CELL & GENE THERAPY INSIGHTS

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SPOTLIGHT

EXPERT INSIGHT

Addressing challenges in meeting chemistry, manufacturing and control regulatory requirements for gene therapy products

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Due to the increasing promise of cell and gene therapy products to address unmet medical needs for the treatment and cure of serious and life threatening diseases, there has been a rapid increase in the number of these products in development. Product developers are utilizing available regulatory pathways to expedite the development and approval of products including the European Medicines Agency's Priority Medicine (PRIME) Scheme, Japan's Ministry of Health, Labour and Welfare's Sakigake strategy and the US Food and Drug Administration's Regenerative Medicine Advanced Therapy (RMAT) designation. To keep pace with the accelerated development of these products, consideration must be given to addressing the unique challenges of meeting regulatory Chemistry, Manufacturing and Control (CMC) requirements. Fundamental cGMP requirements must be met and unique approaches must be taken to ensure the identity, strength, quality, purity, or potency of the product. The regulatory framework for these products is being defined as demonstrated by the European Commission's issuance of Guidelines on Good Manufacturing practice specific to Advanced Therapy Medicinal Products and the US Food and Drug Administration's recent release of draft guidance for gene therapy products. Challenges in meeting CMC regulatory requirements for cell and gene therapy products will be presented and the pursuit of innovative solutions including the development of novel viral vector manufacturing platforms, automated

and closed manufacturing systems and technology focused on the improvement of product yield will be discussed.

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REGULATORY FRAMEWORK FOR GENE THERAPY PRODUCTS

The regulatory framework for the development and manufacturing of drugs and biologics is shaped by regulations, guidance documents and the interpretation of these governing documents by regulators and product sponsors as experience is gained in practice. As a result, approaches to meeting Chemistry, Manufacturing and Control (CMC) regulatory requirements for manufacturing many types of biological products for which there are many commercialized products (e.g., monoclonal antibodies, recombinant proteins and viral vaccines) are well established. However, the pathways to meeting the CMC requirements for gene therapy products are not as clearly defined. Guidelines and guidance documents that reflect the current thinking of regulatory agencies continue to be issued by regulatory agencies at a rapid pace. This is exemplified by the Good Manufacturing Practice (GMP) specific to Advanced Therapy Medicinal Products (ATMPs) guideline adopted by the European Commission on November 22, 2017 [1]. Due to the complexity of ATMPs a separate guideline for GMPs was issued. As stated in the guideline, "ATMPs are complex products and risks may differ according to the type of product, nature/characteristics of the starting materials and level of complexity of the manufacturing process. It is also acknowledged that the finished product may entail some degree of variability due to the use of biological materials and/or complex manipulation steps (e.g., cultivation of cells, manipulations that alter the function of the cells, etc.). In addition, the manufacture and testing of autologous ATMPs (and allogeneic products in a donor-matched scenario) poses specific challenges and the strategies implemented to ensure a high level of quality must be tailored to the constraints of the manufacturing process, limited batch sizes and the inherent variability of the starting material." ATMP manufacturers were required to comply with the guideline no later than May 22, 2018. On July 11, 2018, the US FDA issued six draft guidance documents for gene therapy products [2,3]. FDA Commissioner Scott Gottlieb, M.D. issued a statement regarding the release of the draft guidance documents. Dr Gottlieb stated that, "Today, we're taking a step toward shaping this modern structure for the regulation of gene therapy. The agency is issuing a suite of six scientific guidance documents intended to serve as the building blocks of a modern, comprehensive framework for how we'll help advance the field of gene therapy while making sure new products meet the FDA's gold standard for safety and effectiveness." The European Commission and FDA guidelines reflect the current thinking of these regulatory agencies; however, experience with the manner in which these documents are interpreted and implemented in practice is limited.

Gene therapy products are defined by the US FDA as "all

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products that mediate their effects by transcription or translation of transferred genetic material or by specifically altering host (human) genetic sequences." These products include those directly delivered (*in vivo*) such as nucleic acids and genetically modified microorganisms and *ex vivo* genetically modified human cells such as chimeric antigen receptor-T cells (CAR-T). Dr Peter Marks, Director, CBER provided an example of the complexity of these products at the CASSS Cell and Gene Therapy Products Symposium (2018) by noting that a cell therapy product may contain 3.6×10^6 proteins and 1×10^{14} atoms whereas a single subunit protein product could contain only 1×10^2 atoms. Due to the complexity of these products the tenet that the "process is the product" is highly relevant for ATMPs with manufacturing as a critical element of product development. Dr Gottlieb characterized the manufacturing challenges associated with these products in his comments to the Alliance for Regenerative Medicine in May, 2018 by stating, "In contrast to traditional drug review, where 80 percent of the review is focused on the clinical portion of that process, and maybe 20 percent is focused on the product issues, I'd say that this general principle is almost completely inverted when it comes to cell and gene therapy. The initial clinical efficacy is often established early, and sometimes in small series of patients. The more challenging questions relate to product manufacturing and quality…" Although the basic Current Good Manufacturing Practices (cGMP) are a uniform set of standards for all pharmaceutical products to ensure the identity, strength (including potency), quality and purity of products, there are unique challenges in adhering to cGMP standards for gene therapy products. A significant technical challenge for gene therapy products for which cells are either used to produce a viral vector delivered *in vivo* or human cells are modified *ex vivo* is assuring the quality of raw and starting materials. In addition, establishing a control strategy based on linking critical quality attributes to the mechanism of action and clinical outcomes, without a full understanding of the mechanism of action and limited clinical experience is a major obstacle in developing a robust and reproducible manufacturing process. In addition the development of precise analytical tools to determine the dose and strength of gene therapy products is difficult.

Some of the key challenges and potential means by which to address these CMC challenges for gene therapy products will be explored in this article within the context of guiding regulatory documents. Specific information regarding the quality aspects of vectors used for the direct *in vivo* delivery of nucleic acids or for the *ex vivo* genetic modification of human cells will be provided.

QUALITY RAW MATERIALS

The US FDA summarizes the cGMP standards as "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. This formal system of controls at a pharmaceutical company, if adequately put into practice, helps to prevent instances of contamination, mix-ups, deviations, failures, and errors. This assures that drug products meet their quality standards". The use of raw materials of appropriate quality is therefore one of the pillars of cGMPs and presents some of the most significant challenges in manufacturing gene therapy products. Understanding the requirements for raw materials is complicated by the lack of global harmonization in the terminology used to describe these materials. There is also a lack of clarity in the definition of some labels used to market materials such as 'xeno-free'. In the US, raw materials include starting or source materials, components, ingredients, reagents, ancillary materials, processing aids, formulation components and excipients. Ancillary materials are defined in USP Chapter 1043 Ancillary Materials for Cell, Gene and Tissue-engineered Products [4], as materials used as processing and purification aids or agents that exert their effect on the therapeutic substance but are not intended to be present in the final product. These materials include plasma- or serum-derived products, biological extracts, antibiotics, cytokines, culture media, antibodies, polymeric matrices, separation devices, density gradient media, toxins, conditioned media supplied by 'feeder cell layers', fine chemicals, enzymes, and processing buffers. In the EU, the phrase ancillary materials is not used to describe these materials; however, quality requirements with respect to identity, purity, sterility and biological activity and absence of adventitious agents for these materials are similar to the US requirements.

Several of the key challenges encountered in sourcing and using quality raw materials to manufacture gene therapy products and means to address these challenges are shown in Table 1 [4–19]. These challenges include the use of Research Use Only (RUO) materials such as cytokines and growth factors labeled 'not for clinical use, for research purposes only', use of human and animal-derived materials (e.g., fetal bovine serum [FBS]), the biological complexity of the materials and variable lot-to-lot performance characteristics. Many materials used in the manufacturing of gene therapy products are from single-sources, which presents both logistics challenges in maintaining adequate inventory to support manufacturing demands and complexities in importing these materials if the source is in another country, especially if they are animal-derived. For example, there are a limited number of affinity resins for AAV purification, each of them single sourced, such as AVB sepharose and POROS 8/9. To avoid supply chain disruptions, ensuring that appropriate documentation required for importation of these materials is essential.

A risk-based approach should be used to assess raw materials used to manufacture gene therapy products. Approaches to assessing risk and developing material qualification programs are described in USP Chapter 1043 [4] and European Pharmacopoeia General Chapter 5.2.12 [5]. Raw materials of biological origin for the production of cell-based and gene therapy medicinal products. The risk assessment may result in the requirement to complete additional characterization tests and/

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TABLE 1

Quality raw materials.

Relevant regulatory documents	Challenges	Approaches to address challenges
USP Chapters: <1043> Ancillary Materials for Cell, Gene and Tissue Engineered Products [4] <1046> Cell and Gene Therapy Products [6] <90> Fetal Bovine Serum Quality Attributes and Functionality Tests Attributes and Function Tests [7] <89> Enzymes used as Ancillary Materials in Pharmaceutical Manufacturing [8]	▸ Unique, complex and biologically sourced materials ▸ Single source suppliers ▸ Materials may present infectious disease transmission risks	▸ Use Risk Based Approach to assess raw materials ▸ Establish a comprehensive Raw Material Qualification Program: Identification; Selection; Suitability of use; Characterization; Vendor qualification; and QA/QC data ▸ Set stringent internal specifications
USP Chapters: <92> Growth Factors and Cytokines used in Cell Therapy Manufacturing [9] <130> Protein A Quality Attributes [10]	Animal or human origin materials	▸ Use plant/chemically-synthesized alternatives to animal or human origin materials
E.P 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products [5]	▸ Impact of variation on the quality/safety of product not well understood	▸ Assess lot to lot variability impact on manufacturing process
ICH Q5A(R1) Viral Safety Evaluation of Biotechnology Products Derived from Cell lines of Human or Animal Origin [11]	▸ Lack of simple characterization tests for many materials	▸ Develop well-defined performance assays
ICH Q5B Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Proteins [12]	▸ Reliance on use of research use only and *in vitro* diagnostic use materials	▸ Source materials that are approved or licensed therapeutic products when possible
ICH Q5D Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products [13]	▸ Potential harmful effects if patients are exposed to residual amounts (e.g., adverse immune reactions)	▸ Assess raw material residual levels in the final product and validate removal
9 CFR 113.53. Requirements for ingredients of animal origin used for production of biologics [14] Guidance on the use of bovine serum in the manufacture of human biological medicinal products [15] Minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products [16] CHMP/CAT position statement on Creutzfeldt-Jakob disease and advanced therapy medicinal products [17] Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products [18] Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications [19]		

FIGURE 1

Cocoon Instrument.

or incoming functionality tests for high-risk materials as illustrated in Figure 1 and Table 2.

Lonza is building efficiencies for customers by leveraging experience gained across the Lonza network during the selection of starting materials and during process development work for many of the products that Lonza manufactures. Process development efforts include evaluating alternatives to the use of RUO and animal-derived materials, where possible. The use of licensed/authorized therapeutic products (e.g., Human Serum Albumin, Plasmalyte-A, IVIg) is preferred. Process development efforts are focused on eliminating the use of FBS in media used to cultivate cells during the manufacturing of gene therapy products, and, when FBS is used, gamma irradiated FBS from countries with low BSE risk is sourced. If novel raw material assay methods are required to assess the quality of the material, assay development is initiated as early as possible during product development. Suppliers of raw materials are qualified with an acknowledgement that the phrase 'GMP Grade' is subject to interpretation. A grade is a quality standard defined by specifications, and GMP refers to a quality system. Both aspects must be evaluated during the qualification of the supplier and a material qualification program. A material qualification program with the following elements is followed:

1. Identification:
   a. List of materials, where they are used in the process, source, intended use, quantity, concentration, alternate sources and supply format/formulation (e.g., frozen, lyophilized…)
2. Selection and suitability for use:
   a. Establish and document selection criteria:
      i. assessment of microbiological and chemical purity
      ii. identity
      iii. biological activity pertinent to manufacturing process
   b. If animal origin:
      i. country of origin
      ii. look for plant/chemically synthesized alternatives
      iii. documented chain of custody/material traceability
      iv. look at grades and processing
3. Characterization:
   a. Identity, purity, functionality, freedom from microbial and viral contamination
4. Vendor qualification:
   a. Evaluate vendor cGMP systems, testing program, processing and documentation
   b. Build good working relationships, may provide higher manufacturing standards or custom formulation services

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TABLE 2

High-risk materials [4].

Example	Typical use in cell, gene or tissue-engineered product	Qualification or risk reduction activities
FBS	Cell culture medium additive	Same as in Table 3 plus
Animal-derived (including human) extracts	Cell culture medium additive	Same as in Table 3 plus
Animal-derived polymers, scaffolds, hydrogels	Scaffolds, matrices for immobilized cellular cultivation	Verify traceability to country of origin
Purified enzymes	Process enzyme	Verify traceability to country of origin
Ascites-derived antibodies or proteins	Immunologically targeting specific cell populations for selection or removal	Assure country of origin is qualified as safe with respect to source-relevant animal diseases, including TSE
Animal or human cells used as feeder layers	Cell culture substratum or source of medium components	Assure country of origin is qualified as safe with respect to source-relevant animal diseases, including TSE
Chemical entities with known toxicities (i.e., methotrexate cholera toxin, *Staphylococcus* enterotoxins A and B, toxic shock syndrome toxin)	Selection agents used in cell culture to improve or maintain transgene expression, enhance cellular proliferation, improve cell survival upon cryopreservation, superantigens for the activation of T cells	Adventitious agent testing for animal source-relevant viruses

c. How does vendor prevent cross contamination?
d. Change notification procedures

5. QA/QC – qualification program monitored by quality unit

In addition, importation of materials is a carefully managed process requiring coordination between the project team, supply chain, quality assurance and regulatory affairs.

STARTING MATERIALS

For the majority of gene therapy products (*ex vivo* and *in vivo*), the genetic material is introduced using viral vectors, with less than 20% of the market using plasmids, mRNA, liposomes or bacteria [20]. In addition to the raw materials used in the manufacturing processes for gene therapy products described in the preceding section, critical starting materials typically include Master and Working Cell Banks, Master and Working Virus Banks or plasmids DNA, mRNA constructs, etc. The principles of cGMP practices should be applied during the preparation of cell banks used to produce plasmid DNA. At Lonza, Master and Working Cell and Viral Banks are produced according to cGMP requirements and are extensively characterized. For example, Lonza has produced a platform of well-characterized cell lines that have been used in the cGMP manufacturing of AAV vectors. Both our Sf9 and HEK93 suspension cell banks were developed via single cell cloning, and each passage was documented for

lineage traceability. The cell banks were characterized according to the ICH Guidance on Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products [13], Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications, February 2010 [19], FDA Points to Consider in the Characterization of Cell Lines used to Produce Biologicals (1993) [21] and ICH Guidance on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin [11]. Tests include sterility, mycoplasma, adventitious agents, 9 CFR testing for porcine viruses, determination of cellular morphology and evaluation of growth characteristics [14].

For *ex vivo* genetically modified human cells, there are two potential sources of human cells, allogeneic and autologous. Allogeneic products are derived from healthy donors wherein one donor is typically the starting material for product used to treat a number of patients (e.g., iPSC-derived products). Autologous products are derived from the cells obtained from a single patient, modified and returned to the same patient (e.g., CAR-T products). There are some challenges that are unique to these two sources of human cells. For example, there is variability of starting material for autologous products based on the donor health status of the patient. The ability to meet product specifications (e.g., targeted cell count) can be impacted based on the cell count of the targeted cell population in the starting material and the ability to select/expand the population of interest.

Starting materials used to manufacture allogeneic products have rigorous quality criteria to ensure quality and safety of the final products. The quality and safety standards to ensure the use of safe blood, tissues and cells for starting material for cell therapy products are described in regulations such as 21 CFR part 1271 [22], European Commission Directive 2004/23/EC [23] (complemented by several implementing Commission Directives that specified technical requirements) and US FDA Guidance documents for Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) [24] and Good Tissue Practices [25]. Table 3 [22–29] includes a list of several of the relevant documents, challenges in meeting the requirements and associated strategies to address the challenges.

Lonza works with qualified tissue recovery agencies and licensed tissue establishments that follow applicable regional regulatory requirements to procure starting materials from eligible donors. Donor eligibility criteria are established based on regulatory requirements, plus the specific requirements of the product and current donor screening and testing requirements and recommendations. These requirements change based on the risk of transmission of emerging infectious disease agents through human cells and tissues. In addition, Lonza's manufacturing facilities obtain tissue bank/establishment licenses as required by local and regional authorities and maintains a bone marrow donor program accredited by the American Association of Tissue Banks (AATB). During the process development, appropriate tissue

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TABLE 3

Quality and safety standards for tissue and cell starting materials.

Relevant regulatory documents	Challenges	Approaches to address challenges
21 CFR Part 1271. Human Cells, Tissues, and Cellular and Tissue-Based products [22]	▸ Tissue registration requirements	▸ Assess local and regional requirements and obtain required licenses, registrations and accreditations
FDA Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) [24]	▸ Import/export regulations	▸ Ensure documentation is aligned with requirements to avoid delays during shipment/importation
FDA Guidance for Industry: Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) [25]	▸ Cell and tissue procurement process vary between tissue recovery agencies	▸ Establish Quality Agreements with tissue recovery agencies and conduct periodic audits
EC Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells [23]	▸ Donor eligibility requirements	▸ Develop a donor screening and testing strategy based on monitoring current regional risks and requirements/guidance (e.g., EU restrictions on donation where there is a history of malignant or unknown etiology disease) and specific requirements for the product (e.g., HLA matched) ▸ Use accredited/certified testing laboratories (e.g., CLIA, CE marked testing kits)
EC Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells [26]		
Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells [27]	▸ Record retention ▸ Traceability	▸ Comply with regional requirements (e.g., 10 years for donor records for the US FDA and 30 years for the EMA) ▸ Establish robust traceability system while maintaining donor confidentiality and comply to regional requirements (e.g., Single European Code)
Commission Directive (EU) 2015/565 of 8 April 2015 amending Directive 2006/86/EC as regards certain technical requirements for the coding of human tissues and cells [28]		
Commission Directive 2012/39/EU amending Directive 2006/17/EC as regards certain technical requirements for the testing of human tissues and cells [29]	▸ Quarantine	▸ Develop robust controls for quarantine procedures (storage, shipping, labeling)

receiving conditions including packaging and shipping conditions, temperature, tissue receiving and processing time limits are established to build robust and reproducible GMP manufacturing processes. Donor tissues and cells are transferred from the tissue recovery agency or tissue establishment into a manufacturing facility where the ATMP manufacturing and testing is controlled by a cGMP quality system. Cell banks for allogeneic products are manufactured, tested and released for further manufacturing according to cGMPs and established procedures.

MANUFACTURING STRATEGY

Although some parallels can be drawn between the manufacturing of materials (e.g., viral vectors, plasmid DNA, etc.) for gene therapy products and the manufacturing of other biotechnology products such as mAbs, there are unique challenges for producing these products. For example, there are multiple virus types used for *in vivo* and *ex vivo* gene therapy, each with their own production cell type and process. For a given virus type there may be multiple cell types and processes that can be used for manufacturing. For example, AAV can be produced in HEK293 cells (suspension or adherent) via transient transfection, using plasmids to supply the required AAV genes and gene of interest. Alternatively virus helpers can be used to supply the necessary AAV components, such as the baculovirus/Sf9 system, the HSV/BHK system or the Ad5/HeLa system. Most processes come out of academia and have to be optimized to be suitable for GMP manufacturing. The requirements for process characterization and process validation to establish a robust process control strategy while maintaining cGMP compliance for manufacturing of gene therapy products are the same as those for manufacturing other biological products. However, the complexity of some products, potential cell phenotypic and other changes during the manufacturing process, and limited knowledge of the mechanism of action of some products provide obstacles to following a traditional pathway towards development of a commercial process. Additionally, accelerated pathways to approval are putting pressure on manufacturing timelines and process design. A robust control strategy is established based on identifying Critical Quality Attributes (CQAs) that are linked to clinical outcomes. Meeting acceptance criteria for CQAs is then based on control of process parameters that have an impact on CQAs (Critical Process Parameters [CPPs]). As the field evolves, key and critical quality attributes will be further understood and will be informed by outcomes of clinical studies. CQAs are not as well established for many gene therapy products as they are for typical biologics, and the link to clinical outcomes is often not known. In addition, there is starting material variability for those products that include human cells in the final product, which further complicates the development of a robust manufacturing process. Several of these challenges, means to address them and the associated regulatory framework are summarized in Table 4 [1–3,30–32].

The manufacturing processes for gene therapy products can change during the course of product

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TABLE 4

Process development and manufacturing strategy.

Relevant regulatory documents	Challenges	Approaches to address challenges
EC The Rules Governing Medicinal Products in the European Union Volume 4 Good Manufacturing Practice: Guidelines on Good Manufacturing Practice specific to Advanced [1] Therapy Medicinal Products	▸ Rapid clinical development pathway requires an acceleration of process development and validation activities ▸ Incomplete understanding of the mechanism of action for some products ▸ Limited data available for use linking clinical outcomes to critical quality attributes and process control parameters	▸ Use a quality Target Product Profile (qTPP) to guide process development and characterization ▸ Initiate process characterization activities early in process development ▸ Consider scalability early in process development ▸ Leverage available mechanisms to interact with regulatory agencies to obtain advice on manufacturing and testing issues (e.g., INTERACT, Pre-IND meetings, scientific advice)
Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications [30]	▸ Starting material variability impacts on process performance and product variability (autologous products)	▸ Use pooled, healthy donor material for process validation and consider a hybrid approach of using healthy donor/patient material
	▸ Product yield	▸ Use improved cell lines and media to increase productivity to enhance yields ▸ Develop suspension cell culture processes
Draft Guidance for Industry Chemistry Manufacturing and Control (CMC) Information for Human Gene Therapy Investigation New Drug Applications (INDs) [2]	▸ Robust analytical tools needed to support product characterization, release and comparability testing	▸ Align assay development activities to the qTPP and confirm assay performance early in product development
Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications) [31]	▸ Development and validation of potency assays that are linked to clinical outcomes ▸ Comparability	▸ Initiate evaluation of candidate potency assays early in product development ▸ Develop comparability strategy early in process development and obtain regulatory authority concurrence on the proposed approach
Draft Guidance For Industry Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up [3]	▸ Aseptic processing ▸ Segregation requirements for multi-product facilities	▸ Develop closed and automated processes ▸ Conduct appropriately designed aseptic process simulation studies ▸ Appropriate facility design and a robust contamination prevention control strategy based on engineering and procedural controls
FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products [32]	▸ Chain of custody requirements ▸ Short shelf life and limited volumes for sampling impacts release testing strategy	▸ Use validated patient and product traceability platforms ▸ Develop a conditional release strategy for short shelf life products

FIGURE 2

Nucleofector LV unit.

development. A change in manufacturing sites occurred during the development of the commercial products Kymriah, Yescarta and Luxturna [33]. Manufacturing processes used for the production of early clinical trial material are often developed in an academic setting and/or are at small or pilot scale. Early-phase processes that utilize cell lines typically rely on adherent cell culture methods and the use of FBS in the manufacturing process. Processes that employ downstream processing steps may include unit operations such as clarification via centrifugation, purification via gradient centrifugation and/or manual filling. To address the scalability and process robustness challenges associated with these processes, Lonza has developed a transient transfection production process for AAV using suspension HEK293 cells. The process is suitable for scaling up in single use bioreactors and has been demonstrated multiple times at 50L scale in Hylcone SUB [34]. Process consistency is attained in bioreactor-based processes by continuous monitoring of process parameters such as dissolved oxygen, pH, etc. All unit operations are kept as closed as possible with reliance on single-use tubing with connections made by sterile welders to the extent possible.

When establishing a commercial process there are typically changes made, which can include scale-up or scale-out (depending on the application) to meet commercial product demands and transfer of the process to different manufacturing facilities. As an example, Novartis made changes to the manufacturing process initially developed by the University of Pennsylvania for the manufacturing of Kymriah® (tisagenlecleucel). Novartis took a 'life-cycle' approach to improving the manufacturing process, which included addressing identified process risks (e.g., closing open process steps). The principles in ICH Q5E Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process [35] should be followed when designing an approach to demonstrating product comparability following manufacturing process changes. The strength of a comparability exercise will be based on the analytical tools used to assess the product. Robust analytical methods that are qualified and/or validated early in process development must be developed to support product comparability testing.

Lonza has established phase-appropriate process development and technology transfer procedures and includes comparability as an integral part of the product development life cycle. For each product, a toolbox of analytical methods is developed that is used to assess multiple batches of products before and after manufacturing changes are made. During process development/process characterization studies, critical control points in the process are identified. In process control monitoring strategies are implemented to ensure process robustness and reproducibility. Manufacturing processes are assessed side by side and when possible; split samples are used in a comparability assessment.

For manufacturing processes for gene therapy products that rely on manual processing steps with open manipulations, consideration must be given to the facility design, cleanroom layout, equipment qualification, chain of custody procedures,

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operator training and environmental monitoring as part of developing a robust contamination control strategy. For products that cannot be terminally sterilized, such as products containing human cells or viral vectors that have a diameter greater than 0.22um, adherence to aseptic processing guidelines is essential (e.g., Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice and Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice [36], Appendix A PICS PI007-6, Section 2.3.8 [37]; EU GMPs for ATMPs Section 9.5.2) [1]. The design of Aseptic Process Simulations used to qualify the aseptic portion of manufacturing processes is complicated due to the complexity and length of many of the manufacturing processes for these products. Lonza designs product-specific Aseptic Process Simulations using a risk-based approach. All processing steps that could lead to product exposure are simulated while the time for portions of the process that do not present risk of product exposure (e.g., incubation) is reduced.

Improvements in process robustness and contamination control can be realized using closed and automated manufacturing systems. The Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products [1] has a section dedicated to the requirements for these systems that provides specific recommendations. Implementing automated, closed system unit operations (e.g., computer-controlled bioreactors) is one of the key innovative strategies undertaken during process development at Lonza to minimize open manual processing and establish scalable manufacturing processes. In addition, Lonza is developing manufacturing equipment for use in automated and closed cGMP manufacturing of agene therapy products including the Cocoon™ and the 4D-Nucleofector LV™ (LV). The Cocoon™ is an automated cell processing and expansion instrument that significantly reduces the need for complex manual cell handling procedures.

There are three primary elements that operate together to form the Cocoon™ System:

1. Instrument: provides the process control activities and maintains the required operational environment

2. Software: operates the automated functions of the Cocoon™ instrument

3. Cassette: accommodates the biological process through a 'closed' series of interlinked single use components, which manipulate the cell population to provide the desired number and characteristics specific for the clinical use. Cassette design is tailored for manufacturing processes for *ex vivo* genetically modified cells.

The 4D-Nucleofector LV™ (LV) **(Figure 2)** is Lonza's proprietary gene editing enabling platform used to introduce new genetic material or peptides to cells. The LV can be used for closed unit operation in the manufacturing of gene therapy products and has the potential to be integrated into a fully automated and closed manufacturing platform such as the Cocoon™.

Lonza is using these tools to develop robust gene therapy manufacturing processes for customers that meet regulatory requirements.

Parallels have been drawn between the state of gene therapy manufacturing and testing maturity to that of the monoclonal antibody field 20 years ago. However, with a rapidly changing regulatory framework, the engagement of regulators and numerous companies worldwide dedicated to the development of these promising products, rapid changes are anticipated. It is noteworthy that there is significant sharing of knowledge occurring in this sector in the industry. Meetings such as those held by The American Society of Gene & Cell Therapy (ASGCT), the European Society of Gene & Cell Therapy (ESGCT), CASSS and the Parenteral Drug Association (PDA) and the efforts of organizations such as the Alliance for Regenerative Medicine (ARM) enable the open exchange of information between product developers and regulators. There is an acceleration in developing manufacturing strategies that will ensure that safe and effective products can be manufactured to meet commercial demands in a cost-effective manner. Lonza's approaches to sourcing quality raw and starting materials used in gene therapy manufacturing processes are based on conformance to applicable regulatory requirements. The use of quality raw and starting materials and the development of scalable and robust processes are pillars in Lonza's support of our customer's journeys from concept to commercialization for these promising products.

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The authors are employess of Lonza. No writing assistance was utilized in the production of this manuscript.

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37. Appendix Aiv PICS PI007-6, Section 2.3.8v; EU GMPs for ATMPsvi Section 9.5.2

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