6 Gene Therapy and Viral Vectors: An Overview on Current Trends

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Introduction

The field of regenerative medicine focuses on the development of curative processes or therapeutics, with the end products replacing diseased components or restoring normal function. Advances in our basic understanding of the molecular underpinnings of normal physiology and disease pathophysiology, combined with advances in technology, have paved the way for 22 approved cell and gene therapy (CGT) products developed by academic and industry sponsors.[1] According to the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORT), funding for regenerative medicine-enabling research (**Table 6-1**) has increased over the years.[2]

Gene therapy remains an active field of research, making significant strides with 114 Phase 1, 237 Phase 2, and 72 Phase 3 gene therapy-based clinical trials in 2020.[3] However, the future of gene therapy hinges on the success and safety of the methods by which potentially life-changing technologies are introduced to patients. To this end, this chapter will focus on gene therapy products, with an emphasis on the selection of vectors as a method to deliver genetic material to patients and the regulatory considerations associated with such choices.

Gene Therapy

Gene therapy products intended for human use are regulated by the US Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER). Within CBER, the Office of Tissues and Advanced Therapies (OTAT), previously known as the Office of Cellular, Tissue, and Gene Therapies (OCTGT), evaluates these products. Gene therapy products include[4,5] but are not limited to:

- Non-viral vector-based therapies
- Viral vector-based therapies
- Patient-derived gene-modified cellular products, such as chimeric antigen receptor (CAR) T-cells
- Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas-mediated gene editing
- Oncolytic viruses
- Bacterial vector-based therapies

These gene therapy products encompass the two primary delivery methods to date: *ex vivo* and *in vivo*. With an *ex vivo* delivery strategy, cells are harvested from a patient or donor and subsequently modified or re-engineered (typically by viral vector transduction) and then administered to the patient. In contrast, an *in vivo* delivery system introduces gene therapy products, such

Table 6-1. NIH Funding of Research Areas and Disease Conditions

Research Area/Disease Condition	2008 Funding[a]	2020 Funding[a]	2022 Funding[a,b]
Biotechnology	$5,179	$7,767	$7,912
Cancer Genomics	-	$1,098	$1,152
Gene Therapy	$249	$403	$423
Immunotherapy	-	$1,769	$1,789
Precision Medicine	-	$2,078	$2,167
Regenerative Medicine	$723	$1,126	$1,191
Stem Cell[c]	$1,176	$2,555	$2,707

[a]Funding data for the fiscal year 2008, 2020, and 2022 as dollars in millions.
[b]Estimated funding value for 2022.
[c]Funding for pluripotent stem cell research including embryonic human and non-human sources.
Note: "-" indicates a new category. Funding information is not available.
Source: National Institutes of Health

as viral vectors expressing a therapeutic gene of interest, into the patient directly.[6]

Typical considerations for the vector of choice include the targeted indication and its pathogenesis, identity, and characteristics of the target cells, size of the genetic cargo, and desired duration of gene expression required (sustained or long term). However, insights gleaned from the rapid advancements in the field bear additional concerns, including immunogenicity, cytotoxicity, and genotoxicity, as well as the high cost of manufacturing these gene therapy products at a sufficient scale to meet demand.

Non-Viral Vectors

In gene therapy, non-viral vectors include naked plasmid DNA and chemical-based systems, such as lipids, polymers, and inorganic particles used to introduce the genetic materials inside the cells.[7] These vectors are typically less costly to manufacture, are unencumbered by the size of the nucleic acid to be delivered and may have a better genotoxicity safety profile compared to their viral vector counterparts due to lack of integration into the host genomic DNA. However, a primary issue with the use of non-viral vectors is their low transfection efficiency, which consequently leads to low gene expression. Efforts to circumvent this inefficiency include more efficient delivery methods, such as electroporation, wherein an electrical pulse temporarily destabilizes the cell membrane to increase uptake of the non-viral vector, or through the use of lipid nanoparticle-based technologies.[8] In 2018, FDA approved an infusion treatment based on a lipid nanoparticle delivery system.[9]

Bacterial vectors also are non-viral delivery systems largely used in cancer gene therapy. Attenuated bacterial species, such as Listeria, Escherichia coli, Clostridium, or Salmonella have been used. Bactofection, which is an approach that uses bacteria to deliver therapeutic genes of interest to target cells, has been used successfully in preclinical mouse models of disease. However, despite the promising efficacy of bacterial vectors in the killing of cancer cells in *in vitro* and in *in vivo* animal models, this approach has fallen short in human studies.[10,11]

Viral Vectors

The use of viruses in the delivery of therapeutic genes of interest into target cells, arguably, have been the most commonly used gene therapy approach. Viral vectors regulated by OTAT include[12] but are not limited to:

- Adenovirus
- Adeno-associated virus (AAV)
- Herpes simplex virus
- Lentivirus
- Retrovirus
- Pox virus

Briefly described below are the three most commonly used viral vectors for gene therapy—adenoviruses, AAVs, and lentiviruses.

Adenovirus

Adenoviruses are double-stranded DNA viruses, ~80–100 nm in size and non-enveloped, with an icosahedral capsid structure.[13] Information derived from preclinical and clinical experience indicates that adenoviral vectors have a benign safety profile, with key advantages for their use in gene therapy, such as their ability to infect both dividing and non-dividing cells as well as lack of integration into the host genome.[14] Compared to other common viral vector-based systems, 50% of clinical trials use adenoviruses, while 28% and 22% of clinical trials employ AAVs and lentiviruses, respectively.[15]

While adenoviral vectors have been regarded as an efficient delivery system, a key limitation in their use lies in the transient expression of transgenes and high immunogenicity. Therefore, in order to achieve sustained efficacy, repeat administrations of adenoviral-based gene therapy products may be required, leading to the induction of a strong immune response. Efforts to re-engineer adenoviral vectors to address these limitations and introduce improvements, such as increased capacity, remain an active area of reasearch.[16]

Adeno-Associated Virus (AAV)

AAVs are ~26 nm, single-stranded and non-enveloped viruses with an icosahedral capsid structure.[17] Seminal research in the 1960s exploring their biology has allowed for the continued evolution of their design as vectors in gene therapy, leading to the first application of recombinant AAV (rAAV) in the early 1990s and the first EMA-approved rAAV gene therapy in 2012. **Note**: The first FDA-approved rAAV product, Luxturna, did not receive licensure until 2017.[18] Compared to their wildtype counterpart, rAAVs lack AAV coding sequences. This feature increases the packaging capacity of rAAVs and mitigates some immunogenicity and cytotoxicity *in vivo*. However, key limitations to the use of AAVs as gene therapy vectors remain, including their inability to accommodate >5.0 kb and the incomplete knowledge of the long-term durability of expression.[19] A major challenge also lies in implementing efficient large-scale manufacturing methods to produce these vectors as well as the high cost associated with them.

Lentivirus

Like adenoviruses and AAV, a continued understanding of the biology of lentiviruses has paved the way for their increased use in gene therapy. Lentiviruses are a subtype of retroviruses, which are ~80–120 nm, single-stranded RNA, spherical, and enveloped viruses.[20] As vectors, lentiviruses integrate into the genome, allowing long-term expression of large (up to 9 kb) transgenes and are capable of transducing both proliferating and non-proliferating cells. However, a consequence of long-term gene expression through integration into the host genome is the risk of malignant transformation, which mandates close long-term follow-up of patients who receive lentiviral-based therapies. While continued modifications to lentiviral vectors are underway to achieve better safety profiles, specificity, expression, and transduction efficiency, the process by which lentiviral vectors are manufactured remains a major hurdle. Good manufacturing practice (GMP)-compliant large-scale manufacturing of lentiviral vectors require complex production processes and robust purification methods to generate sufficient vector quantities to meet clinical demand.[21]

Regulatory Considerations

Preclinical Assessment

In November 2013, CBER provided recommendations to sponsors on the required preclinical information to support clinical trials for CGT products under an investigational new drug (IND) application and biologics license application (BLA).[22] Adequate pharmacology and toxicology studies, conducted *in vitro* and in *in vivo* animal models, are needed to ensure sufficient information is available on the safety profile of the investigational gene therapy product prior to its administration to humans. Given the inherent complexity of the products themselves, the innovative processes by which they

are manufactured, and the evolving nature of the field, using the traditional approaches developed for small molecules and devices, for example, may not be appropriate in the evaluation of CGT products. Therefore, early engagement and communication between sponsors and FDA are encouraged. Products that meet the criteria as a regenerative medicine advanced therapy (RMAT) may qualify for expedited development and review by CBER.[23]

Data from preclinical studies are needed to not only support the safety of the CGT product, but also to inform various aspects of the clinical protocol, including dose, dosing regimen, route of administration, inclusion/exclusion criteria, and safety monitoring. Key suggestions or recommendations for the design of these critical studies are briefly outlined below:[24]

- Due to inherent lot-to-lot variations, it is important that the specific lot of the product be well characterized. Therefore, if possible, the lot of CGT product to be administered in humans should be the same as that used in the *in vitro* and *in vivo* preclinical studies in animals, with any differences discussed in the IND. Please note that considerations for the large-scale manufacture of viral vector-based gene therapy products include producing sufficient quantities to be used in key preclinical studies as well as the planned clinical studies.
- To derive pertinent information that can guide the design of the clinical study, appropriate animal species should be used in preclinical studies. Test species that are considered "non-standard" (e.g., transgenic or large animals) may be used if an adequate scientifically based justification is provided. Specific animal models of disease or injury also may be used in preclinical studies to evaluate the mechanism of action, efficacy, and safety of CGT products, as well as inform the safety monitoring criteria in clinical trials.
- Non-GLP proof-of-concept or pilot studies may be conducted prior to definitive preclinical studies. However, a comprehensive safety assessment should be conducted in preclinical, GLP toxicology studies. Key design features of such pivotal studies include the proposed clinical indication; appropriate animal species selected; adequate number of animals per gender for each group; evaluation of safety endpoints, including mortality, body weights and histopathology; and appropriate time points or study duration. Findings from these studies can provide a no observed adverse effect level (NOAEL) to inform the selection of the planned starting dose and dose-escalation schedule.

In addition to the suggestions or recommendations above, specific considerations for gene therapy products include:[25]

- Safety concerns arising from the *ex vivo* and *in vivo* administration of the investigational GT product need to be addressed. Study designs should include evaluations of toxicities due to the route of administration, any immune response towards the vector, and mutagenic/oncogenic potential, to name a few.
- The impact of the conditioning regimen and expression profile of the transgene also should be assessed as persistent expression may lead to 1) overexpression, 2) accumulation, or 3) abnormal immune response. **Note**: Robust and sensitive quantitative methods, such as RT-PCR, should be used to determine transgene expression.
- Characterization of the biodistribution profile of the vector after administration *in vivo* is critical in determining its fate in target and non-target tissues or fluids. The vector's persistence and clearance profile as well as any histopathology findings can inform the dosing schedule and safety monitoring.

Overall, considerations for the preclinical assessment of CGT products for use in humans require a systematic and comprehensive approach to adequately determine their efficacy and safety profile. To the extent possible, the study design of preclinical studies should mimic the design of the clinical trial.

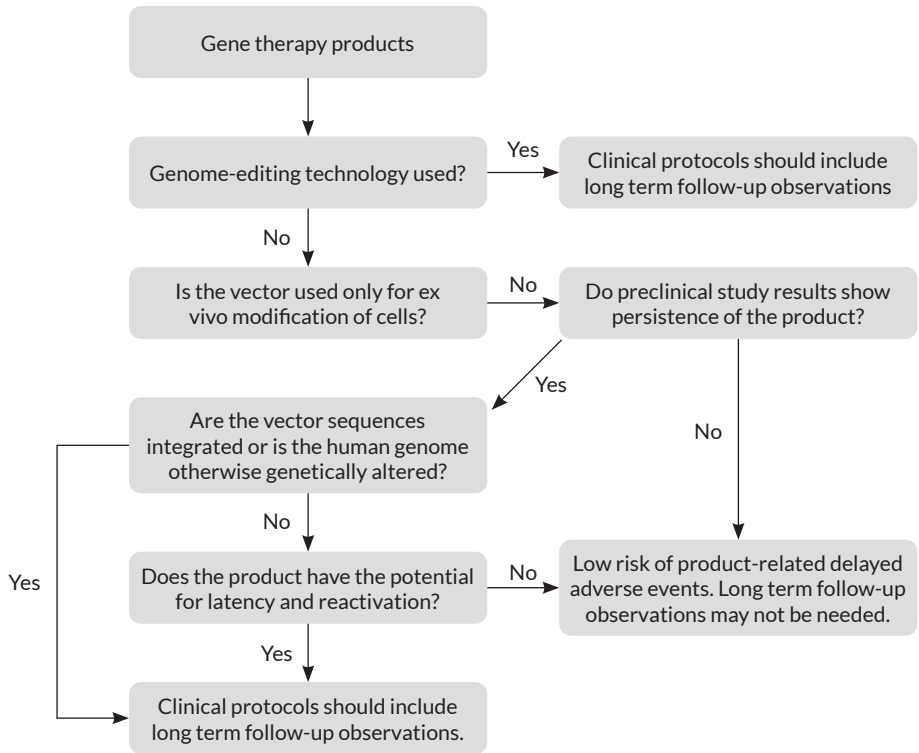
Long-Term Follow-up Studies

In January 2020, CBER provided updated recommendations on the design of long-term follow-up studies (LTFU) following administration of gene therapy products in humans.[26] While a follow-up period is typically described in the main clinical study protocol, long-term follow-up studies are intended to obtain longitudinal adverse event data from patients after the primary follow-up period has ended; adverse events that occur in this timeframe are referred to as delayed adverse events. **Figure 6-1** is adapted from FDA's assessment process.[27] The risks associated with delayed adverse events are largely founded on the biological characteristics of the vectors used in gene therapy products and informed by the extensive preclinical and clinical experience gained to date. These characteristics include the following:[28]

- The capability of vectors to integrate non-specifically to the host genome
- Prolonged or sustained expression of the therapeutic gene of interest may promote unchecked cell growth or latency
- Replication competent viruses and bacteria-based vectors may lead to persistent infection in immunocompromised patients

Note: According to the LTFU guidance, FDA advises observation of subjects for 15 years after exposure to the highest-risk gene therapy products (e.g., integrating viral vectors such as lentivirus), with a minimum of five years of annual examinations and 10 years of annual in-person queries or questionnaires of the study subjects.[29]

Figure 6-1. Framework to Assess the Risk of Gene Therapy-Related Delayed Adverse Events



Note: Please see FDA guidance for industry: long term follow-up after administration of human gene therapy products for nonbinding recommendations and additional considerations for products that may integrate or were designed to facilitate integration; this guidance also includes recommendations on how to perform clinical long-term follow-up observations.

Chemistry, Manufacturing, and Control

Gene therapy products are novel treatments and complex biological products and, as a result, pose multiple challenges for sponsors involved in their development and manufacturing. Establishing controls and standards are the building blocks of developing a robust manufacturing paradigm for these revolutionary therapeutic options. Health authorities across the globe, realizing the potential of gene therapy, have partnered with sponsors and supported the development of gene therapy products by releasing guidance documents with current thinking and recommendations regarding chemistry, manufacturing, and control (CMC) information that is essential for product development and regulatory submissions. Examples of these guidance documents and a brief description of their scope and objective are provided below; guidance also applies to combination products that contain gene therapy in combination with a device, drug, or other biological.

- January 2020 FDA guidance on chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs) provides sponsors developing gene therapy products with recommendations regarding CMC information submitted in an IND. FDA also provides guidance on product development and the expectation around CMC requirements for the investigational product's safety, identity, quality, purity, and strength (including potency). The recommendations are effectively grouped as eCTD modules with appropriate contents to aid sponsors when completing the sections for filing an IND.[30]
- January 2020 FDA guidance on human gene therapy for hemophilia provides recommendations to sponsors mainly on the clinical aspects of developing human gene therapy products for the treatment of hemophilia. The guidance also covers preclinical considerations and CMC expectations to support development of gene therapy products for the treatment of hemophilia. CMC expectations were like those described in the previous guidance and no indication of specific nuances were described.[31]
- January 2020 FDA guidance on human gene therapy for rare diseases provides recommendations to sponsors developing human gene therapy products focusing on a rare disease, which is defined in the US as one that affects fewer than 200,000 people. The guidance outlines CMC, preclinical, and clinical considerations where there may be feasibility issues related to the size of the affected population. Uniqueness of the gene therapy products combined with the limited study population may compound the challenges faced in the development of gene therapy products. While the general philosophy around CMC for developing gene therapy products remain the same as those described in general, aspects such as fewer lots manufactured and insufficient samples make it hard to develop or design critical quality attributes based on clinical outcomes. The guidance also outlines challenges in establishing critical process parameters and the importance of implementing these parameters early in the product development timeline. The preclinical program should be designed to support the development of a robust analytical strategy including potency assays to guide the clinical development of gene therapy products for rare disorders.[32]
- January 2020 FDA guidance on human gene therapy for retinal disorders provides recommendations to sponsors developing human gene therapy products for retinal disorders. In addition to the general CMC guidelines applicable for gene therapy products, special considerations specific to retinal products, such as formulation for small volume and endotoxin limits, are discussed. Purity factors, such as particulate matter, and the evaluation of compatibility of the drug product with the delivery system are also discussed.[33] Other product-specific guidance with CMC recommendations include preparation of IDEs and INDs for products intended to repair or replace knee cartilage and considerations for allogeneic pancreatic islet cell products.[34]

- January 2020 FDA guidance on testing of retroviral vector-based human gene therapy products for replication competent retrovirus during product manufacture and patient follow-up provides guidance on the identification and test methods and patient monitoring after administration of retroviral vector-based gene therapy products.[35]
- September 2016 FDA guidance on recommendations for microbial vectors used for gene therapy provides recommendations on the CMC aspects to be considered for the dose selection and dosing schedule in early-phase clinical trials of products containing microbial vectors.[36]
- January 2011 FDA guidance on potency tests for cellular and gene therapy products provides detailed guidance to manufacturers of CGT products on developing analytical methods to measure product potency, which are applicable to both clinical trial application and eventual license application.[37]
- The European Medicines Agency (EMA) also has provided the industry and CGT developers with scientific guidelines on several topics, including recommendations on quality requirements for investigational and commercialization of advanced therapy medicinal products and gene therapy. In addition to the guidelines, the EMA has provided reflection papers and advice on CMC considerations, such as comparability, in a question-and-answer format.[38]
- Japan's Ministry of Health, Labour, and Welfare also has provided a guideline that lays down basic technicalities required to ensure the quality and safety of *in vivo* gene therapy products and *ex vivo* genetically modified human cell therapy and other regenerative medical products.[39]
- Special designations, such as RMAT[40] in the US and priority medicines (PRIME)[41] in the EU, also provide many opportunities for sponsors to interact with regulatory agencies and provide tools to develop quality aspects of their products. For instance, the EMA has a toolbox guidance on scientific elements and regulatory tools to meeting quality and manufacturing controls and address common challenges in order to support quality data packages for PRIME marketing authorization applications.
- The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) also has provided guidance documents with recommendations toward achieving greater harmonization in the interpretation and application of technical guidelines on quality issues, including but not limited to manufacturing controls and good manufacturing practices, specifications, quality risk management, analytical methods qualification and validation, conducting stability studies, and defining strategies for impurities testing.[42]

Conclusion

This chapter reviewed the advancements in the field of gene therapy and the landscape of vectors used in the clinical space with a special emphasis on viral vectors. Quality and regulatory considerations for the successful development of gene therapy also are discussed. Some of the developmental challenges for gene therapy include safety concerns due to immunogenicity, high manufacturing cost, and the need for long-term follow-up. In spite of the challenges, the field is well poised to deliver potentially life-saving treatments for various unmet medical needs.

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