WHITE PAPER

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# Lessons in Cell and Gene Therapies

## A primer for understanding these remarkable therapies and their impacts in clinical trials.


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## Executive Summary

Cell and gene therapies (CGTs) are a sophisticated and cutting-edge field of therapies. They are considered highly promising, and drug developers' level of interest in these emerging modalities has significantly increased over the past few years with CGTs making up a considerable part of many drug development portfolios. The complexity of these biological products requires an advanced level of understanding in how to successfully advance preclinical and clinical development.

This white paper examines:

- The growth and increasing interest in CGTs
- Preclinical and clinical trial design guidance and planning with an understanding of high impact factors
- How to navigate the regulatory landscape, become familiar with critical guidance and evolving regulations and the importance of early engagement with regulatory agencies
- Considerations for chemistry, manufacturing, and controls (CMC) activities with a focus on the complex and critical nature of potency and purity assays for CGTs

## Understanding These Complex and Innovative Therapies

Cell and gene therapies (CGTs), known in the EU as Advanced Therapy Medicinal Products (ATMPs), are widely considered a ground-breaking field of innovative therapies. While these therapies are viewed to have great promise, they are a complex category of biological products requiring sophisticated preclinical and clinical development. Understanding the unique developmental needs is critical for success. Drug developers can benefit from working with a partner that is well-versed in integrated drug development processes and services, and end-to-end experience, from preclinical to post-approval phases.

Given the relatively new emergence of these therapies, industry analysis shows most CGTs are in the research and preclinical space with the most clinical trials in Phase I/II (early phase). The proportion of late phase trials (Phase III/IV) continues to grow. The total number of CGTs has steadily increased globally. There are currently over 3,600 CGTs in the development pipeline, from the preclinical to pre-registration phases, with 55 percent of these being gene therapies, 23 percent RNA therapies and 22 percent non-genetically modified cells.[1] Oncology and rare disease continue to be the top CGT indications with 98 percent of CAR-T cell therapies being indicated for oncology indications. As of Q2 2022, there are 19 gene therapies, including genetically modified cell therapies, and 18 RNA therapies approved for clinical use, closing the gap on non-genetically modified cell therapies, of which 59 are approved. A complete listing of approved gene and RNA therapies can be found in the Q2 2022 Pharma Intelligence Quarterly Data Report.[2] Ex vivo genetic modification still dominates in vivo genetic modifications and CAR-T still dominates the cell therapy space. The CGT pipeline is rapidly driving partnership models to achieve the expertise needed for successful end-to-end development.

## Clinical Planning and Development for Complex Clinical Trials

The U.S. Food and Drug Administration (FDA) and other regulatory agencies offer guidance for early-phase clinical trial design, providing rationale for how and why clinical trial designs for CGT products often differ from other types of pharmaceutical products. It's important that drug developers have the expertise to properly navigate the complex landscape of CGTs, from early development planning to post-marketing follow-up.

Due to the complexity of these therapies, there are numerous variables that must be evaluated. By demonstrating that genetic modifications introduced are stable within the targeted cells, early phase trials can show proof-of-concept pertaining to genetic engineering, even in cases when they fail to achieve a clear therapeutic benefit. To fully assess safety and durability, long-term follow-up studies can last anywhere from 5-15 years while evaluation of therapeutic effects may last a lifetime.

An early trial of a therapy for X-ALD was a particularly important milestone in the genetically engineered cell therapy space. It showed that lentivirus vectors could be used to transfect hematopoietic stem cells with sustained expression of the healthy ABCD-1 gene and be efficacious in treating an otherwise fatal disease of the central nervous system.

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***"This is important. And in hemophilia, for instance, key stakeholders such as patients and physicians will need to be convinced of durability of constitutive [protein] expression, as well as long-term safety."***

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Currently, there are three key viral vectors used in the development of CGTs including adenoviruses, adeno-associated viruses (AAV), and lentiviruses with nonviral vectors such as lipid nanoparticles gaining significant traction. Each have unique mechanisms, applications and development considerations that need to be addressed in study design and endpoint analysis. Leveraging partners with expertise across the broad spectrum of CGT products, therapeutic indications and the global regulatory space can provide substantial benefits in getting drugs to market.

## The Developing and Growing Regulatory Landscape

The FDA, European Medicines Agency (EMA), International Council for Harmonisation (ICH) and other regional regulatory agencies provide guidance covering validation of traditional pharmacokinetic (PK) and immunogenicity methods.[3,4,5,6,7] Our scientists have led and contributed to numerous white papers in collaboration with key opinion leaders across industry and health authority representatives to gain alignment on expectations in testing and reporting. [8,9,10,11] CGTs have unique nuances that are not yet fully addressed due to the diverse range of investigational gene therapy products and associated biological complexity. The FDA encourages a flexible, data-driven approach based on the novel features of these products. Several white papers are currently followed for best practices when validating assays for a CGT product, particularly when utilizing a platform such as PCR or flow cytometry or assessing exposure which are not covered in current guidance. [12,13,14,15,16] Other factors that result in the need for alternative methods of validation include the lack of reference material. CGT analytical methods frequently measure the downstream product of the therapeutic administered, not the product itself as seen with a traditional monoclonal antibody therapeutic, for example. Conversely, viral vectors do have reference material, which enables more traditional immunogenicity assay development and validation, although often with the added caveat of a significant prevalence of pre-existing antibodies that require novel approaches for both cut point determination and reporting.[17,18] Exposure assessment can be determined by vector copy number and viral shedding and analyzed by PCR methods.

Chromatography and ligand binding assays (LBA) are long-standing and mature technologies routinely used in the bioanalytical space. However, experts in flow cytometry and molecular genomics are required to address the needs of new CGT modalities. The industry needs more time to achieve the level of applied scientific and compliance experience that currently exists in the bioanalytical community.

## Clinical Trial Design Guidance for CGT Products

The FDA first issued industry guidance for human somatic cell therapy and gene therapy in 1998, to replace the 1991 "Points to Consider" document and provide information for production, testing, and administration of recombinant vectors for gene therapy as well as preclinical testing for vectors and cell therapies.

Several guidance documents have since been released, including the 2015 "Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products," focusing on design of Phase I and some Phase 2 CGT clinical trials. The unique characteristics of CGT products, significant morbidity and mortality risks identified with early CGT product trials, the invasive nature of some administration routes, introduction of prolonged biological activity, and enhanced potential for immunogenicity resulted in a need for CGT trials to be designed differently from other products.

Given the rapidly evolving landscape for CGT, sponsors should engage in early and extensive dialogue with regulatory agencies to discuss the following:

- Preclinical studies and proposed clinical study design
- Overall bioanalytical strategy, including the number and applications of biomarkers and proposed endpoints
- Disease-specific clinical response assessment needs and plans for long-term patient follow-up
- Assessment of potential for expedited development (orphan drugs, breakthrough therapies)

A recently updated list of the FDA's Cellular & Gene Therapy Guidances includes indication-specific guidance and draft guidance for CAR-T cell products and human genome editing.

Discussions regarding planning and compliance can significantly impact the development plan. Because there is no one-size-fits-all approach, working with a partner that has strategic, global regulatory and operational expertise can help you properly navigate the regulatory and planning process.

## Recommendations and Guidance for Complex Product Critical Quality Attributes

The FDA, EMA and other national regulatory agencies have also provided guidance for the development, manufacture and evaluation of gene therapy medicinal products (GTMPs). These guidelines ensure appropriate good manufacturing practice (GMP) systems are in place to satisfy the mandates for safety, identity, strength/potency, purity and consistency (all critical quality attributes) of pharmaceutical products.

As more products progress to clinical trials and agencies and the industry gain experience in developing GTMPs, initial, updated draft, and final guidance documents are being issued that reflect the evolving perspectives and expectations agencies have for evaluating these products. However, some areas remain where regulations on specific methodologies are not defined, technologies are considered less than ideal, or where recent developments have added some potential challenges to the products' characterization. The specific areas include methods directed at the quality attributes associated with establishing strength/potency, purity and safety of GTMP products, and especially viral vector-based products.

### 1. Strength/potency

Strength/potency is of paramount importance, not only because these are required under the Code of Federal Regulations (21CFR sections 600.3(s) and 210.3(b)(16)), but because gene therapy vectors are directed at therapeutic, prophylactic, or diagnostic applications and function by inserting or modifying genes in target cells and tissues. If this results in too much expression, there could be significant risks to patient health from possible toxicities. If this results in too little expression, the treatment could fail to offer the intended health benefit.

There is also potential risk both from how it is delivered and what happens to the genetic material after it enters the cells. Further, potential host responses to delivery might significantly impact safety, efficacy and durability of the intended disease treatment.

For typical protein-based biotherapeutic entities, determination of product potency is already complex. There are multiple indicators of product potency, including:

- Physical titer – representing the number of copies of the gene of interest (GOI) within a quantity of the product
- Infectious titer – establishing the concentration of those genomes that can gain entry to the cell and replicate
- Gene expression – demonstration that the gene not only enters the cell, but can direct cellular expression of that genetic material in a way that can be measured and is dose-dependent
- Functional potency – the expression product of the gene is shown to have the GOI's targeted activity

There are also multiple ways of measuring each indicator of potency. FDA guidance acknowledges that "the complexity of CGT products can present significant challenge(s) to establishing potency assays." The guidance goes on to say that meeting all the requirements may not be possible in early phase clinical investigations and that a single test cannot adequately measure the product attributes that predict clinical efficacy. However, data must be available and evidence substantial "to assure the identity, quality, purity and strength … during all phases of clinical study."

Regulatory agencies strongly encourage development and application of expression and functional methods while products are in development to support the investigational new drug (IND) applications and recommend discussions with the appropriate agency as potency measurements are designed, evaluated, and validated.

### 2. Purity and safety of GTMP products

Viral vector products, and recombinant adeno-associated viral (rAAV) vectors in particular, may be a heterogeneous mixture of empty capsids (do not contain DNA), un-infectious particles (contain DNA, but DNA amplification in-vitro is not observed), and infectious particles (enters the cell and transgene expression/DNA amplification is observed in-vitro). Particles that do not result in expression/amplification are considered product-related impurities that can impact product immunogenicity, and need to be quantified.

Production conditions and purification processes can dramatically impact the levels of these impurities. But the regulatory perspective of these particles as contaminants suggests attempts should be made to at least reduce, if not eliminate, non-transgene expressing particles.

Residual nucleic acid material from production cells and/or plasmids/helper viruses present within or external to capsids or other delivery vehicles is also a concern. Both the size and quantity of these contaminants pose risks of either unintended transfer of a gene with functional expression capabilities or genetic material capable of integrating with the chromosomes of recipient cells and altering cell function. Such occurrences could result in adverse events in patients.

Guidance requires selection of cell lines and helper sequences to reduce risk and product-related impurities "be identified and their

levels quantified" and that process-related complexed nucleic acids "be addressed with respect to their impact on safety and performance of the complex when administered to the patients." The FDA recommends testing for such impurities, optimization of manufacturing processes "to reduce non-vector DNA contamination" and to "monitor and control the amount of extraneous nucleic acid sequences."

While the extent of the identification of the impurities required by agencies is still evolving, there are growing indications that agencies have an expectation to at least assess the quantity and the size distribution of non-transgene nucleic acid fragments contained within the product.

**3. Viral vector-based products**

Another safety expectation is for agencies to assess viral vectored gene therapies for the ability to replicate within cells. The FDA has issued specific guidance on these methods for lentiviral vectors, but this expectation applies to all viral vectored products and extends to products that are generated using insect cell/virus platforms. It is a requirement for drug substance lot release and for retro- or lentiviral GTMPs, extends into follow-up assessment in patients. It is unclear whether second- and third-generation vectors designed to prevent spurious recombination and potential vector variant replication can sufficiently demonstrate their ability to eliminate risk and the need for such testing.

While purification processes for standard biological products are validated for their general ability to remove potential adventitious virus particles, viral vectored GTMPs can't do so. GTMPs are generally very complex products that have already shown their potential to dramatically change existing treatment modalities and provide options for rare and genetic diseases that did not previously exist. Like the evolution of regulatory expectations for what are now considered standard biotherapeutic products and the building upon lessons learned from those products, GTMP regulations will also continue to evolve.

That evolution will depend upon safety profiles established from the long-term follow-up of already approved products, responses to issues identified from subsequent authorizations and the results of experimentation reported by developers in their efforts toward characterizing these products for potency, purity and safety. The best recommendation for being aware of these changes is for GTMP drug developers to start communications with regulatory agencies early in the process and to meet regularly to ensure their development plans remain aligned with agency expectations.

## Conclusion

Cell and gene therapy is an exciting and evolving field. Cell and gene therapies continue to show great promise, with trials showing clear clinical successes. As more products progress to clinical trials, and agencies and the industry gain development experience, regulatory organizations are issuing guidance documents that reflect the evolving perspectives and expectations agencies have for evaluating these products. As biopharmaceutical and biotechnology companies expand their capabilities, they should look for a laboratory partner with decades of CGT experience that is equally invested in their success.

PPD® Laboratory services, bioanalytical and GMP labs have supported more than 100 cell and gene therapies spanning the diverse array of therapeutic constructs. Our experts enable biotech and pharmaceutical companies to advance drug development across all areas including manufacturing, laboratory services, early phase to post-approval clinical execution, product development, regulatory strategy, and market access and value assessment.

To learn more about how PPD's laboratory services can enhance your CGT projects, visit https://www.ppd.com/our-solutions/ppd-laboratories/.

## About PPD® Laboratory Services

PPD®, part of Thermo Fisher Scientific, has more than 33,000 employees with 97 offices in 46 countries with over 5,400 employees in PPD® Laboratory services, supporting all aspects of drug development including laboratory services, market access and value assessment, product development and regulatory strategy and early phase to post-approval clinical execution. Since 2016, PPD® Laboratory services have supported more than 60 cell and gene therapies.

PPD® Laboratory services have supported cell and gene therapies for over 20 years with experts in ligand binding assays, activity assays, cell-based assays, chromatography, mass spectrometry, PCR, ELISpot, next-generation sequencing, sanger sequencing, genomic arrays and flow cytometry. Our labs also support extractable/leachable studies for products with unique features and materials associated with production and patient applications associated with CGT products. Our dedicated teams of scientists support CGT programs in our bioanalytical, GMP and central labs, and we are continually expanding our capacity and capabilities to meet the needs of our growing client base and their CGT products. To learn more about PPD's laboratory experience, visit our labs page.

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**For more information:**