and patient outcomes. The end result may enable streamlining of the manufacturing process by pointing out where flexibility lies in the process or the product.

Specific to CAR-T cell products, numerous genotypic, phenotypic, and functional CQAs measured during release and characterization can impact efficacy, safety, and pharmacokinetic profiles at the patient level (Table 10-5). Through correlative analysis, these CQAs and patient-specific characteristics can be examined for their relationship to clinical outcomes of interest (Table 10-6).[44,45]

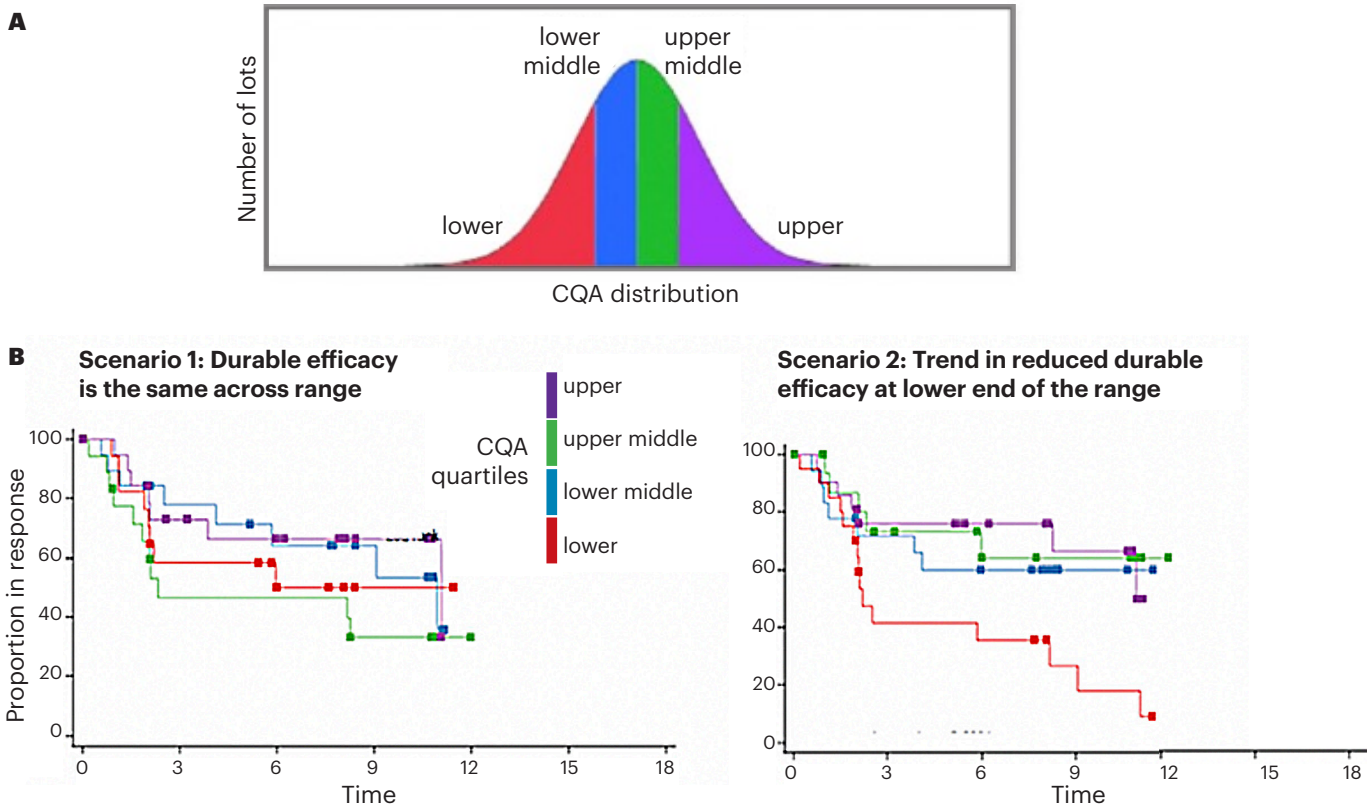
The type and depth (i.e., univariate, multivariate) of correlative analysis performed will depend on the relationship between the CQA and clinical outcome being examined:[45]

- Categorical (e.g., responder/non-responder; cytokine release syndrome [yes/no]; neurotoxicity [yes/no])
- Time-to-event (e.g., progression-free survival; duration of response)
- Continuous (e.g., area under the curve, maximum concentration)

IMPACT OF CQA LEVELS ON DIRECT PATIENT OUTCOMES

The statistical effect size (i.e., magnitude) of the correlative relationship between a CQA and patient outcome and any resultant shift of the risk/benefit profile of the drug product are used to judge the impact and clinical importance of variability within the CQA.[45] Variability can be introduced via patient starting material, manufacturing processes, or analytical methods. Variation in manufacturing processes that seek to clear viral contamination or remove process reagents (e.g., serum) have obvious ties to patient safety, as do analytical methods to detect

Figure 10-9: *Example of correlative analysis for a CQA (a) with two different scenarios illustrated (b).*[45]



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contaminants such as mycoplasma. However, the impact of variation in patient starting materials, which generally represents the largest source of variation in autologous CAR-T cell therapies, is oftentimes less clear.[44] Given that this variability is unique and inherent to the patient, it may not result in an adverse impact on patient safety or product efficacy, even if it results in a related CQA falling outside of the accepted specification range established during preclinical drug development. As such, it may be justifiable to push specification limits for certain CQAs to the edges of their clinically relevant distribution when it can be shown that no difference in the risk/benefit profile is imparted by attribute levels at the upper or lower extremes of the distribution range.[44,45] An example of this is found in Figure 10-9, which groups CQA distribution by upper and lower quartiles to illustratively determine the overall clinical benefit across the range of clinical experience. Scenario 1 of Section B of the figure demonstrates an example where a specification may be justified by the entire range of clinical experience due to similar clinical benefit. Conversely, Scenario 2 of Section B may represent a situation where a tighter specification may be required to maximize patient benefit based on clinical benefit distributions. By expanding the specifications to the broader ranges of clinical experience, delay of product delivery caused by unnecessarily stringent specifications can be prevented.

Several examples illustrate the value of performing correlative analysis and considering wider acceptance criteria for certain CQAs. Out-of-specification cell viability did not compromise the clinical safety or efficacy of tisagenlecleucel in patients with acute lymphoblastic leukemia, non-Hodgkin lymphoma,[46] or diffuse large B-cell lymphoma.[47] Similarly, no significant difference in cell viability was seen among responders and nonresponders to axi-cel therapy among patients with relapsed/refractory large B-cell lymphoma.[48] Broader specification limits for VCN, which is often kept below 5 due to concern for random integration and insertional oncogenesis,[49,50] may be appropriate when a robust understanding of the vector's insertion sites is present and when correlative data show that clinical safety is not compromised.[44,51]

DIVERGENCE BETWEEN AUTOLOGOUS AND ALLOGENEIC APPROACHES

Correlative analytical strategies for autologous and allogeneic CAR-T cell products take somewhat different approaches, owing largely to the unique challenges to efficacy and safety that each pose. With allogeneic CAR-T cell products, optimization of attributes that impact clinical efficacy focus largely on those related to potency, particularly given the extended shelf life of allogeneic products. Additionally, pharmacodynamic considerations such as how quickly the allogeneic CAR-T cell product is cleared by the recipient's immune system can significantly impact efficacy. Following lymphodepletion, infusion of allogeneic CAR-T cell products may be followed by host rejection and a corresponding loss of clinical effect. As such, the degree of lymphodepletion present in recipients and the rapidity of immune reconstitution can significantly impact efficacy. From a safety standpoint, correlative analyses focus on allogeneic product quality attributes that could potentiate GvHD or infectious disease transmission.

IMPROVEMENTS IN CQA UNDERSTANDING THROUGHOUT THE PRODUCT LIFE CYCLE

Initial CQA specifications for a drug product are based on limited manufacturing and preclinical data. As experience grows with the product through clinical production and process and product characterization studies, and as clinical data begins surfacing in pre-pivotal trials, enhanced understanding of CQAs and their appropriate specification levels will follow. As development proceeds toward commercialization and product knowledge is gained, regulatory agencies generally expect to see tightening of acceptance criteria as part of the refined control strategy,[44] though this may be less applicable and feasible with autologous cell-based products. As such, proposals for broader acceptance criteria should be accompanied by extensive correlative data that justifies the request. In these cases, it may be advantageous to engage in direct regulatory interactions to have a robust scientific discussion about proposed specifications, risk/benefit profiles for patients, and potential impact on timely infusion to patients with poor disease prognosis.

Figure 10-10: *Components of a total integrated control strategy.*[52]

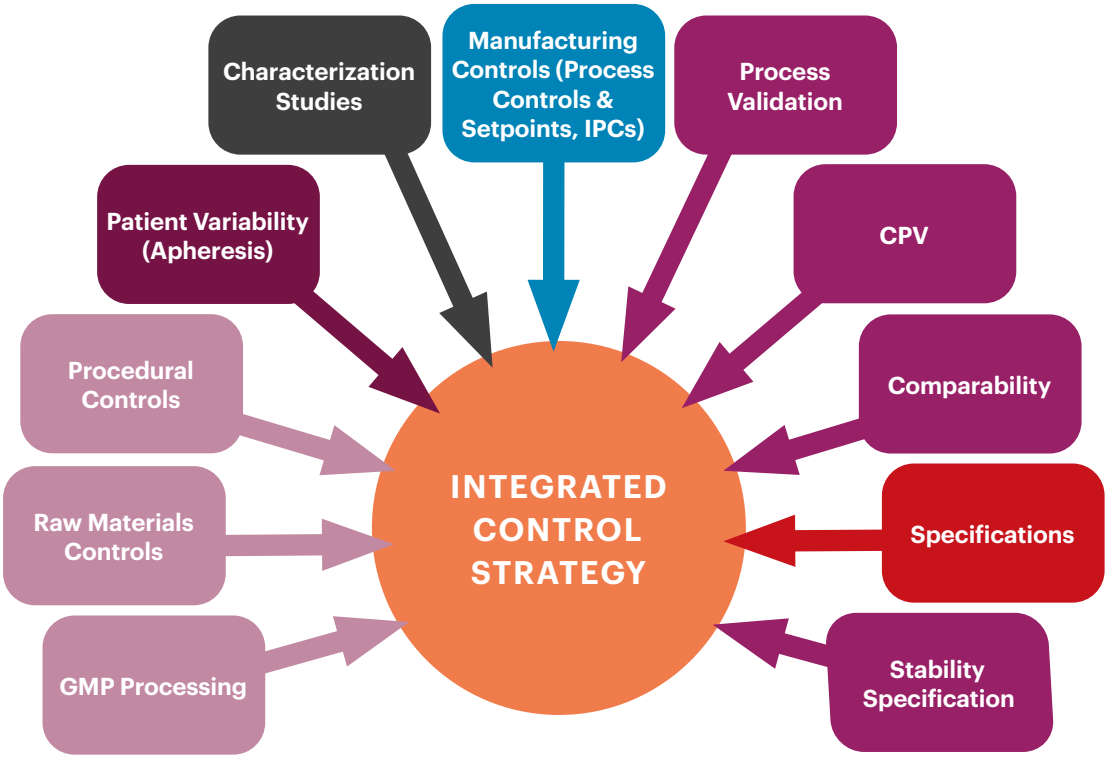

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Figure 10-11: *The integrated control elements matrix is used to score occurrence and detection of each attribute.*

Process knowledge determines the likelihood (occurrence) that the attribute will be present in sufficient quantity in the drug product to cause the effect considered when assigning the severity score.

The Proposed Control Strategy's ability to detect and control of the attribute are considered

Quality attribute	Experience with commercial process	Process knowledge	Raw material testing	DP release testing	IPC action limit/ acceptance criteria	Process validation	Extended characterization	Stability
Attribute 1	x	x		x				x
Attribute 2	x	x	x			x		
Attribute 3	x	x					x	
Attribute 4				x				x

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Evaluation of Total Integrated Control Strategy

The culmination of product development integrates an entire control system that supports a comprehensive control strategy to ensure product quality and drug product reliability to serve the needs of patients. Presenting a total integrated control strategy (ICS) to the health authorities demonstrates a cumulative control of the entire manufacturing process, including GMP procedural and facility controls, raw material controls, robust process characterization to drive meaningful in-process controls, periodic testing controls (e.g., PPQ, continued process verification, comparability, stability), as well as routine testing during drug product release. All these controls, in addition to the target patient population, disease indication, potential impact of variability from the starting patient material, and correlation to clinical outcomes can be used to demonstrate a holistic control of CQAs well beyond just the release specification (Figure 10-10). The

Figure 10-12: *The product quality risk assessment determines the final residual risk to patients from each attribute.*

The Severity score and the Occurrence score are multiplied to arrive at a Preliminary Hazard level. This reflects the risk to patient without consideration of the testing program.

The Preliminary Hazard Level is multiplied by the detection score to determine the final residual risk to patients from each attribute.

Quality attribute	Severity score	Occurrence score	Preliminary hazard level	Detection	Final residual risk
Attribute 1	**3**	**5**	**15**	**1**	**15**
Attribute 2	**2**	**7**	**14**	**3**	**42**
Attribute 3	**1**	**1**	**1**	**2**	**2**
Attribute 4	**6**	**7**	**42**	**1**	**42**

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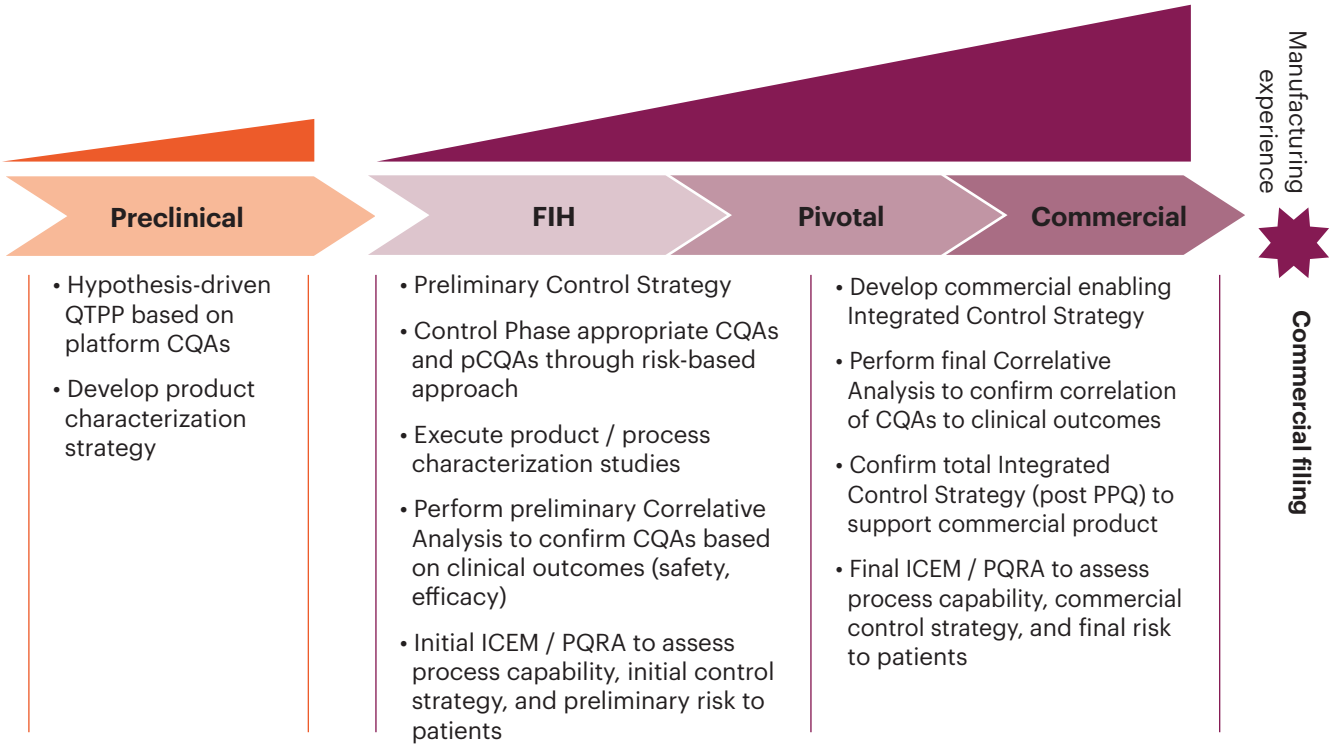
Figure 10-13: *Utilizing quality by design principles to develop an integrated control strategy.*



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total ICS should reflect a risk-based approach to establishing and assessing the overall risk posed by various attributes to patient safety and product quality. As such, the ICS helps to identify attributes that need to be evaluated to demonstrate process control and consistency.

TOOLS TO DEVELOP AN INTEGRATED CONTROL STRATEGY

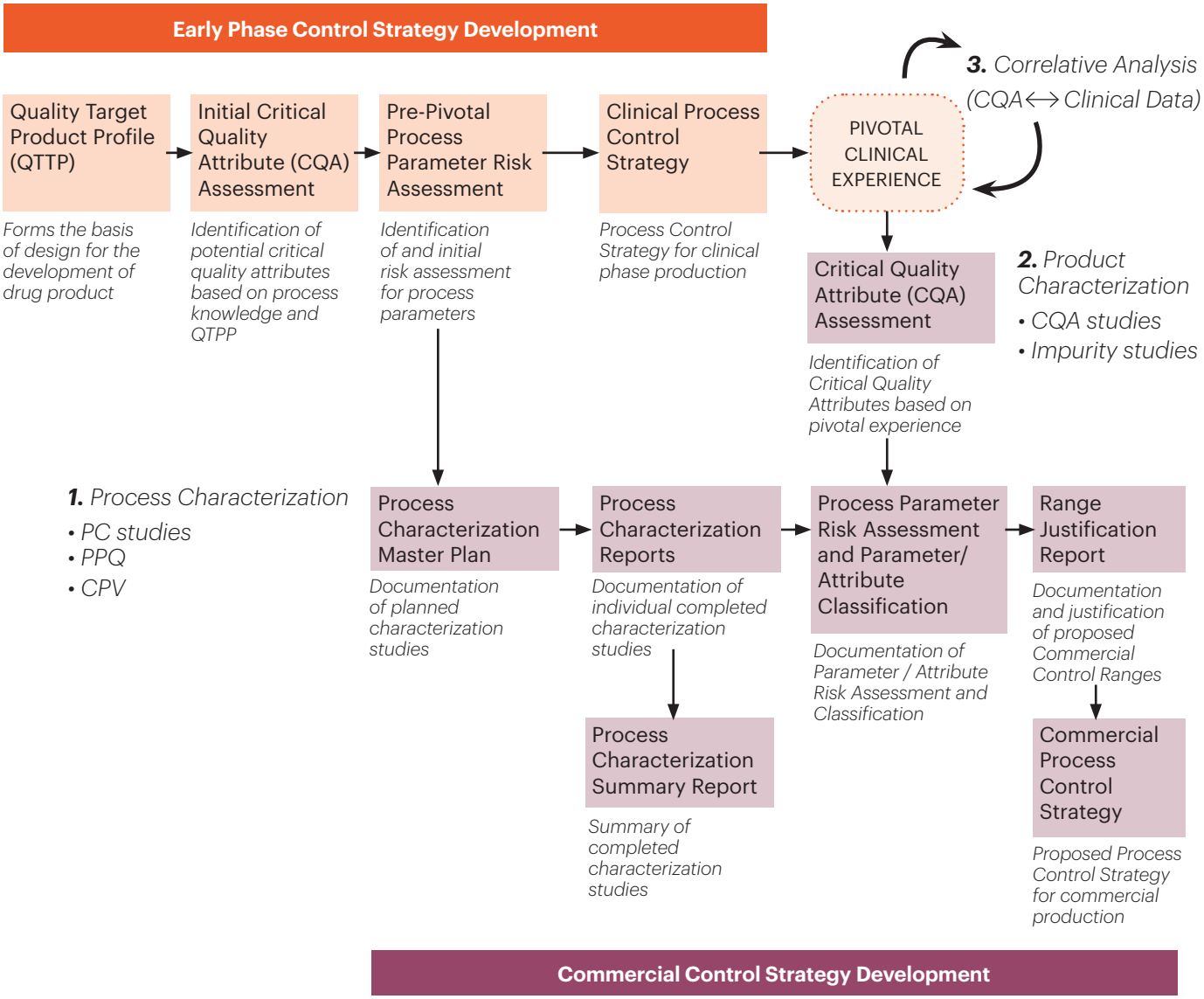
An integrated control elements matrix (ICEM) captures the impact of process elements on product quality attributes, compiles control elements and defines where they are applied in the process, and ultimately serves to define the control strategy. In combination with a product quality risk assessment (PQRA), the ICEM applies the principles of ICH Q9: Quality Risk Management to the ICS. The ICS contained within the ICEM is integrated in the sense that it accounts for attribute criticality, process understanding, and testing controls.[52]

Following a similar approach to FMEA (discussed earlier in the chapter), the combined ICEM/PQRA approach evaluates the ICS for residual risk to patients posed by various attributes. First, the ICEM is used to score occurrence and detection of the attribute based on process understanding and analytical method capabilities (Figure 10-11). The PQRA assigns a severity score based on potential impact to patient safety and product efficacy to the attribute and then determines the overall residual risk to the patient by considering severity, occurrence, and detection (Figure 10-12).[52]

CONTROL STRATEGY EVOLUTION FROM CLINICAL TO COMMERCIAL DEVELOPMENT

According to ICH Q9,[15] consideration should be given to improving the control strategy over the life cycle of product development. The unique aspects of CAR-T cell therapies requires that the industry leverage tools such as ICEM and PQRA to develop phase-appropriate control strategies that adapt over time (Figure 10-13). Early-phase control strategies are reflective of historical/initial knowledge of both the manufacturing

Figure 10-14: *Pathway from early-phase to commercial control strategy.*



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process and the drug product characteristics. The commercial control strategy is refined based on an evolving knowledge gleaned from product/process characterization, characterization of patient variability, and correlative analyses that tie product quality attributes directly to clinical outcomes in the pivotal clinical trials. Establishment and utilization of early-phase control strategies can be used to guide and provide structure to internal product development stages, whereas a refined commercial control strategy is an essential component of any biologics license application filing. An overview of the pathway followed to develop phase-appropriate control strategies is contained in Figure 10-14.

Importantly, different control strategies could be applied at different sites during both early-phase and commercial development.[15] Differences could be due to equipment, facilities, systems, and CDMO-related confidentiality considerations. Where CDMOs are involved, seamless knowledge transfer between the CDMO and sponsor (in both directions) is essential.

Establishing Meaningful Specifications

CAR-T cell products represent a relatively new therapeutic modality. As such, literature on specification development strategies for CAR-T cell therapies is lacking. Guidance can be gleaned from ICH Q6B,[35] which discusses general approaches to specification setting for biological products. A specification, as defined by ICH Q6B, is a list of tests, references to analytical procedures, and appropriate acceptance criteria that describe the numerical limits, ranges, or other criteria for the tests described. Specifications are critical quality standards proposed and justified by the manufacturer and considered as conditions of approval by regulatory bodies. As such, specifications are a key part of a total control strategy that seeks to ensure drug product quality and consistency. The establishment of specifications should consider manufacturing processes, stability of the drug substance and drug product, data from preclinical and clinical studies, and the capabilities of analytical procedures.[35]

EARLY-STAGE DEVELOPMENT

Like all elements of the control strategy, specification development takes on a life cycle approach. Early specifications are based on limited manufacturing experience and/or historical data from experiences with similar products.

Table 10-7: *Examples of early-phase autologous CAR-T cell product specifications.*[52]

Quality Attribute	Parameter	Methodology	Specification
Appearance	Color	Compendial	Description of color
	Clarity	Compendial	Description of turbidity
Identity	Confirmation of identity	Flow cytometry	Anti-XXX CAR+ cells detected (identity confirmed)
Purity	Cell viability	Fluorescent microscopy and image analysis	Initial specifications based on platform knowledge (product, process), patient population, health authority guidance, and risk-based approach
	T-cell purity/ immunophenotype	Flow cytometry	
	Product-related impurities	Flow cytometry	
	Process-related impurities	ELISA or other suitable methodologies	Initial specifications based on historical process understanding, initial impurity risk assessment or tox assessment
Strength	CAR + viable T cells	Flow cytometry	> XX CAR+ cells/mL (strength may be used in lieu of potency)
Safety	Transduction controls	qPCR	Initial specifications based on platform knowledge (product, process), patient population, health authority guidance, and risk-based approach. Strength alternative orthogonal control, develop in later phase of development
	Endotoxin	Compendial	XX EU/mL
	Mycoplasma	Compendial	Not detected
	Sterility	Compendial	No growth

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Table 10-8: *Examples of commercial-phase autologous CAR-T cell specifications.*[52]

Quality Attribute	Parameter	Methodology	Specification
Appearance	Color	Compendial	Description of color
	Clarity	Compendial	Description of turbidity
Identity	Confirmation of identity	Flow cytometry	Anti-XXX CAR+ cells detected (identity confirmed)
Purity	Cell viability	Fluorescent microscopy and image analysis	Meaningful specification established per clinical correlative analysis
	T-cell purity/ immunophenotype	Flow cytometry	
	Product-related impurities	Flow cytometry	
	Process-related impurities	ELISA or other suitable methodologies	Meaningful specification established per process characterization/ impurity risk assessment
Strength	CAR + viable T cells	Flow cytometry	> XX CAR+ cells/mL
Potency	Antigen-specific function	Bioassay	Product-specific acceptance criteria established per clinical correlative analysis (potency, transduction controls)
Safety	Transduction controls	qPCR	
	Endotoxin	Compendial	XX EU/mL
	Mycoplasma	Compendial	Not detected
	Sterility	Compendial	No growth

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They may also take into consideration knowledge of the patient population that the product is designed to treat, guidance from the health authorities, and initial assessments of risk posed by product- or process-related impurities. Specifications for CAR-T cell products generally center around the quality attributes of appearance, identity, purity, strength, and safety (Table 10-7).[44,52]

CLINICAL-TO-COMMERCIAL DEVELOPMENT

As development programs progress through clinical trials and toward commercialization, regulatory bodies generally expect specification acceptance criteria to be tightened. This is often accomplished via a tolerance interval-based approach that is founded on an understanding of process capability. However, process capability should not be the sole determinant of specification acceptance criteria. As discussed earlier in the chapter, acceptance criteria employed at the commercial stage should strongly consider data gleaned from correlative analyses that bridge clinical outcomes to the specifications themselves, with the ultimate goal of setting patient-centric specifications (Table 10-8).[44,52]

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CHAPTER 11

Manufacturing Strategy, Facility Design, and Manufacturing Operations

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Introduction

The growth of the cell and gene therapy (CGT) sector has led to an increasing demand for facilities suitable for manufacturing these products at a clinical and commercial scale. The options for cell-based therapy developers are to manufacture in-house through use of existing facilities or building new facilities; outsource manufacturing to a contract development and manufacturing organization (CDMO); or rent current good manufacturing practice (CGMP) space in a hybrid model where the cell therapy manufacturer utilizes their own employees in a facility overseen by the facility owner.

In contrast to more traditional biologic or vaccine manufacturing facilities, which are typically designed to accommodate large-scale processes using equipment such as bioreactors, stainless steel vessels, and chromatography systems, cell-based therapy manufacturing processes are traditionally more akin to research laboratory scale with equipment such as biosafety cabinets, centrifuges, microscopes, and single-use plasticware to execute operations, especially for autologous products. Allogeneic products produced on a larger scale are on the horizon and utilize bioreactor-based processes with predominantly single-use systems. Many cell-based therapy product developers are in the process of establishing in-house manufacturing facilities to contend with the increasing demand for cell-based therapy manufacturing

capacity, the relative lag and lack of suitable capacity availability from CDMOs and rentable spaces, and to provide flexibility of supply and retention of intellectual property.

Designing and establishing new facilities for cell-based therapy manufacturing that meet required regulatory standards is a challenging task. It is often one of the largest capital expenditures related to cell-based therapy product development due to factors such as the need for highly specialized equipment, the need for facility flexibility to accommodate current and future manufacturing processes, and evolving process scales in anticipation of increased market demand. A key feature of cell-based therapy product manufacturing is the requirement for full aseptic processing rather than sterile filtration or final product terminal sterilization as in the case of traditional biologics. Therefore, the facility design must minimize the risk of product contamination throughout the process from incoming raw materials to final product storage to ensure product quality and patient safety. Autologous processes pose design challenges to concurrent manufacturing of multiple, small-scale, patient-specific batches, where mid to large-scale allogeneic processes require the design to accommodate processes at the boundary of manual processing capability and future introduction of automated technologies that are still in development.

The goal of all biopharmaceutical manufacturing facilities is to produce consistent, compliant products that meet regulatory requirements at a sustainable cost. The facility design process translates process, operational, engineering, and regulatory requirements into a tangible facility and requires dedication of time and resources from all functions of the cell therapy organization coupled with specialist input from architectural, engineering, and construction organizations. The design and construction of a manufacturing facility is integral to the reliable and reproducible execution of the manufacturing process operation and should be defined and controlled in line with qualification and validation principles set out in regulatory guidelines for production of aseptically processed products and CGMP.

This chapter describes an overview of the facility design process, key regulatory requirements, design layout, operational design considerations, and qualification, validation, and manufacturing start-up processes to evolve initial facility requirements into a fit-for-purpose operational facility for cell-based therapies. While some of the concepts presented here (e.g., equipment and room arrangement considerations) are geared towards autologous therapies, discussions relating to risk assessment and management are applicable to other types of cell-based therapies.

Facility Design Process Overview

Designing CAR-T and cellular therapy facilities to be fit-for-purpose requires an integrated approach through which the facility is designed to meet the manufacturing process, operational, regulatory, and business requirements. The facility design ultimately contributes to the consistency of delivering conforming products through facilitation of the manufacturing operation and activities as well as driving the capital and operating costs. Typically, architectural and engineering biopharmaceutical design specialists are engaged by cell-based therapy developers to lead the design process. This begins with the definition of requirements, includes translation of operational and regulatory needs, and ends with the final agreed design. Successful facility design requires expert input and dedication of time from multiple functions within the cell-based therapy developers including manufacturing operations, quality, regulatory, engineering, project management, and business management functions. The resourcing and management of a facility design process should therefore be carefully planned and considered. The evolution from facility design to manufacturing of regulated products can be broadly described in the following stages—definition; design; build, commissioning, qualification and validation (CQV); manufacturing start-up and manufacturing operation—as described below and summarized in Figure 11-1.

DEFINITION

All facility design processes should begin with a *User Requirement Brief (URB)* that contains a clear definition of the business objectives and purpose of the facility. This includes the activities and functions within the facility such as manufacturing, R&D, administration, and quality control as well as details on location, capacity,

throughput, product lines (single or multiple), and future requirements. Information on the processes and analytical testing requirements for the products intended to be manufactured in the facility, and utility, automation, safety, and maintenance requirements should also be included. In addition, a *detailed process definition* describing the manufacturing processes should be developed. These documents set the foundation for the design effort. At this stage, cross-functional engagement and internal alignment from the cell-based therapy developer is important to minimize changes and impacts on time, cost, and quality.

A *feasibility study* may be used to assess and refine the requirements and can provide high-level facility design options and associated estimated delivery costs. This may include assessment of different site locations and types such as greenfield, brownfield, and retrofits of existing buildings.

For many cell and gene therapy organizations, the manufacturing processes are not always completely defined when entering into the facility design process. In this case, particular focus on areas of the process that impact the design in terms of cost, compliance, and ease of scaled operations should be considered in line with proactive discussion with Process Development functions.

It has been common to design facilities with manually operated open processes and benchtop-oriented equipment which can be adequate for early- to mid-stage clinical development programs. However, later-stage manufacturing demands should be considered at the definition stage to ensure that the facility can accommodate future scaled-up and scaled-out manufacturing processes and meet regulatory expectations, particularly with regard to compliance with CGMP standards. A challenge for cell-based therapy developers is to balance the needs of the business at the time of the definition stage and future needs if products prove to be successful through clinical trials. Designing for future expansion without impacting ongoing manufacturing should be considered to balance initial capital outlay with potential future disruption to operations. In addition, best practice and regulatory trends are moving towards adoption of closed systems wherever possible; increased utilization of single-use equipment, process aids and automation to reduce variability and contamination risks associated with manual operation steps; and monitoring specifically designed to ensure the maintenance of aseptic processing. The URB should also consider the manufacturing process evolution in line with future regulatory expectations.

An additional consideration at the facility definition stage is whether different/multiple products are to be manufactured in the facility. In this case, facilities must be designed with appropriate segregation, containment level, and cleaning/disinfection procedures to mitigate opportunities for possible cross-contamination. Further consideration for appropriate containment and biosafety requirements should be taken into account particularly when viral vectors are used in the manufacturing process such as in CAR-T therapies.

DESIGN

The facility design process evolves the URB and process definition into a blueprint used to build the facility. Several design phases can be adopted to translate the requirements into a functional design meeting operational, regulatory, and business needs. Biopharmaceutical specialists including architects and process, mechanical, civil, electrical, and automation engineers work alongside the cell-based therapy developer specialists during the design stage. Typical design phases are:

- ***Preliminary/Concept Design:*** This phase of design translates the URB and process definition document into a practical facility layout that satisfies the needs of all end-users including manufacturing, quality control, engineering, facility, quality assurance, regulatory, and senior management functions. An important first step at this stage is to develop the URB and process definition into an agreed scope of requirements and agreed process definitions for the facility that forms the basis of the design. The process definition is often accompanied by process flow diagrams and block flow diagrams that outline the processes to be manufactured in the facility and aid the development of the facility layout and operations. Different design options are discussed at this stage to produce a conceptual facility layout with associated drawings showing the flow of personnel, materials,

and waste as well as cleanroom classifications and pressurization cascades. For facilities that handle biological agents with a potential hazard to human health (e.g., viral vectors), biosafety requirements are also assessed and included at this design stage along with general Environmental, Health and Safety (EHS) requirements. Cost estimates and project schedules are also produced at this stage. Facility operational philosophy documents are developed including segregation and cross-contamination control, facility flows, gowning, biosafety, and supportive heating, ventilation, and air conditioning (HVAC) and automation philosophies, which feed the facility layout development to a conceptual design. From the process definition, equipment lists and utility and electrical requirements are produced. Identification of back-up and emergency power requirements (e.g., uninterrupted power supplies) for the building and critical equipment are also considered at this stage. Typically, system impact assessment and component criticality assessments are also undertaken to identify equipment and systems that have a direct, non-direct, or no impact on the quality of the product. These assessments form the foundation for the commissioning, qualification, and validation stage.

- ***Detailed Design:*** This phase of design translates the concept design package into an architectural and engineering package for construction. User requirements specifications (URSs) and functional design specifications for the facility, equipment, and utilities are produced that specify in detail what is required and how the systems should work. The URSs may also be produced at the end of the concept design phase depending on the needs of the cell-based therapy developer. These documents and the concept design package form the basis for development of detailed engineering drawings such as piping and instrumentations diagrams (P&IDs), electrical wiring, and HVAC design, as well as enabling the equipment and materials procurement process. Some equipment and materials with long lead times may be procured in the concept phase to enable the installation schedules to be met. It is typically during this phase that the local jurisdiction is engaged and drawing plans are submitted for construction permit approval. Final cost estimates and project timelines are also provided at this stage for executive management review.

Design reviews and contamination control risk assessment

At each design phase, design reviews should be conducted to ensure that the proposed design meets the operational, safety, and regulatory requirements. This includes assessment for compliance with CGMP regulatory guidelines, manufacturing operability, good engineering practice, and safety and maintenance operability reviews. Given that product safety is of primary importance with respect to the eventual delivery of cell-based therapy products to patients, the design review should also be supported by a contamination and cross-contamination control risk assessment.

The contamination control strategy should take into account the aspects of the process design that are meant to protect the product from contamination. Contamination control measures should be designed into each part of the production process, featuring control procedures such as cleaning, decontamination, sterilization, and transfer methods for primary packaging materials, consumables, product intermediates, and waste. The process design should also consider the implementation of closed processing systems or isolators to drive containment to the equipment level and put less stress on maintaining a high classification of the background manufacturing environment.

Manufacturers should comprehensively assess risks associated with possible sources of contamination and should, subsequently, implement measures to prevent contamination commensurate with the risks. This may include revising the design to reduce the risk or prospectively developing acceptable operational measures in conjunction with the operations and quality assurance teams to mitigate the risk.

It should be noted that the regulatory, operability, and safety reviews are live documents and should be reviewed and updated on a periodic basis through the process and facility lifecycle.

BUILD, COMMISSIONING, QUALIFICATION, AND VALIDATION

Facility construction is conducted by qualified specialist contractors who should have experience with the specific requirements for construction of biopharmaceutical facilities. The construction begins with the preparation of the selected site, building the superstructure, making the facility watertight, installing utilities, and then installing the HVAC, cleanrooms, equipment, and internal components with appropriate waste containment strategies to achieve a fully functioning facility. An "as-built" drawing for the constructed facility is provided in a handover package.

Following the construction phase, the facility is commissioned, qualified, and validated (CQV) to demonstrate that the facility with equipment is fit for the intended purpose. The CQV process systematically demonstrates that a facility being installed will offer a high degree of quality assurance such that manufactured products will consistently meet quality requirements through Installation Qualification, Operational Qualification, and Performance Qualification (IQ/OQ/PQ). Installation Qualification (IQ) verifies that the facility is installed in accordance with the detailed design; Operational Qualification (OQ) ensures that the facility operates in accordance with the functional design specification; and Performance Qualification (PQ) ensures the facility performs in accordance with the user requirements specification. The CQV process is conducted through documented test procedures and the level of testing and documentation is determined by the system impact assessments, which identify the impact of the system on the process. Associated Standard Operating Procedures (SOPs) are also developed for operation of the facility, utilities, and equipment to support the CQV process and typically forms the start of the personnel training process.

MANUFACTURING START-UP TO MANUFACTURING OPERATION

Following the handover of the qualified facility to the end-user, operational start-up activities, process qualifications, and validations need to be performed before entering manufacturing operations that can provide data packages to support regulatory licensure and approval to produce clinical or commercial products. This includes completion of critical elements of a quality management system (QMS) that include SOPs, establishing an environmental monitoring baseline, training of manufacturing and facility maintenance operators, process simulations, and cleaning validations.

Construction Methods and Design Layout

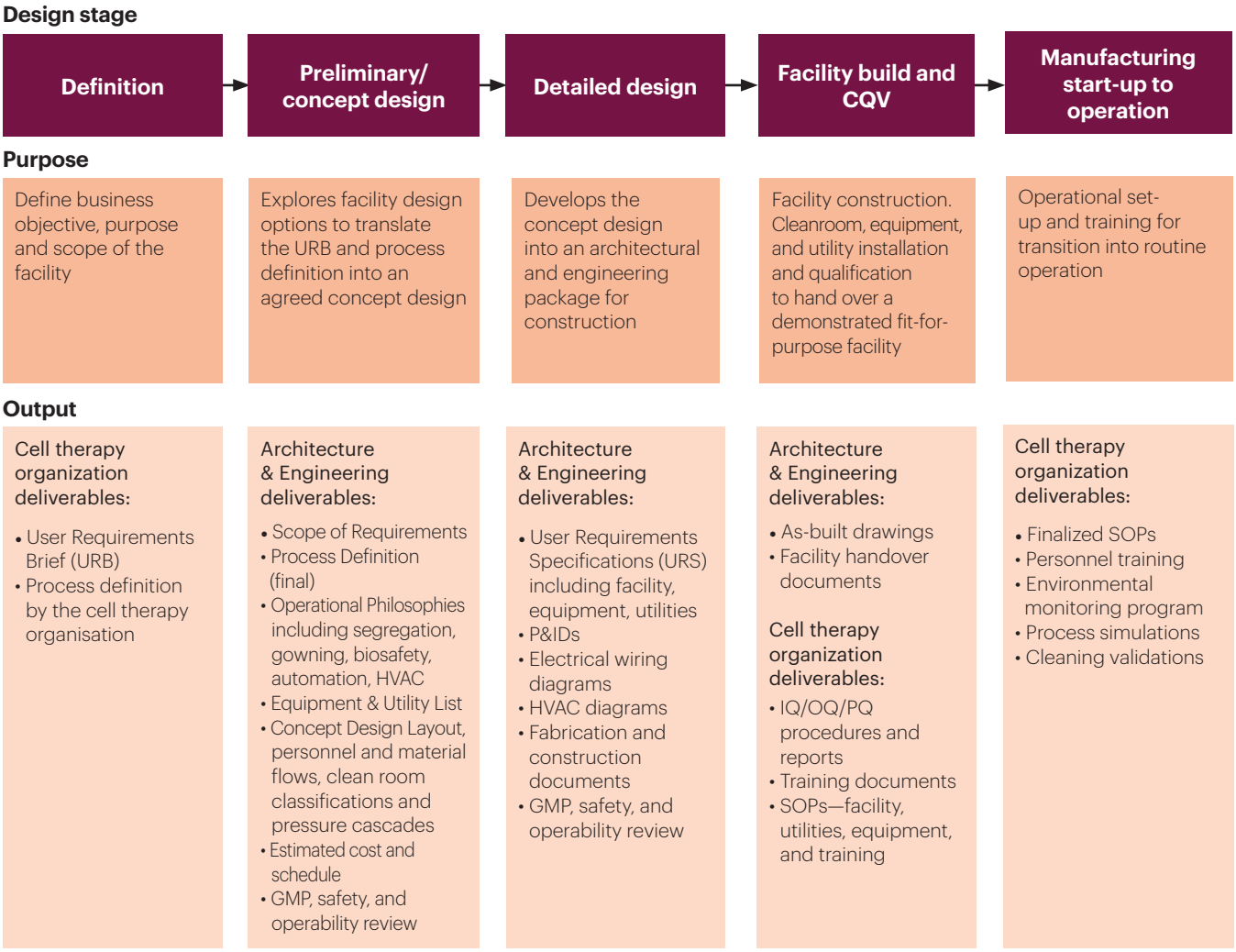
There are a number of construction methods and design layout options that can be used to meet the requirements of the facility set out in the definition phase. These influence the design, cost, construction time, and operability of the final facility. Key factors include the method of construction for the building and cleanrooms; segregation philosophies and flows of people, materials, and equipment through the facility; gowning requirements, cleanroom classifications, and HVAC design and systems to ensure chain-of identity (COI) and chain-of-custody (COC) of the product during processing. There are many design solutions that can meet the requirements and periodic design reviews are undertaken alongside risk assessments governed under the quality risk management (QRM) processes to ensure that the design meets CGMP, aseptic processing, operational, and business requirements. These topics are discussed in more detail in the following sections.

CONSTRUCTION METHODS

Pharmaceutical facilities have traditionally been "stick-built" where they are constructed at the selected site using individual parts (e.g., wooden or steel studs, drywall, brick, and mortar). Selection of building construction material must take into account risks of material decay (rot, mold), which could pose safety concerns to the product. The advantages of a stick-built construction are the flexibility to amend the design after it is built; it is not restricted to pre-determined dimensions, and often has the lowest cost per square foot.

However, for cell-based therapy companies, speed and flexibility for a facility to grow with the evolution of the company are also key drivers in addition to cost. This has led to the more recent adoption of modular construction methods, which employ networks of offsite-constructed, pre-fabricated cleanroom components. This method of delivery especially lends itself to greenfield facilities

Figure 11-1: *Typical facility design stages*



or large, open warehouse retrofits. Using the modular facility approach, cleanrooms can be manufactured using either modular wall panels (which are assembled together onsite to create the entire manufacturing space within the greater facility structure) or fully modular solutions (the entire processing space is manufactured offsite as a whole, and then shipped to the site for installation inside the greater facility structure).

There are several advantages to the modular approach over stick-built. The production site is constructed to facilitate the use of modular units, and the actual cleanrooms are produced simultaneously in another location. Units can be assembled quickly and efficiently at the given facility construction site (which allows for more rapid deployment or removal) and can save several months in overall construction time compared to more traditional methods of biopharmaceutical manufacturing facility construction. Typically, given the parallel nature in which modular delivery and site construction operations can be carried out, the more modular you drive your facility, the more you can compress your construction schedule. Thus, fully modular solutions are generally the fastest way to realize a facility. Also, as the majority of the modular units are constructed offsite, it lends itself to the requirements of a "clean build" to minimize dust and debris and reduce facility cleaning time before operational hand over. It is possible to add or remove modular processing rooms from the facility with minimal interruption to ongoing operations, helping align the facility with manufacturing capacity needs

and market demand. Modules can either be installed in an open shell space or assembled as configurable units to become a biopharmaceutical factory when all of them are put together. In the context of cell-based therapy product manufacturing, which frequently makes use of viral vector technologies and patient cells, modular units are typically tighter in terms of construction and airflow control, potentially further enhancing segregation and containment particularly for processes utilizing biological agents requiring the implementation of higher biosafety levels (e.g., BSL3).

When considering modular construction methods, consider dimensional constraints that are associated with standardized modules and if this is suitable for the overall facility design. During the construction phase, there may be a slightly higher onsite construction risk in terms of component fit, as the modular components may be difficult to adapt to unforeseen site constraints. In this case, the duration of shutdown for repairs and degree of invasive procedures for repair (e.g., particle generation from on-site adjustment) should be further considered. However, this risk is usually offset in a modular approach with a reduction in onsite safety risks (due to the volume of offsite construction) and a reduction in onsite startup risks (modular components can be pre-tested offsite prior to shipment). Modular approaches are also typically more costly than a stick-built approach, so the balance of speed, cost, and contamination potential must be considered.

While a modular approach can, in theory, be applied at any conceivable scale, in practice, the number of modular systems and transportation difficulties associated with larger scale facilities (e.g., high floor to ceiling heights, overly large ballrooms) renders the fully modular approach to be most suitable for processes with smaller footprint requirements. Autologous processes and facilities that are designed to manufacture individual patient batches in dedicated processing rooms, for example, tend to require smaller operating spaces, and thus lend themselves especially well to fully modular solutions.

The use of modular designs, in particular fully modular processing rooms, lends itself to standardization. A modular approach can offer manufacturers a framework to establish the production spaces in their design as "optimized templates" that can be consistently produced and deployed as facility demands increase and production space scale-out is required. This becomes especially useful in cases where consistency is of paramount importance, and leveraging a known design for production expansion brings with it strategic alignment for manufacturing operations. This can be an advantage within sites, between sites, and to help training, operations, and technology transfer of processes.

Table 11-1: *Construction Method Comparison*

	Stick-Built	Modular Panel System	Fully Modular
Schedule	High	Medium	Low
Cost	Low	Medium	High
Flexibility	High	Medium	Low
On-site Construction Risk	High	Medium	Low

Processing room layout options

There are various options for the layout of the processing rooms for cell-based therapy manufacturing processes. When designing the layout, operational, quality, and regulatory requirements, in particular CGMPs, must be considered to enable repeatable delivery of compliant products. As cell therapies cannot be sterile filtered or terminally sterilized, the layout of the processing and support rooms must consider the needs of aseptic processing. The chosen layout should also mitigate the risk of contamination and cross-contamination through appropriate segregation of processes and flows of people, products, materials, and waste.

Ideally, the facility design should be based around the rigorous definition of the processes and the operations to be performed, with associated QRM-based policies. Often processes are not finalized at the time of facility design, however, developers should be able to reference well-defined operational criteria that can serve as the basis for the definitions of the processes to be performed, while maintaining capacity for process

improvements and equipment replacement as part of a quality by design strategy. Details that can directly contribute to facility design considerations and requirements include the identity and scale of the unit operations to be performed; the duration and proximity requirements of each unit operation; the materials to be used in the process; whether operations are open, fully closed, or functionally closed; personnel and required waste flows; and the required proximity of support operations such as testing in-process control samples, filling, and cryopreservation. A risk assessment (discussed in Chapter 4) should be performed with regard to the operational details of manufacturing to document and justify the facility design requirements.

Facility designs must meet CGMPs as described in the applicable country regulations and regulatory guidelines. From a regulatory perspective, facility designs that promote process movement throughout the facility that mitigate the risk of mix-ups and cross-contamination are preferred. To this end, the following factors should be taken into account: whether the process is to be performed in a single room for the process duration; whether the production room is dedicated to a single patient for autologous products; and, if the process is to be performed in a larger space, whether there are dedicated workstations for the duration of the process, or whether the process moves through the space as it is performed. Risk assessments and QRM-based policies should inform all aspects of the approach to facility design.

Additionally, the planned utilization of space and equipment should be carefully considered. In the case of autologous therapy production, operations cannot be scaled up beyond the scope of a single patient. Instead, operating spaces must be scaled out to accommodate larger patient populations and achieve greater facility throughput capacities. Inefficiencies resulting from suboptimal floorplan and equipment-related design could, therefore, be correspondingly scaled out, resulting in larger and more expensive spaces than otherwise required. Thus, it is important to properly define efficient working designs that can be copied and scaled out to meet product capacity needs. In the case of allogeneic therapies which are typically campaign manufactured at larger scale, it is important to define efficient working designs that can be scaled up and maximize the utilization of the space in the facility.

Some common facility design approaches are discussed below. It should be noted that a facility design need not commit entirely to one approach or another. The facility design should be born out of an evaluation of the intended process or processes to be performed, the risks associated with each, and the risk-based management approach for the site. In many cases, a combination of the following approaches may be used to create an effective and efficient manufacturing facility design.

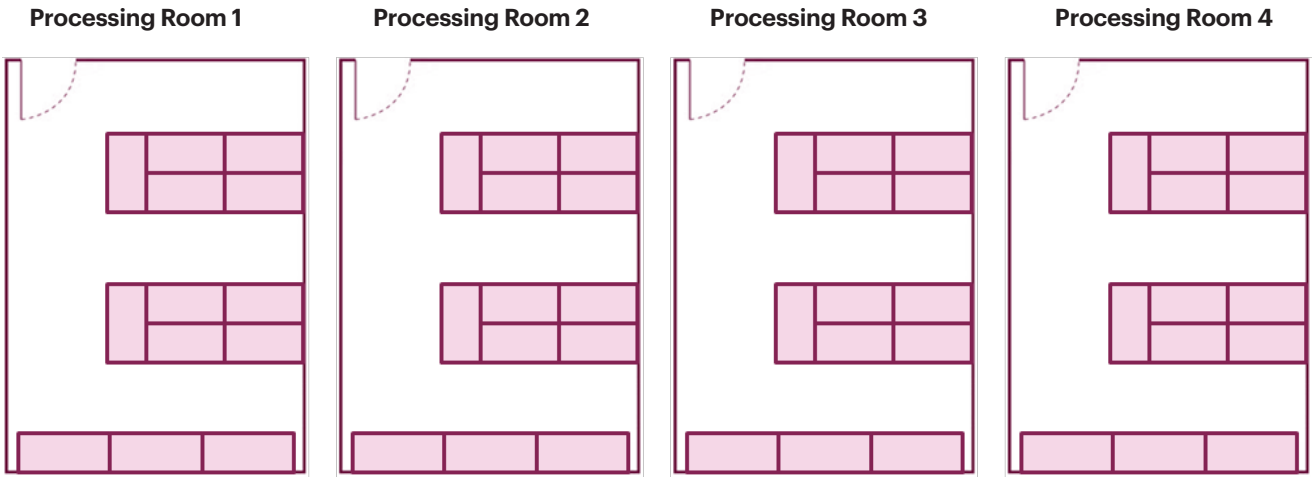
Dedicated production space

In light of the processing demands for autologous cell-based therapies where segregation of individual patient batches is paramount, processing rooms dedicated to the production of a single patient lot have become a common facility approach for early clinical-stage production. Since each room is separated from every other room, this design approach maximizes process segregation by eliminating (as much as possible) the potential for cross-contamination between patient lots or mix-up of operational materials (Figure 11-2).

For autologous therapies, because the production space is dedicated for the duration of the lot and the equipment and footprint cannot be reused for other patient lots during processing hold steps or downtime, dedicated production spaces are inefficient in terms of space and equipment utilization in the facility. In addition, dedicated process room designs are typically used for end-to-end production of autologous therapies that may include both open and closed processes. In this case, open processes are often carried out in Biological Safety Cabinets (BSCs) and require a higher background cleanroom classification compared to fully closed processes as they incur higher operational costs. If isolators are used to contain the open processes and allow for a lower background cleanroom classification, the capital and maintenance cost of the isolator as well as ease of operation need to be evaluated, especially if a scaled-out production model is being considered. While the dedicated production space is most utilized for autologous approaches, this design may also be suitable for allogeneic approaches unless the scale is too great for the room size restrictions.

Figure 11-2: *Example of Dedicated Production Space design*

Each processing room can accommodate an end-to-end autologous process.



Ballroom design

The ballroom concept refers to a large, open manufacturing area that has the flexibility to accommodate different types of processes and equipment with minimal structural segregation (Figure 11-3). Due to the larger footprint and tendency toward flexibility, the ballroom design lends itself to processes and products that are likely to change over time.

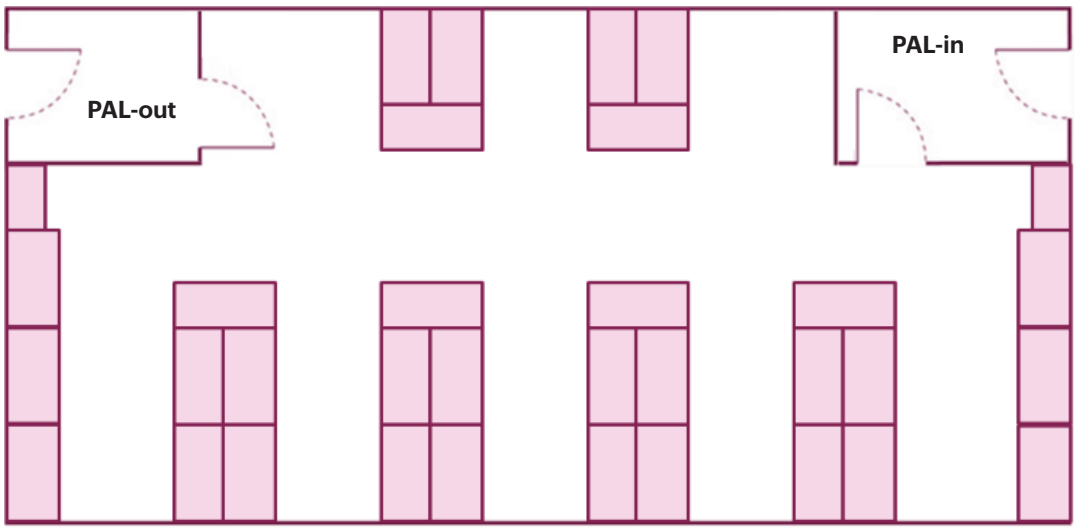
For autologous processes, multiple workstations, each used for an individual patient lot, are required to enable the most efficient use of the ballroom design, and can be more space and cost efficient compared to utilizing dedicated production rooms. However, the operation of this design is more prone to mix-ups and cross-contamination as there are likely multiple operations being staged or performed within the same room at the same time. For this reason, in support of robust QRM, ballrooms are especially conducive for the adoption, implementation, and enhancement of process closure where possible and the use of isolators where process closure is not possible. Because closed systems are characterized by total separation from the surrounding environment, closed equipment may be co-located in the ballroom space, allowing for more production per square foot of processing room. Process steps that are prone to, or pose higher contamination risks that may impact adjacent operations should be segregated, either within specifically designed containment equipment or with performance conducted outside the ballroom in a specifically designed separate space (see *Dedicated production space*). When designing the workstation layout and detailed operations in a ballroom design, rigorous attention should be focused on COI and COC to ensure segregation of materials and patient batches. Particular attention should be focused on any common equipment such as in-process testing stations, incubators, and cold storage that are used to house materials for multiple patient batches and should be governed by the QRM.

For allogeneic products that are likely to utilize a campaign manufacturing approach, the ballroom has the advantage of flexibility with the large open manufacturing space and ability to house different equipment and processes. Suitable, validated cleaning activities between campaigns and consideration of equipment validation processes in the master validation plan should also be investigated if equipment is to be changed between campaigns. If the process has open processing steps, consideration should be given to the use of isolators to enable a lower cleanroom background classification. If BSCs are utilized with the required higher cleanroom classifications, the larger footprint ballroom may incur significantly increased operational costs, and the risks surrounding cross-talk between BSCs should be carefully identified and managed.

Because of the inherent features of ballroom facility design, less facility airlocks are needed (compared to

Figure 11-3: *Example of ballroom design*

For autologous processes each workbench is typically used for a single patient batch with appropriate segregation. For allogeneic processes each workbench may house different unit operations. (PAL: Personnel Air Lock)



other design approaches) and all equipment can be placed and operated in one large, flexible space. Thus, ballroom facilities allow for a measure of creativity in developing a variety of production scenarios. As a result, the ballroom design lends itself well to scale up of allogeneic processes as well as scaling-out of autologous processes as organizations progress through clinical trials to commercial scale. Ballroom spaces that feature a high degree of process closure and automation can facilitate reduction of environmental classification, reduce operator requirements, and allow for greater facility throughput, improved quality, and consistency in production. Additionally, from the standpoint of sustainability, facilities utilizing ballroom spaces have the potential to generate smaller overall footprints.

Dance-floor design (segregated unit operations)

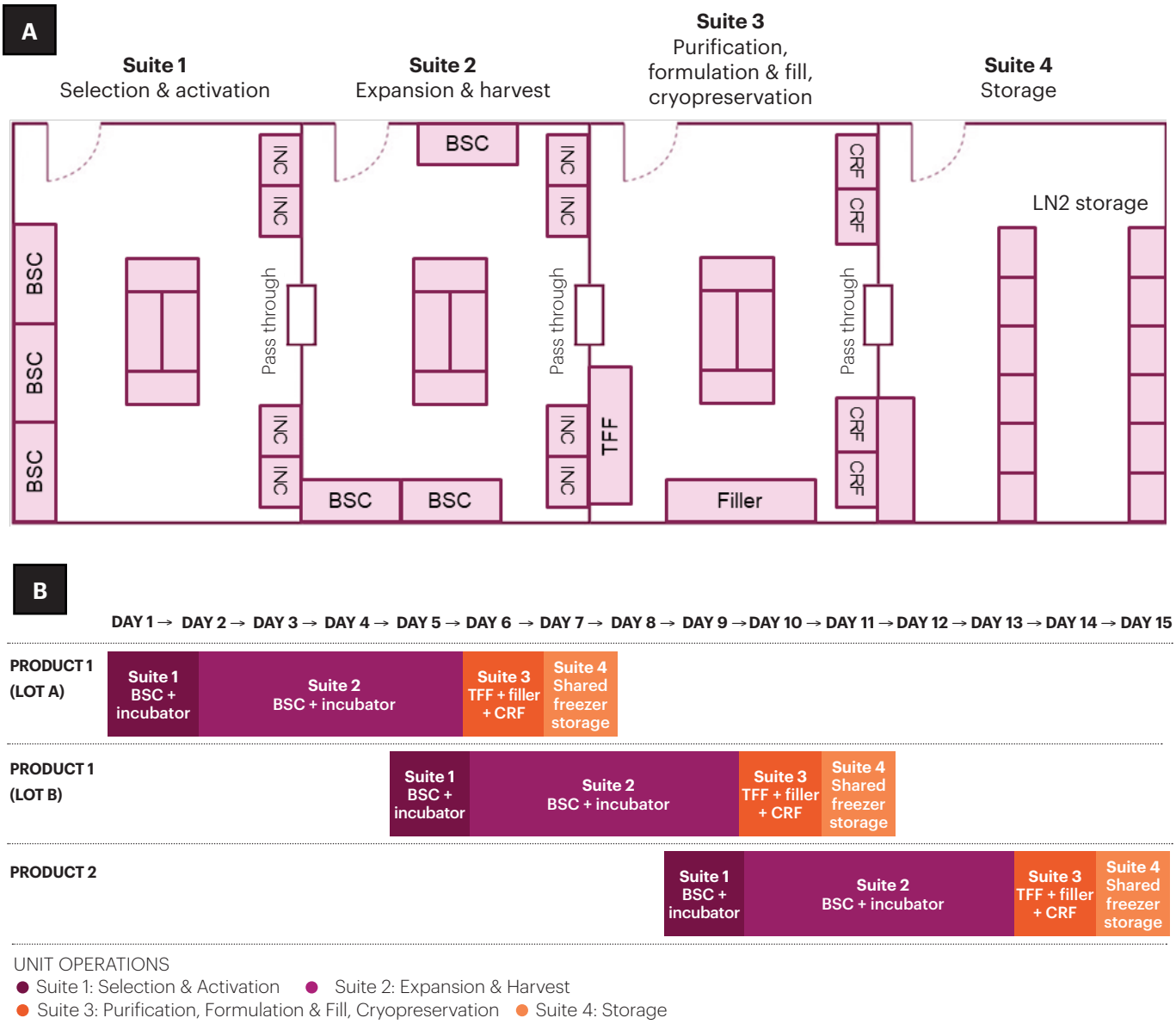
The dance-floor approach is a hybrid between the utilization of dedicated rooms to segregate different batches in a scale-out operating model and the ballroom design where segregation is achieved through closed processes and operational procedures to provide a flexible manufacturing space that lends itself to a scale-up operating model. In the dance-floor design approach, the production unit operations are divided across a number of adjacent smaller spaces. The individual rooms can be connected through the walls or passthroughs to maintain specific process segregation or by common corridors where appropriate operational procedures driven by the QRM would need to be in place to ensure segregation. The dance-floor design allows unit operations to be segregated, and can maximize equipment and space utilization by decontaminating and turning over the space for the next patient or process (Figure 11-4). This configuration allows different levels of cleanroom grades to be applied to each room depending on the process requirements (opened/closed) and allows for appropriate containment of process steps, such as when handling viral vectors.

Due to the increased level of subdivision for the production space, the dance-floor approach does not offer as much flexibility as the ballroom approach and will likely incur increased airlock requirements over a single open ballroom space due to the required transitions to and from each individual room. It may also require greater operational planning and coordination on decontamination between patient lots. Therefore, dance floors are most appropriate when processes use the same facility, equipment, and platform technologies in different locations or when only performing a single process. While the dance-floor approach has segregation advantages over the ballroom design, it does not provide the segregation of the fully dedicated processing room design to maximally implement COI and COC.

The dance-floor design could be suitable for

Figure 11-4: *Example of: (A) Dance floor facility layout, and (B) schematic of the flow of multiple product lots using a dance floor philosophy.*

Suites are arranged based on unit operations, where each suite is dedicated for specific process steps and is connected to adjacent suite by passthroughs. Product lot flows are processed in each suite after appropriate cleaning and line clearance. Note that not all manufacturing unit operations are presented in this diagram. (BSC: Biological Safety Cabinet; INC: incubator; TFF: Tangential Flow Filtration; CRF: Controlled Rate Freezer; LN2: liquid nitrogen).



allogeneic processes if the individual rooms are sized appropriately for the unit operations. The initial isolation and expansion process steps for both allogeneic and autologous processes are often a similar scale with the differences in scale and space required occurring at the later expansion, formulation, and filling process steps. Therefore, the dance-floor design can be space efficient and operationally efficient for cell-based therapy companies planning to manufacture allogeneic and autologous products.

Support rooms

Supporting areas for the processing rooms include warehousing, gowning, cold and frozen storage, personnel

Table 11-2: *Comparison of Facility Designs*

	Segregation	Process Closure	Footprint Utilization	Capital Cost	Operational Cost	Flexibility
Dedicated	Full segregation	Open (most suitable) or closed	Least efficient	High	High	Within each room. Suitable for scale-out. Autologous and allogeneic (space permitting)
Ballroom	Segregation by process closure and operational procedure	Closed (most suitable) or open processes contained by equipment	Most efficient	Low	Low/Middle	Within large room. Suitable for scale-up. Autologous and allogeneic
Dance Floor	Unit operations segregated	Open or closed – adapted by room	Moderate efficiency	Middle	Middle	Cleanroom grades can be adapted by room. Autologous and allogeneic

and material airlocks, and cleaning storage. These must be designed in conjunction with the processing rooms to ensure appropriate segregation, mitigate the risk of contamination and cross-contamination, and facilitate operationally efficient flows for people, materials, and product through the facility.

The *warehouse* should be appropriately sized for the needs of the facility and in line with the supply chain and logistics strategy. It should have appropriate systems for tracking materials and quarantine status and have procedures that minimize opportunities for mix ups, especially if the facility supports multiple products. The most common strategies are either to receive, store, test, and release materials in an area connected to the main production facility or to carry out these activities offsite and have a smaller short-term warehousing area within the production facility. For both cases, the warehousing space design should consider areas for inspection, sampling, and testing of incoming materials; quarantine, released, and rejected material and consumables storage at each required temperature; final product storage; and transportation packaging areas.

If liquid nitrogen (LN_2) is being utilized for product storage or transportation, correct ventilation and operations health and safety requirements need to be implemented. Spaces for cryostorage should be O_2 monitored and have sufficient warning and mitigation systems. Selection of flooring material is important where LN_2 is used as some flooring materials (e.g., epoxy resin flooring) can crack on contact with LN_2 spills compared to other flooring materials (e.g., polished concrete) that are more resistant to cracking.

Gowning specific activities should have their own dedicated spaces within the facility, such as locker rooms and personnel air locks (PALs). Design of these spaces should consider the quality of environment appropriate for the gowning being performed, and the number of personnel required to be able to perform gowning concurrently. These spaces should consider operational aspects such as shift changes, total personnel the facility is designed to accommodate, and the number of gowning components and steps performed. The gowning process itself is inherently associated with high numbers of particulate shedding from operators and non-processing room clothing; HVAC for gowning spaces should anticipate the number of personnel concurrently gowning to ensure gowning-generated particulates can be effectively flushed from the space to maintain the desired environment classification. If improperly sized, gowning spaces can impede the gowning procedures and ultimately bottleneck facility operations. Within the gowning rooms, storage of the gowning wear (overgowns, head covers, hair nets, shoe covers, gloves, etc.) and waste disposal of packaging and sanitizing materials should be considered

for optimal operation. People are often the most common cause of contamination, so adequate space to allow for correct gowning before handling the product is critical.

Personnel and material airlocks are utilized in facilities to transition between different classifications of cleanrooms and typically require some level of room pressure rebalancing. Transitioning through airlocks can require significant time and therefore spaces should be sized so that the movement of people and materials to the end destination meets the needs of the manufacturing program and any operational working hour constraints. Modeling software can be employed to determine the optimum balance of airlock size and personnel flow. When exiting process spaces where open operations are performed, a best practice is to have de-gowning activities occur in a separate airlock from gown-in activities. This mitigates any process materials on the operators' gowns from cross-contaminating other operators coming into the processing space. For material airlocks, sufficient space should be allowed for laydown areas to clean materials, the decontamination materials themselves, and waste disposal for spent cleaning materials and discarded packaging.

Cleaning and decontamination storage areas can be overlooked in initial facility designs but are critical to enabling the required cleaning processes. The room should be designed for adequate storage and preparation of cleaning agents and cleaning equipment as well as waste disposal. Cleaning storage rooms should be located in the facility to enable unidirectional cleaning from higher classifications to lower classifications if possible. If there are steps in the production process that are specifically segregated due to higher risk of cross-contamination, cleaning of these spaces should be evaluated and may require additional procedures or flows. The location should also consider any needs for sinks, drains, and supply of potable and compendial water, which can be a source of contamination.

Storage rooms for environmental monitoring equipment such as air samplers are also required and the locations need to be considered as part of the design.

Media preparation rooms generally require BSCs for manual open additions to media (such as cytokines and serum) and an appropriate supportive background environment. Depending on the utilization of the space, it is often used for other open-process support activities such as sterile container change and sterile tubing preparation.

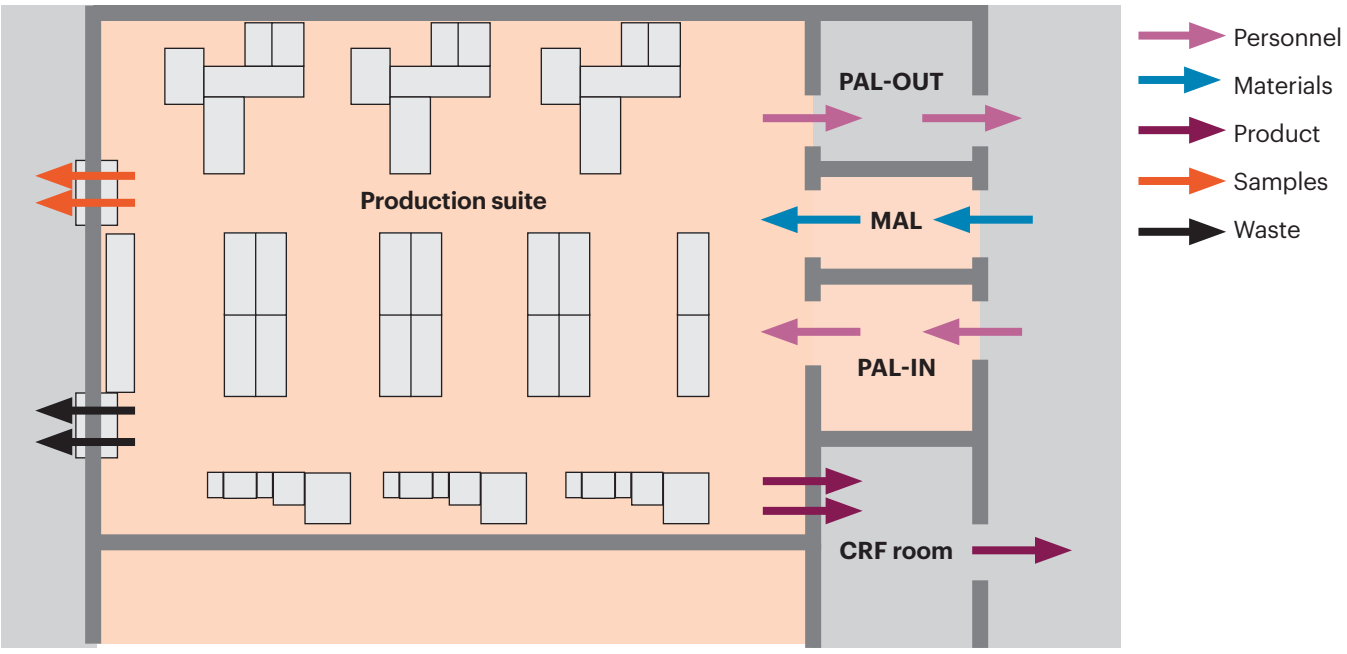
Facility personnel and material flows

The process rooms and support rooms must be designed so that personnel and materials can flow through a facility to minimize the risk of contamination. With each evolution of design layout considered through the facility design phase, flow diagrams are produced to communicate the operational intent for the manufacturing facility. Personnel flow diagrams show personnel entry into the designated controlled manufacturing area through gowning and de-gowning stages to exit from the controlled area. This includes personnel entering the facility to operate the process and to clean the facility. Material flows show entry of raw materials and consumables into the facility, through the manufacturing areas and exit route of materials as process waste, cleaning waste, or as part of the product, including flows through labelling, storage, and final packaging prior to shipping out of the facility. The flow of any intermediates and quality control (QC) samples for in-process and outsourced testing are also produced. Manufacturers of cell-based therapy products are also required to specifically show the movement of all patient cellular materials. In this way, the robustness of the design can be thoroughly interrogated and whether risks for contamination or cross-contamination have been properly identified, understood, and suitably mitigated. An example facility flow diagram is shown in Figure 11-5 below.

Unidirectional flows and facility design

To minimize the risk of contamination in cell-based therapy manufacturing facilities, the use of unidirectional flows is often employed. A unidirectional flow of personnel, raw materials, intermediates, products, equipment, and waste can minimize the risk of confusion between different products or product components, avoid the risk of cross-contamination, and minimize the potential for omission or wrong application of manufacturing or control steps. In general, in a strictly unidirectional workflow scenario, after personnel change out of street clothes and

Figure 11-5: *Example facility flow diagram showing flow of personnel, materials, product, and waste.*



don facility uniforms or scrubs in a changing room, the flow should start with a PAL for personnel to gown and prepare for entering the production space. Within the production space, appropriate restrictions should be in place to control movement between different manufacturing rooms, and entry or reentry into classified areas. Parallel processing rooms should be separated by ingoing and outgoing PALs and clean corridors should be clearly distinguished from dirty ones based on the facility flow QRM. There should be separated areas designated for receipt and storage of materials and reagents, quality testing, and manufacturing processes. The exit should be through a de-gowning room to prevent personnel from passing back into the clean gowning area after exiting. It should be noted that access to and from the main gowning room depends on many factors, but it is commonly through a single vestibule.

However, it should be noted that unidirectional flow at all times and across all aspects of the facility is space intensive, not always buildable, and not strictly required from a regulatory standpoint. Manufacturing spaces should be assessed for factors like what processes are to be performed within, the level of segregation required, the degree of process closure, and the potential implication to the operator and their external gowning through process performance. For areas where less stringent requirements may be allowed, alternative facility designs can create more efficient space usage and flows. For example, unidirectional flow may be established specifically for critical process rooms, with bi-directional flow and less stringent controls for less critical support spaces. Dedicated gown-in and gown-out PALs that connect to an open common corridor may be employed where operators are not deemed to be a source of cross contamination. If unidirectional flow is not feasible in the facility, segregation in time for movement of starting materials, product, and waste should be implemented in conjunction with appropriate risk assessment.

Equipment selection and workstation layout

The layout of equipment in each cleanroom space should be designed to enable consistent operations, mitigate the risk of cross-contamination, and optimize cleanroom space. As cell-based therapy manufacturing processes can be in early stages of development and scale when designing facilities, there should be particular focus on areas of the process that are not conducive to meeting these needs. For autologous processes that are replicated throughout facilities, exacerbation of operational inefficiencies and oversized facilities should be avoided.

Workstations are defined here as specific sets of equipment chosen and arranged to facilitate an intended manufacturing operation. This may be manual or automated processes including single or multiple unit operations. For autologous processes it is more common for workstations to include multiple unit operations for a single patient batch. For allogeneic processes, it is more common for workstations to be segregated by unit operation due to the larger scale. Depending on the complexity of the workstation, equipment can include any number of components such as biosafety cabinets (BCSs), incubators, centrifuges, microscopes, electroporators, cell separators, environmental isolators, and automated cell processors.

Equipment selection should consider compatibility with cleaning and sanitization agents, auditable data collection systems to meet data integrity guidelines, and connectivity with other equipment. Heat loads and particulate generation from equipment should also be assessed to ensure that cleanroom environments can be maintained within specification when all equipment in an area is fully operational.

Design practices for workstations

The layout and arrangement of equipment within the production space can be critically important to the overall success of the manufacturing operation and reproducible production of the process. The workflow of activities in cell-based therapy manufacturing processing rooms must be organized in a purposeful and methodical manner. The flows of personnel, materials, samples, and waste should be identified and designed to ensure that conditions that promote opportunities for cross-contamination or material mix-ups are removed.

Personnel circulation, not just at the equipment but from process step to process step as well as general circulation through the room, must be accounted for in the overall space design. As manufacturing operations have reasonably predictable durations and typically are conducted in a methodical series of process steps, temporal segregation can be leveraged to aid circulation. The design should account for areas for preparation of incoming materials and consumables, laydown areas, in-process sample transfer, and waste removal to reduce overlapping flows during operations. Waste handling and flows should be segregated from the handling and transport of any incoming raw or in-process materials to mitigate cross-contamination opportunities. Particular attention should be paid to waste liquid removal as there are often limitations on sinks or drains in the area where there are unit operations that require water (e.g., incubators).

Equipment should be arranged to provide adequate room to conduct planned operations and should account for maintenance access and cleanability. Equipment on wheels or mounted on a cleanroom cart that can be moved to provide accessibility, and connectivity to adjacent equipment, walls, or panels (process tubing, power cabling, utility connections, etc.) should be considered. Equipment located in classified cleanrooms will require routine exterior decontamination and cleaning, the level of which will depend on the environmental classification and the site procedures. Sufficient space should be given around the systems (for example, from an adjacent wall) to allow proper cleaning and decontamination procedures.

Placement of equipment within a cleanroom space can significantly impact the air flows within the cleanroom. In processing spaces where open aseptic operations are conducted (for example, within a BSC), control and protection of the airflow in the critical zone is paramount to mitigating contamination of the process material. It is critical for these spaces that the arrangement of equipment works to maintain both the critical zone airflow pattern and also the intended airflow within the supporting processing room environment. Equipment must not be in positions where they will disrupt air velocities across the face of the critical zone (for example, the sash of a BSC) or where they will impede airflow into HVAC low wall return ducts and disrupt the overall room airflow patterns. Considerations should be taken to ensure processing spaces with high loads of large equipment are able to maintain environmental conditions and airflow patterns appropriate to the required classification. Smoke studies may be used to confirm that airflow is not being impeded. Production spaces with mobile equipment or cleanroom carts should have designated locations for stowing both while in and out of operation to mitigate chances of them being in front of a low wall return and disrupting room conditions.

Specific considerations for commonly used equipment

Biological safety cabinets and incubators are commonly used critical equipment in cell-based therapy manufacturing. Specific considerations and best practices are described below for these items of equipment.

Biological safety cabinets

A biological safety cabinet (BSC) provides a cost-effective means to deliver the highest classified environment to protect open processes used in cell-based therapy manufacturing from contamination. Due to the high risk of contamination posed by open processes, the ease of use and placement of a BSC in a cleanroom is paramount.

The effectiveness of a BSC is highly dependent on its positioning within its surrounding environment as disruptions to the sash airflow can impact the critical zone environment internal to the BSC. Careful evaluation must be taken when locating a BSC within a processing space. Adjacent equipment and workspaces should be evaluated on their likeliness to disrupt and impact room air flow and quality, and therefore their potential to impact the environment internal to the BSC. Ideally, BSCs should be positioned with the sash facing away from doors, personnel circulation zones, or other equipment or spaces expected to create irregular airflow conditions (i.e., rocking or rotating equipment, incubators, other BSCs). If such an arrangement is unavoidable, an appropriate distance should be given between the sash and the opposite disturbance to allow for room driven HEPA airflow to return to a regular airflow pattern at the face of the BSC.

Incubators

Incubators maintain an optimal cell growth environment by controlling temperature, humidity, and carbon dioxide concentration. Incubators typically maintain the atmosphere to contain 5% CO_2. CO_2 can be supplied from local, stand-alone cylinders near to the incubators or from a centralized distribution system with piping runs to supply the CO_2 to the incubators across the facility. In general, it is best to have the CO_2 sources located outside the sterile area to mitigate environmental contamination risks when performing change-overs or refills. If cylinders are used to supply gas, they should be securely anchored, clearly labelled, and have the correct regulating valves attached. The tubing should be appropriate for the pressure of the gas used and securely fastened to avoid any leakage of carbon dioxide. In all cases, a 0.22-μm porosity, non-wettable filter should be used on the input gas lines. Though major deviations in CO_2 levels may occur due to door opening activities or a change in pH of the medium, CO_2 levels should be checked monthly using a calibrated CO_2 meter. To manage risk for contamination, the incubator should be emptied, dried, and cleaned with appropriate, non-corrosive disinfectant at a frequency determined by risk assessment. There are several incubator designs and consideration should be given to those that have multiple small inner doors to reduce the change in conditions in the incubator when the door is open, individual trays where material containers can be easily moved in and out of the incubator, and enclosed water reservoirs used for humidification. Risk of contamination from the humidifying water can be further controlled by adding antibacterial or antifungal agents or planned routine disinfection, though these should be assessed to ensure that the agents are validated to be nontoxic with respect to the cell-based therapy product material.

Cleanrooms and classification

Cell-based therapy products cannot be sterile filtered or terminally sterilized, and therefore must be manufactured in cleanrooms using aseptic processes. Cleanrooms are designed to mitigate the risk of contamination of products through control of the concentration of airborne particles and viable organisms that pose risks to product quality and sterility. The appropriate selection of the cleanroom environment to mitigate the risk of contamination is critical for successful manufacturing.

Regulatory authorities such as the FDA (U.S.), MHRA (U.K.), and EMA (EU) specify the standards that characterize the different tiers of cleanroom environments and are an integral part of CGMP guidelines. The CGMP standards are based on the allowable number of various sized particles, defined in size category stages from 0.1 microns to 5 microns, in a given volume of airspace within a cleanroom. They also provide guidance of the cleanroom air quality expected for different types of processes.

Different regulatory authorities have different naming terminology for the cleanroom environment tiers and there are some slight differences between the cleanroom

specification and monitoring requirements, which are summarized in Table 11-3 below. The ISO-14644-1 classification system (as defined by the International Organization for Standardization) provides a set of standards for cleanroom particle levels that may be referenced by manufacturers worldwide, thereby allowing facilities to calibrate cleanrooms using established benchmarks. For manufacturers in the U.S. market, facilities are currently designed in accordance with the ISO standard, which in 2001 replaced the previously utilized cleanroom Class standard denoted by Federal Standard 209E.

It should be noted that ISO-14644-1 uses metric units and references a standard particle diameter starting at 0.1 microns, while Federal Standard 209E, the previous cleanroom standard, used imperial units and referenced a standard particle diameter starting of 0.5 microns. The two sets of standards are often easily confused (even in manufacturer spec sheets), and even though legally cancelled, Federal Standard 209E is still widely, though erroneously, used. If Federal Standard 209E is encountered, developers should keep in mind that a Class 10,000 (Federal Standard 209E) cleanroom corresponds to the particle threshold equivalent of an ISO Class 7 (ISO-14644-1) cleanroom. Nevertheless, the ISO standard should be the reference for all new facility spaces being designed to accommodate U.S. markets.

For manufacturers operating in the EU market, cleanroom standards are defined in terms of Grades under EU GMP standards as outlined in EudraLex Volume 4, Annex 1. Cleanrooms are certified as being Grade A, B, C, or D with Grade A cleanrooms having the strictest environment (in terms of air cleanliness), and Grade D cleanrooms having the least strict environment.

Where cell-based therapy companies are seeking product licensing from different countries, it is advised to design and operate the facilities to the strictest applicable guidelines.

Cleanroom classification for open and closed processes

Open manufacturing processing steps must be performed within the highest cleanroom ISO 5/Grade A in operation environment for adequate aseptic processing conditions. If the ISO 5 critical working zone is maintained within a BSC or a restricted access barrier system (RABS), the supporting background environment must be classified ISO 7/Grade B.

A difference between the EU (EMA) and U.S. (FDA) requirements is that if the ISO 5 critical working zone is maintained within a closed system isolator, the cleanroom background environment may have a supporting background environment of Grade D with appropriate risk assessment and validation. In contrast, the FDA requires ISO 8 depending on the operation.

Regardless of process closure, the regulatory expectation is that aseptic processing steps take place within a cleanroom environment. Therefore, processes contained within either closed systems or isolators are expected to be

Table 11-3: *Comparison of EU vs. U.S. GMP Cleanroom Standards*

Cleanroom Standard				Max number of particles/m³			
U.S. (FDA)		U.K. (MHRA) / EU (EMA)		At rest ≥ 0.5 µm	At rest ≥ 5 µm	In operation ≥ 0.5 µm	In operation ≥ 5 µm
Class	**ISO**	**EU GMP At rest**	**EU GMP In operation**				
100	ISO Class 5	A/B	A	3 520	20	3 520	20
1 000	ISO Class 6						
10 000	ISO Class 7	C	B	3 520	29	3 520	2 900
100 000	ISO Class 8	D	C	352 000	2 900	3 520 000	29 000
			D	3 520 000	29 000	Not defined	Not Defined

Table 11-4: *EU vs. U.S. GMP Requirements for Microbial Contamination in Operation*

ISO Class/ Annex 1 Grade	FDA		EU GMP Annex 1	
	Active air sampling, CFU/m³	Settle plates (diameter 90 mm/ 4 hours)	Active air sampling, CFU/m³	Settle plates (diameter 90 mm/ 4 hours)
5/A	1	1	<1	<1
6	7	3	-	-
7/B[1]	10	5	10	5
8/C	100	50	100	50
-/D	-	-	200	100

[1] Annex 1 Grade B cleanroom dynamics are roughly comparable to ISO Class 7 cleanrooms for microbial contamination in operation. At rest, Annex 1 Grade B are cleaner than ISO Class 7 cleanrooms and are more comparable to ISO Class 5 cleanrooms.

supported by a minimum of an ISO 8 environment. These include processes that use closed system equipment and sterile connectors, tube welders, and sealers to connect within and between unit operations.

Cleanroom environmental monitoring

Periodic monitoring of cleanrooms is required to demonstrate control over both viable and non-viable particles found in critical areas of the operation. The cleanroom air, critical cleanroom surfaces, and personnel working in the cleanrooms should be monitored as necessary. Viable and non-viable particulates are monitored using a combination of settle plates, contact plates, and air sampling.

Settle plates are considered the standard passive air sampling method and make use of petri dishes that contain sterile growth media (Tryptic Soy Agar) and measure viable particulates in the cleanroom air. The plates are placed in designated locations in the cleanrooms for a designated time before processing in quality control laboratories and assessment for viable particulate growth, measured as colony forming units/m³ (CFU/m³).

Active air samplers draw in a defined volume of air that passes over a media plate. This plate is incubated and will reveal viable organisms and indicate the number of viable particles per cubic feet or liter. Non-viable particulates are measured using particulate counters that draw in a defined volume of air, which is analyzed in real time using laser-diode technology to count the number of non-viable particulates.

Contact plates also contain sterile growth media in a petri dish and are designed for dabbing onto critical cleanroom surfaces or areas of the operators' gowning that are near critical processes (e.g., fingers and wrists).

The locations and frequency of the sampling and monitoring are determined through risk-based analysis and through the results obtained during the cleanroom qualification. It should be noted that the requirements of the FDA and EMA differ slightly in the acceptable limits for microbial contamination for ISO 5/grade A environments (Table 11-4 and Table 11-5). The stricter requirements should be utilized if companies are licensing products in both jurisdictions.

Table 11-5: *EU GMP Recommended Limits for Microbiological Monitoring of Clean Areas During Operation*

Grade	Air sample, CFU/m³	Settle plates (diameter 90 mm), CFU/4 hours	Contact plates (diameter 55 mm), CFU/plate	Glove print (5 fingers), CFU/glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

ISO-14698 Biocontamination Control describes standards for systems to control, monitor, and evaluate biocontamination in cleanrooms. It should be noted that the ISO-14644-1 and ISO-14698 classification systems do not provide limits on viable particles, leaving action and target values for the user to set as appropriate. For manufacturers in the U.K. and EU markets, ISO-14698 has been superseded by EN 17141, which is considered to contain more detail on the areas covered by ISO-14698. EN 17141 has an emphasis on microbiological control rather than biocontamination control and describes potential sources of microbial contamination, the use of the risk assessment approach, and developing an effective environmental monitoring plan. There is also greater emphasis on data recording, trending, alert and action limits, and data integrity.

Heating, ventilation, and air conditioning (HVAC)

Cell-based therapy facilities must be designed, constructed, and used to minimize the possibility for the introduction and retention of particles inside rooms where aseptic processing is to occur to mitigate the potential for contamination. HVAC systems control airflow supply rates and airflow patterns in the cleanrooms to eliminate sub-micron airborne contamination originating from people, processes, and equipment and maintain the requirements of the cleanroom classification. The HVAC system works to control the number of particulates, microbial load, temperature, humidity, and pressure.

HVAC systems utilize air handling units (AHUs) to draw in air (outside or recirculated) that is filtered, cooled, or heated and processed to remove any excess humidity. The air is then supplied to the cleanrooms through high efficiency particulate air (HEPA) filters that are designed to retain a very high degree (typically 99.995%) of particulates. The air in the cleanroom is then returned to the HVAC system through in-room exhaust ducts. There are several HVAC design options and an initial HVAC philosophy should be developed at the concept design stage to consider factors such as the air recirculation rate, AHU zoning, and HVAC maintenance.

The air exhausted from the cleanroom area can either be recirculated and mixed with the outside air before returning to the cleanroom, or it can be a single pass system where the air is completely exhausted. Single pass air removes the risk of any process related contaminants in the cleanroom being transferred back into the cleanroom or to other cleanrooms in the same AHU zone of the facility, but is more energy intensive and therefore more costly to operate than a recirculated system as outside air must be continuously treated for temperature and humidity. Recirculated air is commonly utilized in aseptic facilities, but manufacturers must consider the risk of transferring process contaminants from a cleanroom that may not be removed by HEPA filters. Single pass air is generally considered for rooms that are processing high-risk materials (for example, viral vectors), for biological safety level (BSL) containment, or as a zoning segregation strategy (may be less expensive to single pass a small room than carry another dedicated AHU). The use of single pass air should be evaluated as part of the total cleanroom HVAC design strategy.

The location of exhaust and intake for AHUs should be considered. AHUs can be dedicated to each cleanroom in a facility or zoned where one AHU services multiple rooms. During the facility design phase, HVAC diagrams that indicate AHU zoning, room pressurization, and room classifications are developed. The design of the AHUs to serve dedicated cleanrooms or multiple cleanrooms should primarily be based on mitigating the risk of cross-contamination in the facility. The risk level varies across different facilities and depends on factors such as the total volume of air to be processed by the AHU, the number of concurrent products planned to be manufactured, the effectiveness of the gowning protocol, the specific manufacturing processes, and the equipment employed. It should be noted that when developing a comprehensive facility HVAC scheme, manufacturing cleanrooms and facility areas outside the cleanroom should never be tied in and served by the same AHU. Dedicated AHUs are usually smaller and have (at an individual level) reduced capital cost per unit compared to larger AHUs that serve multiple areas of a facility. However, more dedicated units are required, and operational costs can be greater as there are more units to test and maintain compared to a zoned design.

The HVAC system also controls the pressurization between cleanrooms and the pressurization strategy acts as another measure to maintain the cleanroom environment and reduce the risk of cross-contamination.

Pressure cascades are designed on the principle to facilitate the flow of particulates away from areas where the product and process are most prone to contamination to protect the product and maintain the required cleanroom classifications. In practice, pressurization schemes facilitate flow away from the highest classified cleanrooms and areas of high particulate generation (e.g., gowning rooms) to lower classified cleanrooms. Where there are pass-throughs between cleanrooms HEPA filtration should also be used to create "active" pass-throughs for material transfer into or between critical areas, especially if there is a change in classification between the rooms and pressure cascades applied. The CGMP regulations provide guidance for pressure differentials between different grade cleanrooms that are adjacent to each other. Pressure differentials are also utilized for health and safety purposes to facilitate the requirements of rooms with BSL designations where infectious materials or organisms may be handled.

Finally, to maximize the effectiveness of the HVAC in higher classified spaces, air flows within the cleanroom should be designed to be directional where possible, where the air flows down through the ceiling HEPAs and out of the room through low-level exhaust ducts to sweep down and out any particulates and contaminants. The positioning of equipment in the cleanrooms should be considered in combination with the locations of the low-level exhaust ducting to enable unobstructed air flow. In general, it is good practice to locate large equipment away from the exhaust ducts and not directly underneath supply ducts. Equipment that is known to generate particulates, such as tube welders, should be located in the airflow path if possible. Smoke studies are required to demonstrate adequate air flow through the cleanrooms to mitigate the risk of contamination.

In case of AHU failure, measures such as the use of redundant AHUs, fail-safe isolation valves at critical duct branching points, and a standby power supply should be in place to re-establish containment. HVACs supplying airflow to critical operating spaces, such as ISO 5 or Grade A spaces for open aseptic operations, should be powered by uninterruptible power to reduce the risk of potential process contamination in the event of site power loss.

Testing and maintenance of the HVAC system, and in particular the HEPA filters, is required on a periodic basis to ensure the continued control of cleanroom air and classification. During the design phase, manufacturers must consider the access to the HVAC system and the type of HEPA filter installation, which can be either accessed for testing and maintenance from inside the cleanroom or via walkable ceilings outside of the cleanroom. Walkable ceilings reduce the need for maintenance personnel and tools to be taken into the cleanroom area but are more costly to install. The selection of the type of HEPA filter installation should consider the disruption and risk to the operation.

OPERATIONAL DESIGN CONSIDERATIONS

In parallel with the physical layout and engineering design factors, operational aspects that enable the practical and efficient operation of a facility should be considered at the design stage. Cell-based therapy product manufacturing for autologous processes involves many separate unit operations that require manufacture of multiple patient lots from the same product line potentially within the same production space. Allogeneic products, although not patient-specific, also have multiple batches manufactured within the same production space and an increasing number of cell-based therapy developers have requirements for manufacture of both autologous and allogeneic product lines in the same facility. Regardless of the type of product, the design of the facility must always maintain conditions to ensure the safety, efficacy, and quality of products and minimization of the risk of cross contamination. Tracking and segregation strategies should be implemented with respect to factors including (but not limited to) adventitious agent contamination, viral vector contamination control, cell material COI and COC, raw materials, consumables, and specific product and process considerations. For manufacturers utilizing viral vectors in transfection or transduction steps, the degree of process closure being implemented should also be factored into the overall evaluation of the necessary level of segregation required. Key operational design considerations for segregation in multi-patient and multi-product facilities, COI and COC are discussed here. Operational gowning considerations are also discussed as personnel are integral to the manufacturing operation but present the largest risk of contamination to the product.

Gowning operations

To minimize the risk of contamination introduced by humans, personnel must adhere to well-defined gowning protocols commensurate with the requirements of the cleanroom classification. For higher classifications, such as Grade B/ISO 7, aseptic gowning is required. Aseptic gowning practice is fundamentally the process of "wrapping" personnel to isolate the body from the cleanroom environment and keep individuals from shedding particulate contaminants into the cleanroom environment (to the extent that is possible). This wrapping consists of layers of gloves, masks, and covers that are specially designed to minimize the amount of particulate that enters the cleanroom environment. Thus, specialized cleanroom garments help to ensure that the particles emitted by the bodies of personnel do not diminish the sterility of the environment.

Access to cleanrooms of higher classifications should be strictly limited to trained personnel wearing proper gowning in accordance with the facility protocol. Gowning apparel should not be worn outside of the cleanroom. The exact garment system should be selected based on specifications for the particular cleanroom applications. Often, personnel must not wear gowning apparel over street clothes, but must instead remove their street clothes and don a polyester under-suit. Gloves, facemasks, and goggles are put on after the under-suit and outer gown have been donned. A further aspect of the gowning system is the maximum length of time that a gown can be worn. In ISO 7/Grade B areas, gowns are typically worn only for the length of the operator shift, either to be discarded if single-use or submitted for laundering if reusable. In lower grade cleanrooms, the same gowning apparel might be worn by an operator for several sessions throughout the day. Generally, as individuals perspire and stress the gowning materials through manual operations, the integrity of the gown weakens.

Gowning apparel can be either reusable or single-use/disposable. Reusable gowns are usually manufactured using 100% continuous filament polyester or continuous filament polyester/carbon combination yarns and must be re-laundered as specified by a protocol. Disposable garments are non-woven and are constructed of polyolefin. All cleanroom gowning garments are designed to allow air to pass through, but retain particulates and thus, functionally act as filters. Efficacy is comparable between disposable and non-disposable gowns; the choice to use either depends largely on economic and practical considerations. Important factors to keep in mind when choosing appropriate gowning materials include thickness, weight, flexibility, filtering properties, durability, and comfort. The nature of the tasks involved in the production process, regulatory requirements, and any specific customer requirements will also impact choices.

In addition to gowning procedures in the gowning area of the facility, it must be noted that gowning procedures truly begin at home, with adequate bathing or showering, shaving, brushing of teeth, and maintenance of hair. If needed, non-silicone skin moisturizers should be applied to reduce skin flakes. Cleanroom environments are prohibitive for the use of make-up, hair gels, hair sprays, perfumes, aromatic after-shave lotions, or body lotions. While working, all employees must wash their hands with soap and water before entering the cleanroom environment. Personnel must repeat gowning procedures if re-entering after exiting (such as after eating or using the toilet).

For general reference, IEST-RP-CC003.3 contains guidelines for gowning configurations and suggested frequency for change of garments based on the requirements of the ISO 14644 classifications.

Chain of identity and chain of custody

Chain of identity (COI) and chain of custody (COC) are key records that form part of a manufacturing lot genealogy required for all pharmaceutical products to ensure patient safety and product quality as set out in regulatory guidance. COI and COC is of particular importance for cell therapies as it is critical that patients are treated with the product intended due to the presence of living cells donated from the patient themselves, in the case of autologous therapies, or from donors, in the case of allogeneic therapies. COI is a record associating a donor's unique identifiers connected with the sample being processed from order through collection, manufacturing, administration, and post-treatment monitoring. For allogeneic therapies manufactured from cell banks, the link to the donor is through the lot number for the cell bank used for manufacturing. COC is a record of data points from collection through product administration of all

actions performed, all staff involved, and the associated locations, dates, and times.

In facilities that process patient material, as operations move through the manufacturing process from workstation component to workstation component or from workstation to workstation, it is critical that all raw and starting materials, intermediates, QC samples, final products, and product- or batch-specific components and equipment are tracked accurately to prevent mix-ups or cross-contamination. This tracking should, therefore, be robust enough to establish a COI and COC that, in both dedicated and multi-product facilities, should support segregation and ease of identification between different lots. Special attention must be paid when cryogenic storage is critical to the process and timing of product delivery to patients. This includes the flow of product out of the processing area into the storage area and through shipment, as well as stored product that requires further processing in the production areas (e.g., master cell banks used in the production of working cell banks). Tracking can be paper-based or through validated electronic traceability systems (or a combination of both). For example, a validated electronic traceability program based on barcoding could be used, in which each printed label contains multiple unique identifiers (e.g., patient ID, lot #, or process #) and is reconciled as part of line clearance. Meanwhile, a COC documentation system can be used to track and document the movement, handling, and temperature of materials through every step in the manufacturing workflow and logistics chain.

Segregation

Processing areas should be segregated with respect to incoming and outgoing streams of raw materials, consumables, intermediates, final product, and QC samples. Separate areas should be in place for unloading, storage, and movement between cleanrooms. The most important segregation should, arguably, be between the route for incoming process materials and the route for outgoing process waste. There should be designated personnel for waste collection and transfer, dedicated secondary containers for material/product/waste transport through shared spaces, and a dedicated waste disposal area based on waste type and hazard level. Ultimately the manufacturing plan in terms of physical segregations, flows of personnel and materials, and any temporal segregations should be supported by the site QRM policy and justified by associated risk assessments.

All raw materials, consumables, and cellular product material should be tracked and checked before entering or exiting a controlled area. For processes where incoming cellular material is processed to isolate the relevant cells for further processing, a dedicated room for starting material isolation should be considered. If this is a required manufacturing step for an allogeneic process or for allogeneic processes that utilize cell banks, the cells may then be placed in freezers arranged or installed with appropriate controls (such as with an automated system) to ensure separation between the starting cells and any final frozen products. When ready for use in production, cellular starting material should be transferred in temperature-controlled containers into the intended processing rooms. Regardless of process closure, operations should be carefully evaluated for potential risks for contamination and cross-contamination, and the operational space should be designed to mitigate those risks. Regulatory guidance and QRM should also be referenced to determine which processes can be performed side-by-side in the same room and which processes must be conducted in segregated areas of the facility. After processing, the final cell product should be transferred out of the processing room (for example, via a pass-through) and transported to an inspection area. The status of the material should be tracked through all stages of the production process using the conceptual frameworks of COI and COC.

Multi-patient and multi-product facilities

In multi-patient facilities, stringent spatial segregation of processing areas must be implemented to minimize the potential for product mix-up or cross contamination. If any amount of parallel processing is being considered, risks for cross-contamination should be evaluated. For example, open transduction of one product generally should not be performed within the same room as open processing of another product line (it should be noted, however, that there exist ways of potentially mitigating risks, such as the use of closed processing). Overall, criteria for segregation may be based on factors such as product types, origin of starting material, BSLs, required

storage conditions, and processing materials (e.g., use of viral vectors for transduction). Procedures such as fumigation of manufacturing rooms, enhanced physical segregation within the manufacturing area, single-pass filtered air, and HEPA filtration of exhaust air may be required, especially if products involve the use of potentially infectious viral vectors. Additionally, emphasis should be placed on using, wherever possible, single-use consumables and equipment, product-dedicated equipment, and automated closed systems. Cleaning of the manufacturing area and equipment must be validated to demonstrate adequate removal of any active viral vector particles and any byproducts of patient-individualized production processes.

If the facility is intended to support manufacturing of multiple products (e.g., viral vectors and the final cell product in the case of CAR-T manufacturing), a careful assessment of cross-contamination risks and biosafety requirements should be performed, with mitigation measures integrated into the facility design. A major design factor is typically whether the facility is intending to support multiple products through temporally segregated production campaigns, or concurrent production with facility elements in place to ensure process segregation. If planning for temporal segregation in production, a robust sanitization and product turnover procedure will need to be developed to provide segregation between different products. If planning for concurrent manufacturing, a robust QRM strategy will need to be developed and documented to ensure appropriate mitigation measures are in place to protect against cross contamination and mix-up events between product materials.

Manufacturing Start-Up and Operation

Following handover of the facility after construction and facility validation, several manufacturing start-up activities are required before production of qualification batches to produce data for regulatory licensure can proceed. These include training of personnel, running process simulations, validating aseptic and cleaning processes, establishing line clearance processes to segregate batches, and establishing environmental monitoring baselines and programs. All these activities form part of the overarching contamination control strategy and contribute towards the repeatable production of compliant products.

Personnel training and monitoring

Personnel conducting manual operations present a significant risk of contamination in all production processes. Therefore, personnel training and behavior are key to the implementation of an effective contamination control strategy. Personnel training should be frequently updated as required and should consist of a curriculum that covers theoretical, practical, and CGMP aspects of basic microbiology, personal hygiene, and aseptic techniques. Records of personnel training should be maintained in a controlled document management system. Further, given that appropriate and effective gowning is essential in all facilities where aseptic techniques are required, personnel must be trained on the methods and importance of good gowning techniques. Gowning techniques must be monitored and assessed regularly through practices such as visual observation and contact plate sampling and is a requirement for Grade B/ISO 7 areas.

As specified in regulatory guidance for aseptic filling, personnel monitoring is required in facilities engaging in aseptic processing. The purpose of personnel monitoring is to ensure minimal introduction of particulate contamination into the cleanroom and to demonstrate and confirm that personnel comply with aseptic technique procedures. This monitoring is particularly important because most cleanroom contamination can be traced to humans (personnel shed large numbers of skin cells as skin flakes); monitoring is especially critical for situations where maintenance of aseptic conditions can be especially tricky, such as during planned open operations (that frequently occur during cell-based therapy product manufacturing). Ultimately, the most important outcome of personnel monitoring is to minimize the risk to processes, products, and patients. However, it is important to keep in mind that, because of a relative lack of high-precision counting methods and limited sample volumes, monitoring is a semi-quantitative exercise and cannot identify and quantify all contaminants that might be present.

The most common practice to assess the risk of contaminants introduced by personnel is to use agar contact plates to take samples from gowns and gloves worn by persons as they leave the aseptic area. Typical locations

monitored include the fingertips, the top of the head, the left and right arms, the torso, and the left and right legs. This is carried out at defined intervals as part of the training and gowning qualification for personnel entering the controlled manufacturing areas. To account for the occurrence of coughing or sneezing, it is also typical to sample the facemask. Most frequently, 55 mm plates with TDA/SCDA media are used. The plate is pressed against the sampling location for a specified time period (typically 3-5 seconds). Afterwards, the gown must be discarded due to the potential reduction of gown integrity upon contact with the plates. For Grade B/ISO Class 7 areas, the same limits should be applied to finger-sample plates as to gown-sample plates. In addition to gowning control, procedures should be established for personnel to be able to notify staff about health conditions. Staff displaying signs of illness, open lesions, shedding skin conditions, or gastric upsets should not enter cleanrooms. Appropriate job role duplication/mirroring must be in place to establish a sustained labor force for continual and uninterrupted processing.

Process simulation and aseptic process validation

For cell-based therapy manufacturing processes, contamination control risk assessment should be informed by the performance of process simulations, in which a liquid sterile microbiological nutrient medium replaces product material in the process and final filled units are incubated for the purpose of detecting microbial contamination. The process is run as closely as possible to the routine process, taking into account interventions known to occur during normal production, as well as projected worst-case contamination risk conditions. It is important that process simulations include an assessment of the worst-case processing parameters that might lead to microbial contamination events. Process simulations should be performed as part of the initial validation of a facility to establish baseline expectations for risk and should be performed routinely thereafter at intervals of a frequency determined by risk management assessment to monitor the effectiveness of contamination controls in the facility. Process simulations should also be performed after any significant modification to the HVAC system, facility, equipment, or manufacturing process.

Line clearance

Line clearance refers to the clearance of residue after a given manufacturing process step and before initiation of a new manufacturing process step. The primary purpose of line clearance is to prevent mix-ups and cross-contamination between manufacturing operations. In the context of cell-based therapy product manufacturing facilities, line clearance of workstations and manufacturing rooms is to be done whether the process change-over is batch-to-batch or product-to-product. All materials and waste associated with the previous lot should be cleared, with attention given to the particularities of the equipment and with respect to quality assurance, materials labelling, and documentation. This is particularly important in autologous cell-based therapy manufacturing settings, where each lot corresponds to an individual patient. In such cases, though multiple patient batches can be concurrently manufactured at different workstations in the same room, only one patient batch can be processed at a time in a workstation-based BSC; thus, proper line clearance must be done after each batch is processed. All aspects of line clearance should be supported by quality risk assessments and validation based on facility and cleanroom-specific microbial flora profiles. Line clearance procedures should be performed by trained manufacturing operators and independently verified.

Cleaning and disinfection

Cleaning and disinfection of equipment and areas used within the facility should be conducted according to a defined schedule. Disinfectants should be capable of destroying bacterial endospores and fungal spores and, according to common practice, at least two different disinfectants should be used in rotation. Disinfectants and detergents used for cleaning must be validated prior to use through measures such as surface challenge testing. The frequency of cleaning and disinfection should be commensurate with the manufacturing environmental requirements and production risk assessment established at the time of facility start-up and should be regularly reviewed and updated as needed. Personnel responsible for cleaning and disinfection should be trained to competently work in a manner to minimize the risk of contamination.

Additionally, protocols should also be in place for cases of viral vector or cell product spillages. Viral

vector spillages are of particular concern (especially in multi-product facilities) due to the presence of infection-competent and potentially replication-competent viral particles (which may lead to undesired genetic transduction events), and the potential for contamination of the final product lot, while product spillages (especially in the context of autologous CAR-T manufacturing) may result in inter-patient product contamination.

When closed processes are employed, leakages of process material may also occur due to compromised integrity of single-use plastic components of the product apparatuses, which pose similar risks as spillages during more conventional open operations.

Cleaning validation

In GMP facilities, all equipment and tools associated with the manufacturing process and the cleanrooms themselves must be properly cleaned, sanitized, and maintained to prevent contamination or process failures that could affect the quality, safety, or efficacy of the final product. Cleaning validation should be performed for the purpose of confirming the effectiveness of cleaning procedures employed at the facility. It should be carried out for surfaces making direct contact with drug substances and drug products and for indirect product-contact surfaces that have the potential to come in contact with surface contaminants. Single-use items such beakers, pipettes, weigh boats, silicone tubing, sample tubes, storage and bioreactor bags, and normal-flow filtration filters are typically not subject to cleaning validation. Typical process contaminants for which cleaning is necessary include media, buffers, storage solutions, cellular material from patients, and viral vector particles.

Cleaning may consist of automated, semi-automated, or manual processes although the latter are more difficult to validate given the variability of manual cleaning processes. Cleaning validation refers to establishing documented evidence that provides assurance that the specific cleaning process being employed produces consistent and reproducible results that comply with set standards. To avoid unnecessary troubleshooting and expenditures, cleaning validation should be preceded by extensive consideration of cleaning process design studies, cleaning sample assay validation, suitability of sampling, and recovery studies for assay and sampling methods.

Cleaning validation should be focused on the specific areas where cleaning is to occur, the equipment to be cleaned, the equipment to be used for cleaning, and any detergents and decontaminants to be used. It should take into account process parameters including substance concentrations, contact times, microbicidal efficacy of disinfectants, methods for removal of detergents, and standards for qualification and training of operators. Additionally, manufacturers should account for whether cleaning agents used are caustic, acidic, neutral, or oxidizing. Before conducting cleaning validation, installation qualification and operational qualification must be performed on the process components to be cleaned and on the equipment to be used for cleaning. For multi-product facilities, particular attention should be focused on any equipment that is used to process multiple products.

Common cleaning validation procedures include active residue identification, active residue detection, method selection, sampling method selection, the establishment of residue acceptance criteria, methods validation, and recovery studies. To directly demonstrate the effectiveness of a cleaning process, the processes can be challenged. Typically, a process challenge consists of at least three consecutive successful cleaning process runs, after which residues are measured and results are compared to predetermined acceptance criteria. A mock soiling procedure can also be performed. Mock soiling is the soiling of equipment by a process other than routine manufacturing that dirties the equipment similarly to routine manufacturing. Mock soiling procedures must adequately simulate normal manufacturing processes. To facilitate the conducting of validation, matrices and tables are often used as tools to ensure proper preparation and implementation of cleaning validation procedures.

Environmental monitoring program

An environmental monitoring program can be used to monitor and determine the type and level of contamination present in manufacturing facility environments. The purpose of an environmental monitoring program is to gather data that can be used to assess effectiveness of established contamination controls to identify trends that can allow for proactive interventions to prevent a contamination event from impacting product quality.

Environmental monitoring is commonly performed by exposing nutrient medium plates to sample areas in the facility that pose the highest contamination risks. The sampling locations should be selected using a documented risk-based approach that is reviewed and repeated periodically to account for any changes in the manufacturing process or the facility.

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Appendix: List of Acronyms

AHU	Air Handling Unit
AOQ	Average Outgoing Quality
BSC	Biological Safety Cabinet
CDMO	Contract Development and Manufacturing Organization
CGMP	Current Good Manufacturing Practice
COC	Chain of Custody
COI	Chain of Identity
CQV	Commissioning, Qualification, and Validation
EHS	Environmental, Health, and Safety
GMP	Good Manufacturing Practice
HVAC	Heating, Ventilation, and Air Conditioning
IQ	Installation Qualification
OQ	Operational Qualification
PAL	Personnel Air Lock
PQ	Performance Qualification
P&ID	Piping and Instrumentation Diagram
QMS	Quality Management System
QRM	Quality Risk Management
SOP	Standard Operating Procedure
URB	User Requirement Brief
URS	User Requirements Specification