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Key chemistry, manufacturing, and controls strategies for the development of cell and gene therapies

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Cell and gene therapies (CGTs) continue to address unmet medical needs and improve the standard of care. More than 2,500 drug development sponsors are involved in the CGT landscape with 1,894 clinical trials[1] and 4,047 therapies in development worldwide.[2]

While the investment in these structurally complex, diverse and personalized treatments is promising, drug development sponsors must contend with an integral development constraint: Chemistry, Manufacturing and Controls (CMC). This article provides an overview of how CMC differs for CGTs and discusses the complexities and challenges during development and some potential strategies to overcome these complications.

Comparing conventional therapies with CGTs

At a high level, conventional therapies focus on a broad spectrum of disease pathways and symptoms. CGTs can offer highly targeted approaches by addressing the root causes of diseases at the cellular or genetic level with the potential for long-term or permanent therapeutic effects. Table 1 summarizes the comparison of conventional therapies with CGTs.

Table 1: Comparing conventional therapies with CGTs

Parameter	Conventional therapy	Cell and gene therapy
Mechanism of action	**Uses small molecules, peptides or proteins** Treatment mimics or disrupts processes associated with a condition or disease.	**Uses DNA, RNA, cells or viral vectors** Reprograms the body to reverse or fight the disease, a personalized approach to therapy.
Treatment paradigm	**Chronic therapy** Treatments are in the form of a tablet, injection or infusion and taken on a long-term, repeated dosing basis.	**One-time treatment** Generally, these are single treatment (potentially "one and done") regimens.
Disease management	**Manage or treat symptoms long-term** Relieve the signs and symptoms of disease; treatment effect usually stops once the medication is stopped; broad biomarker strategies.	**Potentially curative** Transformative therapy, halt progress or alleviate underlying cause of disease; complex; treatment- or patient-specific biomarker.
Logistics	**Standard logistics** Typically shipped with other clinical trial samples in ambient packaging.	**Complex logistics** Requires coordinated operations with advanced planning, complex manufacturing, adherence to storage and shipping conditions, stringent chain of identity and chain of custody.
Development timelines	**Standard clinical trial timelines** Phase-approach for research and development; well-defined regulatory environment with clinical development process driven by therapeutic area.	**Rapid timelines** Accelerated approval timelines, complex development process, maturing regulatory landscape and specialty clinical delivery/operational needs.

Understanding cell and gene therapy categories

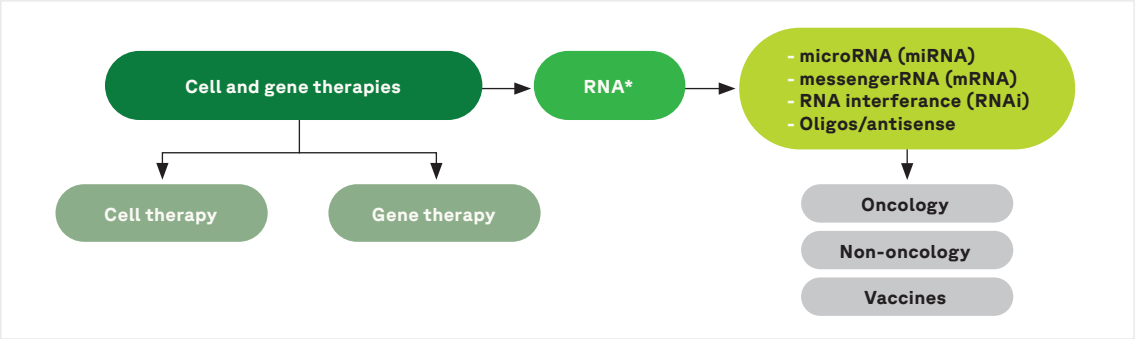
A cell therapy is any living cell administered to a patient; these cells are expected to differentiate into another cell type for therapeutic effect (e.g., stem cells, induced pluripotent stem cells), including cells that are genetically modified for a therapeutic effect.

A gene therapy is an investigational therapeutic approach that aims to add, delete, or correct genetic material to treat a disease through one of the following methods:

- Gene replacement or silencing
- Expression of a therapeutic gene
- Gene editing

The CGT categories are summarized in Figure 1.

Figure 1: Cell and gene therapy categories

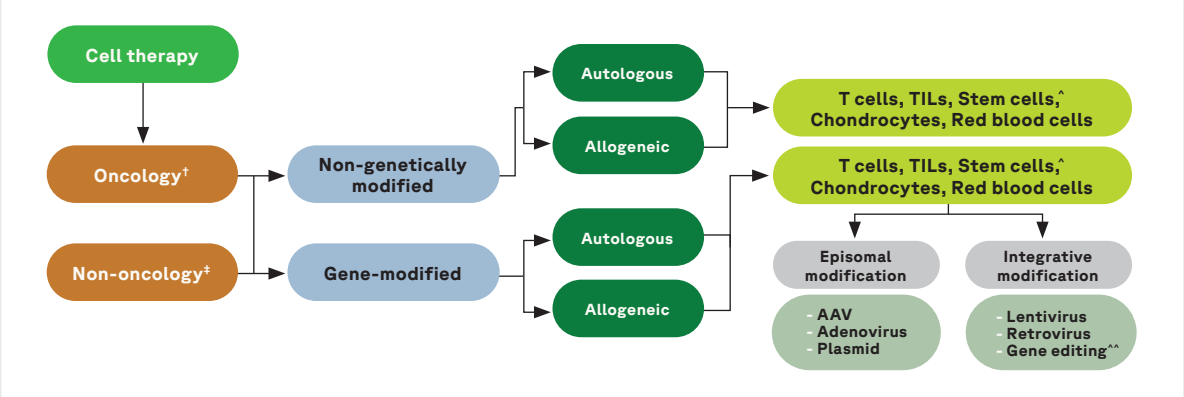


*It is important to note that most RNA-based therapies (and some DNA vaccines) are not classified as gene therapies by the U.S. FDA or as advanced therapy medicinal products by the European Medicines Agency.

Cell therapy modalities

The cell therapies are broadly classified under oncology and non-oncology applications. A summary of these modalities is presented in Figure 2. These can be stem cell therapies (regenerative medicines) that promote the repair response of diseased, dysfunctional or injured tissue, or immune cell therapy (adoptive cell therapy) using the cells of the immune system to eliminate cancer and other indications, e.g., macrophage, dendritic, T or natural killer cells. Because chimeric antigen receptors (CARs) are currently the most commonly used immuno-oncology cell therapy with several approved products, they will be used as an exemplar in later discussions.

Figure 2: Cell therapy modalities

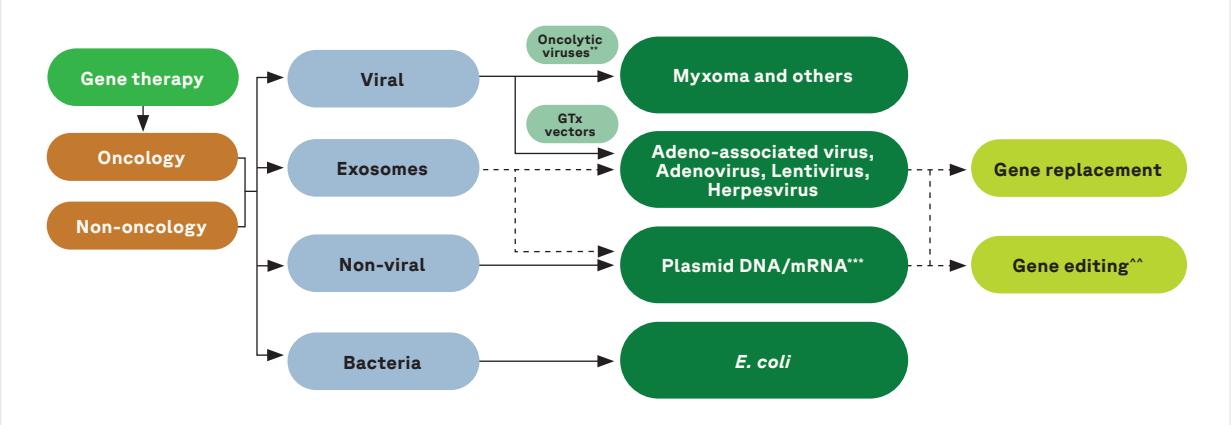


† Inclusive of immunotherapies for oncology targets
‡ Inclusive of immunotherapies for non-oncology targets (e.g., autoimmune diseases)
^ Stem cells include hematological stem cell, mesenchymal stem cell, neural stem cell, induce pluripotent cell, and embryonic stem cell-derived cells, adult stem cells, etc.
^^ Gene editing technologies include, but are not limited to, CRISPR, TALENS, zinc finger, transposons and meganucleases
TILs = tumor-infiltrating lymphocytes

Gene therapy modalities (vectors)

A summary of the gene therapy modalities is presented in Figure 3. A number of different modalities are in development as gene delivery vehicles with viral vectors currently the most commonly utilized. Because adeno-associated virus (AAV) is the most commonly used vector in gene therapy applications with several approved products, it is used as an exemplar in later discussions.

Figure 3: Gene therapy modalities



** Oncolytic viruses that are not modified are classified as gene therapies by the U.S. FDA
*** mRNA-carrying, e.g., CRISPR elements, would be classified as a gene therapy
^^ Gene editing technologies include but are not limited to, CRISPR, TALENS, zinc finger, transposons, meganucleases and vesicular stomatitis virus

Manufacturing CAR T-cells

At a high level, Good Manufacturing Practice (GMP) for a typical clinical scale autologous CAR T-cell therapy[4] is presented in Table 2.

Table 2: GMP of a typical clinical scale autologous CAR T-cell therapy

Leukapheresis	This includes the isolation of human peripheral blood mononuclear cells (PBMCs) in an accredited apheresis unit.
Elutriation	It is a process performed on the PBMCs to reduce unwanted contaminating cells, e.g., red blood cells.
Cell separation	This includes selection of specific T cell types within the PBMCs.
Transfection or transduction	The cells are genetically modified using a gene delivery method (e.g., a viral vector) to express the CAR.
Expansion	The cell population is then expanded in the presence of cytokines to a dose suitable for administration into patients.
Formulation	The cells are stored in a cryopreservation medium containing a cryoprotectant to reduce intracellular ice formation and osmotic stress during freezing, e.g., dimethyl sulfoxide.
Release testing, shipment and thawing	The process involves quality control (including stability testing) and quality assurance lot release; and shipment of the cells to treatment locations, where the product is thawed and used.

Manufacturing a gene therapy product

AAV-based gene therapy production follows a process that can require a few weeks, depending on the scale of the product and specific requirements of the gene therapy product as well as regulatory requirements.[5,6] The most commonly used manufacturing method involves several steps: plasmid preparation and construction; cell transfection; viral production; harvesting; purification; concentration and formulation; quality control testing; storage; and distribution.

Recognizing CMC complexities and challenges during development

Compared to conventional therapies, the CMC process is more complex for cell therapies, which must account for the challenge of working with living cells while the modalities (vectors) of gene therapy involve modified viruses or non-viral vectors for gene replacement or editing.

Cell therapy production challenges

The key challenges in developing a cell therapy product are summarized in Table 3.

Table 3: Cell therapy production challenges

Challenge	Explanation
Cell type	Phenotype, desired cell population, genetic modification, in vitro cell expansion and subsequent patient infusion (e.g., CAR T-cells).
Cell sourcing	Matching compatibility and consideration of potential immunosuppression (e.g., allogeneic therapies).
Manufacturing process	Heterologous population and small batch size, no filtration or final sterilization.
Potency assay	Mechanism of action often not fully known (e.g., unmodified cells), assay duration versus shelf-life.
Stability	To develop assays that predict stability over extended periods when assessing living cells.
New analytical requirements	Single-cell analysis and next-generation sequencing.
Limited, precious material	Working with the patient's own cells/product, analysts face pressure to perform accurate processing the first time.
Reference/control material (e.g., CAR T-cells)	The patient's T cells are the therapy and healthy donors/cells should be representative of patient material.
Scheduling and resources	Sponsors need to consider the vein-to-vein time. Speed is the key, from 5 to 10 days following receipt of Certificates of Analysis.

Gene therapy production challenges

The common challenges in developing a gene therapy product are presented in Table 4.

Table 4: Gene therapy production challenges

Challenge	Explanation
Raw, starting and ancillary materials	- Variability can affect the safety, purity and potency of products - Qualification of cell banks
Manufacturing procedure	- Selection of a suitable vector (e.g., consider potential immune responses) - Cell culture characteristics (e.g., adherent vs suspension cells) - Cell lines for vector manufacturing and scale up - Potential variability during production (e.g., viral vectors) - Capacity for hold steps - Technology transfer - Scalability of processes to meet clinical/commercial dosing requirements
Quality control	- Definition of the drug substance, the drug product and their characterization - Sensitivity and nature of analytical methods (e.g., potency assay: matrix approach; not correlated to expected clinical response) - Product and process-related impurities - Consistency between batches and justification of specifications
Comparability	- Changes in the materials, manufacturing processes or analytical methods
Reference materials	- Amount and availability of reference materials
Stability	- Product reconstitution and stability
Delivery device	- Compatibility with container and closure system and delivery device
Logistics and timelines	- Complex supply chain - Rapid development timelines

Strategies for overcoming cell and gene therapy development challenges

Some of the CMC strategies for overcoming CGT development challenges are presented in Table 5.

Table 5: Strategies for overcoming CGT development challenges

Challenge	Explanation
Raw and starting materials	- Use of the highest or clinical grade materials (e.g., fetal bovine serum, growth factors, digestive enzymes, other non-animal derived products) - Establish a qualification strategy: safety analysis (e.g., endotoxin), functional analysis and purity testing - Identify critical material attributes
Cell sourcing	- **Autologous cells** (patient) • Establish acceptable population doubling time, cell counts and their viability • Ensure validated transportation method • Achieve desired vein-to-vein time by selecting manufacturing and clinical sites in close proximity - **Allogeneic cells** (healthy volunteer) • Follow donor eligibility requirements (e.g., safety testing in Clinical Laboratory Improvement Amendments labs) • Create a traceability plan
Manufacturing process	- Ensure a scalable GMP vector manufacturing while maintaining quality - For host cell transfection, establish stable transfected DNA integrated into the genome - Optimal culture conditions are required for high yield (e.g., viral vector) - Adopt closed and automated processes and incorporate single-use disposables - Identify in-process controls (IPCs), critical process parameters (CPPs) and critical quality attributes (CQAs) - Optimal and scalable downstream process is required for consistency
Analytics reliability	- The control strategy for characterization, stability and batch release should be established throughout the development; the acceptance criteria can be refined as the development progresses - Any manufacturing process changes should be supported by comparability studies to confirm that product quality and safety is not affected - Use statistical techniques for data analysis
Other considerations	- CMC development should be aligned with clinical development - Risk-based justifications or approach - Timely interactions with regulators is immensely helpful - Noticing where the guidance does not fit - Consider implementing a commercial (or near-commercial) manufacturing process early in product development to reduce the need for process changes and further analytics (e.g., comparability studies)

Analytical methods

The control strategy for characterization, stability and batch release should be established throughout the manufacturing process. This can be done by the selection of CPPs, IPCs and CQAs. The acceptance criteria can be refined as the development progresses.

"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."

- Scott Gottlieb, Former U.S. FDA Commissioner

Analytical strategy: CQAs for cell and gene therapy products

Some of the common CQAs for CGTs are presented in Table 6.

Table 6: CQAs for cell and gene therapy products

Question	CQA	Description	AAV vectors	CAR T-cell
How much do you have?	Quantity	Quantity and titer	- Genomic titer (ddPCR) - Capsid titer (ELISA)	- Vector titer (flow and qPCR)* - Viability (flow/ViCell)** - Cell count and VCN (Flow and ddPCR)**
How potent is it?	Potency	Biological activity, transduction and potency	- Cell-based assay with ELISA or other functional assay endpoint; - Infectious titer (cell-based qPCR endpoint)	- PBMC transduction* - Target binding, tumor cell killing, inflammatory cytokine secretion (IFNγ, TNFα, IL-2), proliferation and transduction efficiency - CAR expression (flow)**
How safe is it?	Safety	Safety testing	- Safety testing - rcAAV	- Safety testing* - RCR/RCL* - Sterility, mycoplasma and endotoxin**
How pure is it?	Purity	Purity and impurities	- HCPs and HCDNA, plasmid DNA (ELISA and qPCR) - BSA, benzonase (ELISA) - Capsid purity (CE, MS) - Empty vs. full (AUC, TEM)	- HCP and HCDNA (ELISA and qPCR)* - RCR/RCL* - Vector (qPCR)** - Beads (Microscopy)**
How to identify it?	Identity	Identification	Sequencing, mass spectroscopy	- Sequencing* - qPCR**

*Retroviral/lentiviral vector **Active substance/drug product; AUC = analytical ultracentrifuge; AAV = adeno-associated virus; BSA = bovine serum albumin; CAR T = chimeric antigen receptor-T; CE = capillary electrophoresis; CQA = critical quality attribute; ddPCR = droplet digital polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; HCP = host cell protein; HCDNA = host cell DNA; MS = mass spectroscopy; PBMC = peripheral blood mononuclear cells; qPCR = quantitative polymerase chain reaction; rcAAV = replication-competent AAV; RCR = replication competent retrovirus; RCL = replication competent lentiviruses; TEM = transmission electron microscopy; VCN = vector copy number

Following a risk-based approach

Agency guidelines can support CMC efforts for CGTs. In particular, the European Medicines Agency document "ICH Q14 Analytical procedure development - Scientific guideline"[3] can be viewed as an industry differentiator. The latest guidance emphasizes development work prior to validation and the use of concepts like quality-by-design. The guidance focuses on creating an analytical target profile outlining requirements before development to enhance method understanding and robustness, define the appropriate performance criteria and transfer/design/development and validation, which are closely interrelated.

The guidance also focuses on robustness testing prior to validation to include stability-indicating parameters. It is important to drive harmonization across multiple laboratories in this process. A central analytical lab can derisk the analytical package as assays are performed within a single lab with phase-appropriate method development and validation is expected along with eliminating the movement of analytical tests.

Conclusion

CGTs address unmet medical needs for diseases in which the standard of care is insufficient. However, as technology and methodologies are improving, regulatory agencies' expectations are shifting and increasing. Chemistry, manufacturing and control of CGTs represent a key constraint in the industry. CMC strategies should be developed while keeping the end goal in mind, knowing that "one size does not fit all."

A prudent CMC strategy must meet regulatory expectations and provide in-depth knowledge of the product. Sponsors can rely on guidance documents to provide a framework, where the current development focus is highly scientific. Early interactions with regulators are key to understanding agency expectations and proactively addressing the unique challenges faced in clinical development with these highly technical and extremely specialized products.

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