Virus-Mediated Gene Delivery:
A Cornerstone of Modern Gene Therapy

VMGD is central to improving the safety, efficiency, and precision of therapeutic interventions.

Introduction

Virus-mediated gene delivery (VMGD) uses adenoviruses, adeno-associated viruses (AAVs), lentiviruses, and other common human viral pathogens to deliver genes into cells. While VMGD provides a facile avenue to novel, research-worthy cell lines, its highest-value application has been treating gene-based illnesses. Approximately 70% of gene therapy clinical trials now use viral vectors[1]. The remainder utilize non-viral methods like liposomes, lipid nanoparticles, highly branched poly(β-amino ester), single-chain cyclic polymer, poly(amidoamine), dendrimers, or polyethyleneimine.

Therapeutic VMGD often incorporates a step to render the virus incapable of causing illness while retaining its proliferative capabilities. This step is not required for AAVs since they generally do not cause human disease[2]. However, all viral vectors have the potential to elicit potentially harmful immune responses in patients[3].

VMGD was originally conceived as a potential treatment for heritable genetic disorders like cystic fibrosis. However, clinical applications have expanded to include neurologic conditions, diseases of the eyes and cardiovascular system, and cancer[4].

As of March 2024, the U.S. Food and Drug Administration (FDA) had approved 36 gene therapies, with approximately 500 under development. Segal, a consulting firm, predicts up to 20 new gene therapy approvals per year beginning in 2025[5].

TABLE 1. SOME FDA-APPROVED THERAPIES UTILIZING VIRAL VECTORS

THERAPY NAME	CONDITION TREATED	VIRAL VECTOR	APPROVAL DATE	MANUFACTURER	NOTES
Luxturna	Biallelic RPE65 mutation-associated retinal dystrophy	AAV2	Dec 2017	Spark Therapeutics	First *in vivo* gene therapy approved by the FDA.
Zolgensma	Spinal muscular atrophy (SMA) in pediatric patients under two years old with bi-allelic mutations in the SMN1 gene	AAV9	May 2019	Novartis	One-time intravenous infusion therapy.
Kymriah	Relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) in patients up to 25 years old	Lentivirus	Aug 2017	Novartis	First FDA-approved CAR T-cell therapy.
Yescarta	Relapsed or refractory large B-cell lymphoma in adults	Retrovirus	Oct 2017	Kite Pharma (Gilead Sciences)	Second CAR T-cell therapy approved by the FDA.

Viral Vector Overview

Most experimental VMGD are based on just four viruses: adeno-associated virus, adenovirus (AV), lentiviruses, and retroviruses[6]. Developers select these viruses based on tropism or target cells, size of the delivered gene, and safety.

- AAV, the workhorse VMGD virus, targets many tissue and cell types and shows minimal immunogenicity, particularly for AAV2, AAV8, and AAV5 serotypes[7]. On the downside, the carrying capacity of AAVs is limited to about 4.7 kilobases (kb). AAV safety has been demonstrated in more than 120 clinical trials, with only mild, transient side effects reported[8].
- Adenoviruses also show tropism toward a wide range of cells and tissues[9], and a much higher gene-carrying capacity compared with AAVs—up to 36 kb[10], with high functional titers. Adenoviruses are generally safe but their association with undesirable immune responses limits their utility for treatments requiring multiple or periodic dosing.
- Lentiviruses, which operate on both dividing and non-dividing cells, have an intermediate carrying capacity of up to 10 kb and lower immunogenicity than adenoviruses. Lentivirus show limited tropism, which may be modified by engineering viral surface proteins, or pseudotyping[11]. Along with typical immunogenicity concerns, lentiviruses carry the capacity to integrate into the host genome[12]. This beneficial feature enables to construct stable mutation cell lines. But it carries risk of insertional mutagenesis when one wishes to use lentiviruses in clinics.Lentiviruses have undergone significant design improvements to address this issue, including the incorporation of integration-deficient vectors[13]. Current-generation lentivirus gene delivery is now considered among the safest VMGD approaches.The first lentivirus VMGD treatment, Kymriah® (tisagenlecleucel), was approved in the United States in 2017 for treating pediatric acute lymphoblastic leukemia, and other similar treatments are in late-phase development[14]. Although insertional mutagenesis remains a possibility with these vectors, no cases have been reported to date.
- Retroviruses, of which lentivirus is one example, are used in VMGD to integrate genetic material into host genomes, including those of dividing and non-dividing cells[15]. Other retroviruses suitable for VMGD are the mouse-derived gamma-retrovirus (mostly used for hematopoietic stem cell treatments), alpharetrovirus, murine leukemia virus, human modified immunodeficiency virus type 1 (especially for non-dividing cells), and spleen necrosis virus[16].

Retrovirus packaging capacity is similar to that of lentivirus, and these vectors show generally low immunogenicity. As with lentiviruses, retroviruses are capable of host genome integration, which is beneficial in some instances but introduces another layer of safety challenges. And among the therapy-worthy VMGD platforms, retroviruses are particularly suited to *ex vivo* treatments[17].

THERAPY NAME	CONDITION TREATED	VIRAL VECTOR	APPROVAL DATE	MANUFACTURER	NOTES
Breyanzi	Relapsed or refractory large B-cell lymphoma in adults	Lentivirus	Feb 2021	Bristol Myers Squibb	Offers a personalized CAR T-cell treatment option.
Abecma	Relapsed or refractory multiple myeloma	Lentivirus	Mar 2021	Bristol Myers Squibb and bluebird bio	First CAR T-cell therapy approved for multiple myeloma.
Roctavian	Hemophilia A	AAV5	Jun 2023	BioMarin Pharmaceutical	Delivers the gene for clotting Factor VIII to reduce uncontrolled bleeding.
Elevidys	Duchenne muscular dtrophy (DMD)	AAVrh74	Jun 2023	Sarepta Therapeutics	First gene therapy approved for DMD.
Kebilidi	Aromatic L-amino acid decarboxylase (AADC) deficiency	rAAV	Nov 2024	PTC Therapeutics	One-time treatment introducing the AADC gene into nerve cells.

TABLE 2. CHARACTERISTICS OF THE MOST COMMONLY USED VIRAL VECTORS IN GENE THERAPY

VIRAL VECTOR	GENOME TYPE	CARRYING CAPACITY	TROPISM	INTEGRATION	IMMUNOGENICITY	KEY FEATURES
Adeno-Associated Virus (AAV)	Single-stranded DNA	Up to 4.7 kb	Broad; varies with serotype	Rare	Low	Non-pathogenic; long-term expression; limited packaging capacity.
Adenovirus	Double-stranded DNA	Up to 36 kb	Broad; infects dividing and non-dividing cells	No	High	High transduction efficiency; transient expression; can elicit strong immune responses.
Lentivirus	Single-stranded RNA	Up to 8 kb	Broad; can be pseudotyped for specificity	Yes	Moderate	Stable integration into host genome; suitable for long-term expression; capable of transducing non-dividing cells.
Gamma-Retrovirus	Single-stranded RNA	Up to 8 kb	Dividing cells	Yes	Moderate	Integrates into host genome; primarily infects dividing cells; risk of insertional mutagenesis.
Herpes Simplex Virus (HSV)	Double-stranded DNA	Up to 150 kb	Neurons	No	Moderate	Large packaging capacity; establishes lifelong latency in neurons; potential for neuronal gene therapy.

Choice of viral vector depends on the disease, treatment goals, target tissue, and desired duration of expression. AAVs, for example, have emerged as the preferred gene transfer vehicle in clinical trials due to their safety and sustained expression, while lentiviruses are preferred for *ex vivo* applications. Developers turn to AVs for transferring very large genes, whereas "target-tunable" retroviruses excel at treatments aimed at specific cell types.

Applications

Through its ability to deliver specific genes to specific cells or tissues, VMGD supports basic and applied research in the life sciences, particularly in developing gene-based therapeutics.

The ability to create human-like phenotypes in cells, tissues, or living animals is another common application of VMGD. At the cellular level viral transduction leads to cell-based test systems for gene function[18], drug discovery[19], and cell-based therapy studies[20].

In functional gene studies, VMGD allows researchers to observe the effects of gene expression on cellular activity, cell viability, and gene-gene interactions. Lentiviral vectors are often selected for these experiments for their ability to integrate into the host genomes of many different cell types. Lentivirus vectors can also be used to over- or under-express genes already present[21].

Similarly, by introducing disease-causing genes into intact animals, researchers can study disease progression and test for responses to gene-based, biological, or small molecule treatments for cancer[22], genetic diseases[23], immune disorders[24], central nervous system diseases[25], and others.

AAV, lentivirus, and adenovirus are common vectors for delivering CRISPR gene-editing components to both cells and organisms.

CRISPR opens avenues to treat genetic diseases by introducing working copies of defective genes into the organism's genome via retroviral transduction. Diseases potentially treated through CRISPR include hemophilia, inherited retinal degeneration, muscular dystrophy, cystic fibrosis, Parkinson's, genetic deafness, and others[26,27].

Through their ability to design custom stem cells, VMGD-created CRISPR technology has many applications in

regenerative medicine, particularly for treating injuries and age-related diseases. For example, CRISPR can edit genes involved in cellular aging with the p16INK4a-pRB and p53-p21 pathways, to delay senescence and enhance cell function. CRISPR can also reactivate silenced stem cell genes to improve those cells' viability[28].

Experimental Workflow: Manufacturing and Production

Choice of viral vector is dictated by factors discussed earlier, namely tropism or target cell/tissue, desired duration of response, immunogenicity, genetic payload size, and whether permanent genomic insertion is desired. One factor not previously mentioned is manufacturability at scale[29].

Manufacturability refers to the potential to produce viral vectors and associated products reliably, safely, and economically sustainably at levels required to meet market demand.

Unlike monoclonal antibodies, whose discovery-through-production lifecycle—including analytics—is characterized by standardized, "platform" unit operations and methods, most steps in viral vector manufacturing require separate, resource-heavy optimization to achieve the desired titers and yields.

The relevant manufacturing steps include plasmid ratios, ratio of DNA to transfection reagents, transfection times, harvest and cell lysis, cell culture clarification, host cell fragment and protein clearance, pre-purification volume management, separation/purification, and sterile finishing—all of which are time- and resource-intensive.

One pitfall common to viral vector production is the tendency to become mired in non-scalable early-stage, "quick-and-dirty" processes and operations. For example, scientists will often use transient transfection to produce a few milligrams of a protein for characterization. However, when it comes time to promote the molecule to preclinical stages, they are no closer to a robust manufacturing process than they were at the beginning of the project.

Conducting early-stage research with manufacturing scale in mind applies not only to production host cells and viral vector strain but also to reagents, starting materials, bioreactor vessels and conditions, and process monitoring methods.

Non-Viral Gene Delivery

Non-viral gene transfer methods remain attractive due to their safety, flexibility in packaging nucleic acids, and low production costs relative to viral vectors[30]. There are, however, some challenges but non-viral transfer methods are undergoing constant improvement and, in some instances, can approach the efficiencies of VMGD[31].

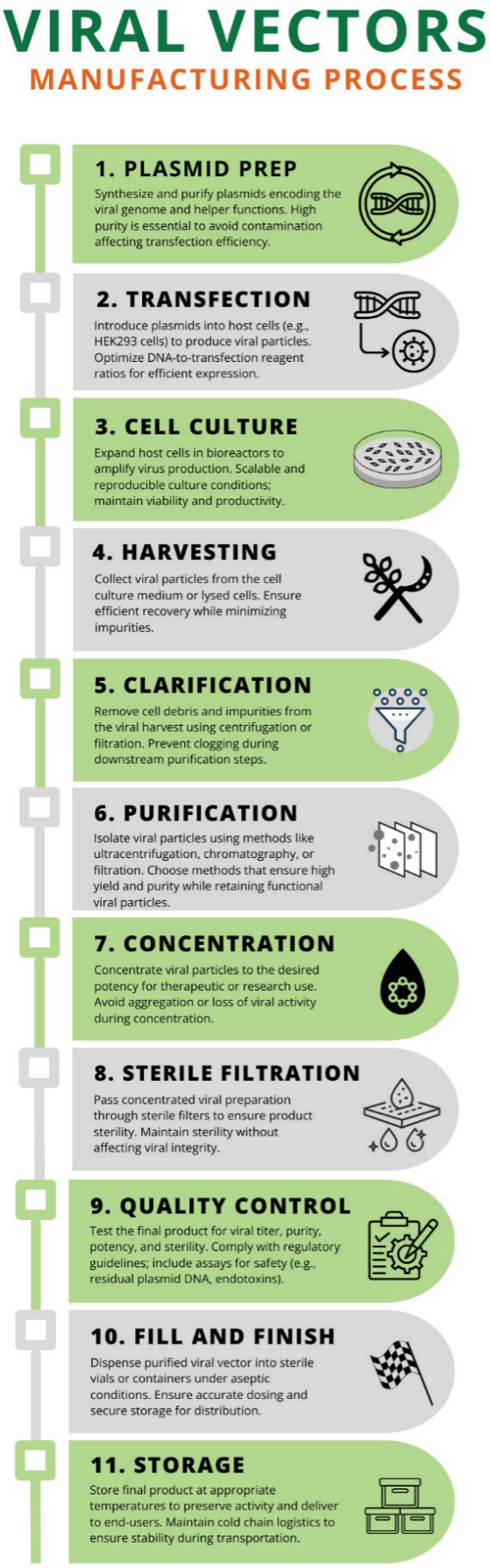

TABLE 3. CHARACTERISTICS OF NON-VIRAL GENE DELIVERY METHODS

METHOD	DELIVERY MECHANISM	PAYLOAD CAPACITY	TARGET SPECIFICITY	IMMUNOGENICITY	ADVANTAGES	LIMITATIONS
Liposomes	Encapsulation of genes in lipid vesicles	High	Moderate; can be modified	Low	Non-pathogenic, biocompatible, can carry large payloads	Mechanically unstable; lacks native cell-specific targeting
Polymeric Nanoparticles	Genes encapsulated in engineered polymers	High	High; biodegradable polymers can be targeted	Low	Controlled release, low immunogenicity, adaptable for various applications	Production complexity; potential toxicity if not biodegradable
Peptide-Based Vectors	Gene conjugation with cell-penetrating peptides	Moderate	High; customizable	Low	High delivery efficiency; adaptable for specific targeting	Limited stability, high cost for covalent strategies
Electroporation	Electric pulses create temporary cell membrane pores	Moderate	High; specific to targeted cells	Low	Precise targeting; suitable for a wide range of cells	Risk of cell damage; poor control over number of gene copies delivered
Sonoporation	Ultrasound creates temporary pores in cell membranes	Moderate	High; location-specific	Low	Enhances tissue generation; non-invasive	Limited clinical validation; may require adjunct agents like microbubbles
Biolistic Delivery	Microprojectiles coated with DNA shot into cells	High	Low	Very Low	Useful for hard-to-transfect cells and organisms	Mechanically invasive; limited use for certain cell types

Liposomes are lipid-based vesicles or bubbles capable of encapsulating genes or other therapeutic payloads[32]. Liposomes deliver genes into cells by fusing with the cell membrane and releasing their contents into the cytoplasm. Liposomes are non-pathogenic, biocompatible, and can carry large genetic payloads, but their mechanical instability and lack of native tropism limits their utility in therapeutic gene delivery.

Conjugating liposomes to lipids or polyethylene glycol can provide some mechanical stability and control over drug release, but that only adds to liposomes high production costs.

Polymeric nanoparticle gene delivery uses engineered polymers to encapsulate genes and deliver them to diseased cells[33]. Polymers may be designed for controlled release—for example to biodegrade at specific times or locations to ensure targeted delivery with low immunogenicity. Therapeutic targets include cancer and regenerative medicine.

Peptide-based vectors use gene-conjugated, cell-penetrating amino acid chains to deliver therapeutic genes to diseased cells[34]. There are two ways to use peptides to deliver genes: with the gene conjugated to the peptide through a spacer (the "covalent strategy" similar to antibody-drug conjugates), or complexed to and carried by a peptide assembly (the "non-covalent strategy," similar to polymeric and lipid nanoparticle delivery).

Electroporation uses microsecond-long electric pulses to create temporary holes or pores in cell membranes, allowing genetic materials to enter cells passively[35]. Since only target cells are affected, electroporation avoids most off-target effects common with VMGD. The potential to fatally damage cells, and poor control over the number of gene copies entering electroporated cells, are electroporation's primary weaknesses. However, the introduction of micro/nano-electroporation have improved transfection

efficiency and improved electroporated cell viability . These modifications have improved prospects for electroporation for both research and therapeutic applications.

Sonoporation, or ultrasound-mediated gene transfer, works similarly to electroporation but uses sound waves rather than electrical current to create pores in cell membranes[37]. Like many non-viral methods, sonoporation works with a wide variety of cells and tissues and is capable of precise targeting. Sonoporation also appears to enhance tissue-generation treatments. Despite these potential benefits, no clinical trials of sonoporation have been reported as of this writing.

Biolistic gene delivery is a purely mechanical method that shoots microscopic DNA-coated particles directly into target cells using a gene gun[38]. Originally intended for genetically engineering plants[39], whose cell walls are more difficult to penetrate than animal cell membranes, gene guns have been adapted for animal cell transfections[40]. Biolistic delivery has a very low risk of immunogenicity and is particularly useful for cells or organisms that are difficult to transfect using viral, chemical, or other mechanical methods.

Conclusion

Developers of gene-based medicines face no shortage of transient or permanent transfection methods. While mechanical and chemical transfections are relatively inexpensive and less prone to induce immunogenic adverse reactions, they lack the potential to target specific cells, particularly those that are inaccessible to biolistics or electroporation, and their gene delivery stoichiometries are less predictable.

And, through a combination of lower transfection efficiency, limited cargo capacity, cellular barriers, inherent toxicities, and limitation to transient expression, their therapeutic effectiveness is limited compared with VMGD[41].

While non-viral transfection is improving every day, so are viral vectors.

For example, the relatively new field of synthetic virology allows creation of viral vectors with enhanced specificity, lower immunogenicity and higher, more effectively controlled, transfection[42]. Synthetic virology also enables development of vectors tailored for specific applications or cells, for example gene editing, vaccines, or cancer treatment.

Ongoing improvements in VMGD promise the broad application of gene-based therapies across disease categories, particularly for diseases lacking effective treatments. They also allow developers to consider an additional avenue for personalized medicine, a goal that has thus far been elusive due to the high cost of individualized treatments with biologics.

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