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NUCLEIC ACID-BASED THERAPEUTIC DELIVERY SYSTEMS: GENE THERAPY PRINCIPLES, DELIVERY STRATEGIES, AND LIPOSOMAL APPROACHES: A REVIEW

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ABSTRACT

Nucleic acid therapeutics, including plasmid DNA, mRNA, siRNA, antisense oligonucleotides, and CRISPR-based systems, have revolutionized modern medicine by enabling targeted treatment of genetic and acquired diseases. Gene therapy involves the delivery of genetic material to correct, replace, silence, or supplement defective genes through ex-vivo or in-vivo approaches. It has shown significant therapeutic potential in inherited disorders such as severe combined immunodeficiency, haemophilia, spinal muscular atrophy, inherited retinal diseases, and various cancers, including CAR-T cell therapy. The success of gene therapy depends on efficient and safe delivery systems. Viral vectors provide high gene transfer efficiency but are limited by immunogenicity, insertional mutagenesis, and manufacturing challenges. Non-viral approaches, including physical methods and chemical carriers, offer improved safety with advancing delivery efficiency. Among these, liposomal systems, particularly ionizable lipid nanoparticles (LNPs), have become clinically established platforms for nucleic acid delivery, as demonstrated by the siRNA drug patisiran and mRNA COVID-19 vaccines. This review summarizes gene therapy strategies, delivery systems, liposomal gene delivery technologies, their clinical applications, and future perspectives.

KEYWORDS: Gene Therapy, Nucleic Acid Delivery, Viral Vectors, Non-viral Vectors, Liposomal Gene Delivery, *Ex-vivo* Gene Therapy, *In-vivo* Gene Therapy, Cationic Liposomes, Lipid Nanoparticles.

INTRODUCTION

Nucleic acid-based therapeutics constitute a distinct class of biopharmaceuticals in which the active agent is genetic material — DNA, RNA, or a gene-editing construct — rather than a small molecule or protein. Unlike conventional drugs that act by modulating pre-existing protein function, nucleic acid therapeutics act upstream, at the level of the genome or transcriptome, either by supplying a functional copy of a defective gene, silencing an aberrantly expressed gene, or directing translation of a therapeutic protein. Gene therapy was first conceptually proposed by Friedmann and Roblin in 1972, and the field achieved its first authorized clinical trial in 1990, when a four-year-old girl with adenosine deaminase deficiency-associated severe combined immunodeficiency (ADA-SCID) received retrovirally transduced autologous T-lymphocytes at the National Institutes of Health.

Over the past three decades, the field has matured from an experimental concept fraught with high-profile safety setbacks — including the 1999 death of Jesse Gelsinger following adenoviral vector administration and the development of leukaemia in several X-linked SCID trial participants due to insertional mutagenesis — into a clinically validated therapeutic modality with more than a dozen approved gene and cell therapy products, including Zolgensma (onasemnogene abeparvovec) for spinal muscular atrophy, Luxturna (voretigene neparvovec) for RPE65-mediated inherited retinal dystrophy, Kymriah and Yescarta (CAR-T cell therapies) for haematological malignancies, and Casgevy, the first

CRISPR/Cas9 gene-edited therapy approved for sickle cell disease and transfusion-dependent beta-thalassaemia.

Milestones in the Development of Gene Therapy

The trajectory of gene therapy from concept to approved medicine has been punctuated by both landmark successes and cautionary setbacks that have shaped current vector design and regulatory practice, as summarised in table below.

Year	Milestone
1972	Friedmann and Roblin propose the concept of human gene therapy
1990	First authorised gene therapy trial: retroviral correction of ADA-SCID
1999	Death of Jesse Gelsinger following high-dose adenoviral vector administration
2002–2003	Leukaemia in X-linked SCID trial participants linked to retroviral insertional mutagenesis
2012	Alipogene tiparvovec (Glybera) becomes first AAV gene therapy approved in the West
2017	Tisagenlecleucel and Luxturna approved by the US FDA
2019	Onasemnogene abeparvovec (Zolgensma) approved for spinal muscular atrophy
2020–2021	mRNA-LNP COVID-19 vaccines authorised for emergency and full use worldwide
2023	Exagamglogene autotemcel (Casgevy), first CRISPR/Cas9-edited therapy, approved
2024	Lenmeldy approved in the United States for early-onset metachromatic leukodystrophy, marking an important advance in ex vivo gene therapy.
2024-2025	Continued expansion of approved gene and cell therapies worldwide, including additional indications for existing products and rapid clinical advancement of in vivo CRISPR, base-editing, and prime-editing technologies.

Ex-vivo Gene Therapy

Ex-vivo gene therapy involves the isolation of target cells — most commonly haematopoietic stem cells, T-lymphocytes, or fibroblasts — from the patient (autologous) or a donor (allogeneic), followed by genetic modification under controlled laboratory conditions, expansion of the modified cell population, and subsequent reinfusion into the patient. The stepwise workflow comprises:[1] apheresis or biopsy to harvest target cells;[2] ex-vivo culture and activation;[3] transduction or transfection with the therapeutic vector, typically a retroviral or lentiviral construct;[4] quality control testing for vector copy number, sterility, and potency; and[5] reinfusion of the modified cell product, often preceded by lymphodepleting conditioning chemotherapy.

This approach is exemplified by chimeric antigen receptor (CAR)-T cell therapy, in which a patient's own T-cells are engineered ex-vivo to express a synthetic receptor targeting a tumour antigen such as CD19, and by the correction of ADA-SCID and X-linked SCID through lentiviral transduction of autologous CD34+ haematopoietic stem cells. The principal advantage of the ex-vivo strategy is the ability to rigorously characterise, select, and quality-test the modified cell population before it is returned to the patient, thereby minimising off-target biodistribution of the vector and permitting the use of higher, more efficient vector doses in a closed system. Limitations include the invasiveness and cost of cell harvesting and ex-vivo manufacturing, the restriction to cell types that can be cultured and expanded outside the body, and the requirement for specialised cell-processing facilities.

***In-vivo* Gene Therapy**

In-vivo gene therapy involves direct administration of the therapeutic vector into the patient, either systemically (intravenous infusion) or locally (intraocular, intramuscular, intrathecal, or intratumoural injection), such that transduction of the target tissue occurs within the living organism. This strategy underlies Luxturna, administered by subretinal injection for inherited retinal dystrophy, Zolgensma, administered as a single intravenous AAV9 infusion for spinal muscular atrophy, and oncolytic viral therapies such as talimogene laherparepvec (T-VEC), injected directly into melanoma lesions.

In-vivo approaches offer the advantages of a simpler, less invasive treatment paradigm that does not require specialised cell-processing infrastructure and is applicable to tissues that cannot be readily harvested and cultured, such as neurons, cardiomyocytes, and retinal photoreceptors. However, in-vivo delivery is constrained by unpredictable biodistribution, the presence of pre-existing neutralising antibodies against common viral capsids, dose-dependent hepatotoxicity and complement activation with high-dose systemic AAV, and comparatively limited control over the transduction event once the vector has been administered.

Feature	Ex-vivo Gene Therapy	In-vivo Gene Therapy
Site of modification	Outside the body (laboratory)	Directly within the patient
Target cells	Readily harvestable/culturable cells (HSCs, T-cells)	Any accessible tissue (liver, eye, muscle, CNS)

Quality control	Extensive pre-infusion testing possible	Limited; occurs after administration
Typical vector	Retrovirus / Lentivirus	AAV / Adenovirus / LNP
Representative example	CAR-T cell therapy (tisagenlecleucel)	Zolgensma, Luxturna
Key limitation	Invasive, costly, complex manufacturing	Unpredictable biodistribution, immune response

Comparison of Ex-vivo and In-vivo Gene Therapy Strategies

Potential Target Diseases for Gene Therapy

The rational selection of a candidate disease for gene therapy intervention depends on the underlying genetic architecture of the disorder, the accessibility of the target tissue, and the therapeutic index achievable with available vector technology. Diseases caused by a single defective gene (monogenic disorders) with a well-defined molecular mechanism represent the most tractable targets, while polygenic and multifactorial conditions such as cancer require more sophisticated combinatorial strategies.

Inherited Disorders

Monogenic inherited disorders were the earliest and remain among the most successful indications for gene therapy, since restoration of a single functional gene copy can be sufficient to normalise the disease phenotype. Key examples include:

- **Severe Combined Immunodeficiency (SCID):** caused by mutations in the adenosine deaminase (ADA) gene or the common gamma chain of the interleukin receptor (X-linked SCID); corrected by ex-vivo retroviral or lentiviral transduction of autologous haematopoietic stem cells.
- **Haemophilia A and B:** deficiency of clotting factor VIII or IX; AAV-mediated liver-directed in-vivo gene transfer (e.g., etranacogene dezaparvovec) restores endogenous factor synthesis, reducing bleeding episodes and factor-replacement dependence.
- **Spinal Muscular Atrophy (SMA):** loss-of-function mutation in the SMN1 gene; onasemnogene abeparvovec delivers a functional SMN1 transgene via AAV9, which crosses the blood–brain barrier following intravenous infusion in infants.
- **Inherited Retinal Dystrophy:** biallelic RPE65 mutations causing Leber congenital amaurosis; voretigene neparvovec restores the visual cycle following subretinal AAV2 injection.
- **Cystic Fibrosis:** CFTR gene mutation; aerosolised non-viral and viral CFTR gene transfer to airway epithelium has been investigated, though transient expression and mucus-barrier penetration remain challenges.
- **Duchenne Muscular Dystrophy and Haemoglobinopathies:** micro-dystrophin AAV gene transfer and CRISPR/Cas9-based re-activation of foetal haemoglobin (exemplified by exagamglogene autotemcel, Casgevy) for sickle cell disease and transfusion-dependent beta-thalassaemia.

CANCER

Cancer represents a genetically heterogeneous and acquired disease target, requiring strategies distinct from the single-gene replacement paradigm used for inherited disorders. Gene-therapy approaches to malignancy include:

- **Tumour-Suppressor Gene Replacement:** reintroduction of functional p53 using a recombinant adenoviral vector (gendicine, approved in China) to restore apoptotic signalling in p53-deficient tumours.
- **Suicide Gene (Prodrug-Activating) Therapy:** delivery of the herpes simplex virus thymidine kinase (HSV-TK) gene into tumour cells, followed by systemic administration of ganciclovir, which is selectively phosphorylated into a cytotoxic metabolite within transduced cells.
- **Oncolytic Virotherapy:** genetically modified viruses (e.g., talimogene laherparepvec, a modified herpes simplex virus expressing GM-CSF) that selectively replicate within and lyse tumour cells while stimulating anti-tumour immunity.
- **Chimeric Antigen Receptor (CAR)-T Cell Therapy:** ex-vivo lentiviral or retroviral engineering of autologous T-cells to express a synthetic receptor targeting tumour-associated antigens such as CD19 (tisagenlecleucel, axicabtagene ciloleucel) or BCMA, achieving durable remission in relapsed/refractory leukaemia, lymphoma, and myeloma.
- **Immunomodulatory and Anti-angiogenic Gene Therapy:** delivery of cytokine genes (IL-2, IL-12) to enhance anti-tumour immune response, or anti-angiogenic genes (endostatin) to restrict tumour vascularisation.

Principal Target Disease Categories for Gene Therapy			
Inherited Immuno-deficiencies (SCID)	Hemophilia & Coagulation Disorders	Neuromuscular Disorders (SMA, DMD)	Inherited Retinal Dystrophies
Hematological Malignancies	Solid Tumors	Haemoglobinopathies (Sickle Cell, Thalassemia)	Infectious Disease (mRNA Vaccines)

Principal Disease Categories Targeted by Nucleic Acid-Based Gene Therapy

Criteria for an Ideal Gene Therapy Candidate Disease

Not every genetic or acquired disease is equally amenable to gene therapy intervention. Favourable candidate diseases generally share several features: a well-characterised, single-gene molecular defect with a clear genotype–phenotype correlation; an accessible target tissue that can be reached by local or systemic vector administration (liver, muscle, eye, and haematopoietic compartments have historically been the most tractable); a disease process that is halted or meaningfully improved by restoring even partial (10–20%) normal gene function, as is the case for haemophilia and SMA; the absence of a requirement for extremely precise, cell-type-specific temporal or quantitative gene regulation; and a severity and natural history that justifies the risk-benefit profile of an invasive, currently high-cost intervention. Diseases that fail to meet these criteria — such as complex polygenic disorders with diffuse tissue involvement — remain considerably more challenging targets for current gene expression technology.

Gene Expression Systems

The therapeutic gene of interest, once selected, must be packaged within a delivery vehicle — collectively termed a gene expression or gene transfer system — capable of protecting the nucleic acid cargo from extracellular and intracellular nuclease degradation, crossing the plasma membrane, escaping the endosomal-lysosomal pathway, and enabling adequate transcription and translation within the target cell nucleus or cytoplasm. Gene expression systems are broadly classified into viral and non-viral vectors, each with a distinct efficiency, safety, and manufacturing profile.

Mechanisms of Gene Expression Systems		
Viral Vectors (Transduction)	**Non-viral Vectors (Transfection)**	**Liposomal Vectors (Lipofection)**
Retrovirus, Lentivirus, Adenovirus, AAV, HSV	Physical & chemical carrier-mediated methods	Cationic lipid–nucleic acid complexes (lipoplex)

Classification of Gene Expression Systems by Mechanism of Cellular Entry

Viral Gene Transfer Systems

Viruses have evolved highly efficient natural machinery for delivering their genetic material into host cells, making recombinant, replication-deficient viral vectors the most widely used and clinically successful gene expression systems to date. The therapeutic transgene replaces viral genes required for replication, rendering the vector capable of transduction but incapable of causing productive infection.

- **Retroviral and Lentiviral Vectors:** derived from murine leukaemia virus (retrovirus) or HIV-1 (lentivirus); integrate stably into the host genome, providing long-term transgene expression in dividing (retrovirus) or both dividing and non-dividing (lentivirus) cells. Packaging capacity is approximately 8 kb. Integration carries a risk of insertional mutagenesis, historically associated with leukaemogenesis in early X-linked SCID trials, though self-inactivating (SIN) vector designs have substantially mitigated this risk.
- **Adenoviral Vectors:** double-stranded DNA viruses that transduce both dividing and non-dividing cells with very high efficiency and large packaging capacity (up to 36 kb in helper-dependent 'gutless' vectors), but remain episomal (non-integrating), resulting in transient expression, and elicit strong innate and adaptive immune responses that limit repeat dosing.
- **Adeno-Associated Viral (AAV) Vectors:** small, non-pathogenic parvoviruses that predominantly persist as episomes, providing sustained expression in post-mitotic tissues (liver, muscle, retina, central nervous system) with a comparatively favourable safety and immunogenicity profile. Limitations include a small packaging capacity (~4.7 kb) and pre-existing neutralising antibodies in a substantial proportion of the population due to natural AAV exposure.
- **Herpes Simplex Virus (HSV) Vectors:** large packaging capacity (>30 kb) and natural neurotropism, making them suitable for suicide-gene cancer therapy and oncolytic virotherapy, as well as gene delivery to the nervous system.

Vector	Genome	Packaging Capacity	Integration	Key Feature
Retrovirus	RNA	~8 kb	Yes (dividing cells)	First clinically used integrating vector
Lentivirus	RNA	~8 kb	(dividing & non-dividing)	Basis of CAR-T manufacturing
Adenovirus	dsDNA	up to 36 kb	No (episomal)	High efficiency, transient, immunogenic
AAV	ssDNA	~4.7 kb	redominantly episomal	Low immunogenicity, sustained expression
HSV	dsDNA	>30 kb	No (episomal)	Neurotropic, large cargo, oncolytic use

Comparative Features of Commonly Used Viral Gene Expression Vectors

Non-viral Gene Transfer Systems

Non-viral gene delivery methods circumvent the immunogenicity, insertional mutagenesis risk, packaging-capacity limitations, and high manufacturing cost associated with viral vectors, at the expense of generally lower transfection efficiency and transient gene expression. Non-viral systems are broadly divided into physical and chemical (carrier-mediated) methods.

- **Naked DNA / Hydrodynamic Injection:** rapid, high-volume intravenous injection of plasmid DNA that transiently increases capillary permeability, predominantly used in preclinical liver-targeted gene transfer.
- **Electroporation:** application of brief high-voltage electrical pulses that transiently permeabilise the cell membrane, permitting entry of plasmid DNA or mRNA; widely used for ex-vivo transfection of T-cells and dendritic cells.
- **Gene Gun (Biolistic Particle Delivery):** DNA-coated gold or tungsten microparticles are propelled into target tissue (typically skin or muscle) using compressed gas, used mainly for DNA vaccination.
- **Sonoporation and Microinjection:** ultrasound-induced transient membrane permeabilisation and direct intracellular/intranuclear injection respectively, the latter restricted to ex-vivo single-cell applications such as embryo manipulation.
- **Cationic Polymers and Dendrimers:** polyethylenimine (PEI), poly-L-lysine, and PAMAM dendrimers electrostatically condense anionic nucleic acids into nanoscale polyplexes, facilitating endocytic uptake and endosomal escape via the 'proton sponge' effect.
- **Cationic Lipid-Based Vectors (Liposomal Systems):** discussed in detail in Section 4, these represent the most clinically advanced non-viral gene expression platform.

Targeting Strategies for Enhanced Gene Delivery

Both viral and non-viral gene expression systems can be engineered with cell- or tissue-specific targeting moieties to improve selectivity and reduce off-target exposure. Viral capsid engineering — including directed evolution and rational mutagenesis of AAV capsid surface loops — has generated novel serotypes (e.g., AAV-PHP.eB) with enhanced central-nervous-system tropism following systemic administration. Non-viral and liposomal carriers can be surface-functionalised with cell-specific ligands such as transferrin (for transferrin-receptor-overexpressing tumour cells), folate, monoclonal antibody fragments, aptamers, or N-acetylgalactosamine (GalNAc, widely used for hepatocyte-selective siRNA delivery). Cell-penetrating peptides (e.g., TAT, penetratin) conjugated to the vector surface further enhance cytoplasmic and nuclear uptake by transiently disrupting membrane order. These active-targeting strategies collectively reduce the required therapeutic dose, limit systemic toxicity, and are increasingly incorporated into next-generation viral, polymeric, and liposomal gene expression systems.

Liposomal Gene Delivery Systems

Liposomal gene delivery systems occupy a pre-eminent position among non-viral vectors, combining the versatility and safety of a synthetic lipid-based carrier with transfection efficiencies that, in optimised formulations, approach those of viral vectors. Their clinical maturity is exemplified by patisiran, the first FDA-approved siRNA therapeutic (delivered as a lipid nanoparticle), and by the ionizable lipid nanoparticle (LNP) mRNA-based COVID-19 vaccines (Comirnaty and Spikevax), which together validate the liposomal platform at global manufacturing scale.

Principle of Liposomal Gene Delivery

Liposomes are self-assembling spherical vesicles composed of one or more lipid bilayers enclosing an aqueous core. In gene delivery applications, cationic (positively charged) lipids — such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol) — are formulated with a neutral 'helper' lipid such as dioleoylphosphatidylethanolamine (DOPE) or cholesterol. The cationic head group electrostatically binds the negatively charged phosphate backbone of DNA, mRNA, or siRNA, spontaneously condensing the nucleic acid into a compact, positively charged lipid–nucleic acid complex known as a lipoplex.

The net positive surface charge of the lipoplex promotes electrostatic adsorption to the anionic proteoglycans of the target cell membrane, triggering cellular internalisation predominantly via endocytosis. Endosomal escape — the rate-limiting step for effective gene transfer — is facilitated by DOPE, which adopts a destabilising hexagonal (HII) phase at endosomal pH, promoting fusion between the lipoplex and the endosomal membrane and release of the nucleic acid cargo into the cytoplasm before lysosomal degradation can occur.

Types of Liposomal Gene Delivery Systems

- **Conventional Cationic Liposomes (Lipoplexes):** simple binary formulations of a cationic lipid and a neutral helper lipid, mixed directly with plasmid DNA or RNA; e.g., Lipofectamine, a widely used research-grade transfection reagent based on DOSPA/DOPE.
- **Liposome–Polycation–DNA (LPD) Complexes:** ternary systems in which DNA is first condensed with a polycation (e.g., protamine or poly-L-lysine) before encapsulation within a cationic liposome shell, improving nuclease protection and compaction efficiency.
- **pH-Sensitive Liposomes:** incorporate lipids that destabilise selectively at acidic endosomal pH,

enhancing endosomal escape while remaining stable at physiological pH during systemic circulation.

- **Stealth (PEGylated) Liposomal Vectors:** surface modification with polyethylene glycol (PEG) reduces opsonisation and reticuloendothelial clearance, prolonging systemic circulation time and improving passive tumour or tissue accumulation, though excessive PEGylation can reduce cellular uptake ('PEG dilemma').
- **Ionizable Lipid Nanoparticles (LNPs):** the current state of the art for mRNA and siRNA delivery; formulated from four components — an ionizable cationic lipid, a helper phospholipid, cholesterol, and a PEGylated lipid — assembled by microfluidic mixing. The ionizable lipid is near-neutral at physiological pH (minimising toxicity) but becomes protonated within the acidic endosome, promoting membrane destabilisation and cytosolic release; this design underlies patisiran and the mRNA-LNP COVID-19 vaccines.

Formulation Methods

Liposomal gene carriers are prepared by thin-film hydration followed by extrusion or sonication to control vesicle size, by ethanol injection, in which a lipid–ethanol solution is rapidly injected into an aqueous nucleic acid solution, or, most commonly for clinical-grade LNPs, by microfluidic (staggered herringbone or T-junction) mixing, which enables precise, reproducible, and scalable control of particle size, polydispersity, and encapsulation efficiency by rapidly mixing an ethanolic lipid stream with an aqueous nucleic acid stream at a defined flow-rate ratio.

Factors Affecting Transfection Efficiency

- **Cationic Lipid: Nucleic Acid Charge Ratio:** an excess positive charge (N/P ratio) is generally required for efficient cell binding and endosomal escape, though excessive positive charge increases cytotoxicity and serum protein aggregation.
- **Helper Lipid Selection:** DOPE promotes membrane fusion and endosomal escape, whereas cholesterol enhances particle stability and rigidity; the ratio of the two is optimised for each nucleic acid cargo.
- **Particle Size and Surface Charge (Zeta Potential):** particles in the 80–150 nm range achieve optimal balance between cellular uptake and circulation stability; zeta potential influences both cellular association and serum-protein-mediated aggregation.
- **Serum Stability:** anionic serum proteins can displace nucleic acid cargo from cationic lipoplexes or induce aggregation, necessitating PEGylation or ionizable-lipid designs for systemic administration.

Clinical Applications and Advantages/Disadvantages

Liposomal and lipid-nanoparticle gene delivery systems have achieved landmark clinical translation: patisiran (Onpattro), an LNP-encapsulated siRNA silencing transthyretin mRNA for hereditary transthyretin-mediated amyloidosis; and the mRNA-LNP COVID-19 vaccines, which delivered spike-protein-encoding mRNA at global scale, demonstrating the manufacturability, stability (with cold-chain requirements), and immunogenic safety profile of the platform. Advantages of liposomal systems include low immunogenicity relative to viral vectors, absence of insertional mutagenesis risk (nucleic acid remains largely episomal/cytoplasmic), scalable and reproducible manufacturing, capacity to encapsulate large or fragile RNA cargo, and the flexibility to incorporate targeting ligands on the liposomal surface. Disadvantages include comparatively lower and more transient gene expression than integrating viral vectors, potential cytotoxicity and inflammatory cytokine induction at high cationic lipid doses, susceptibility to serum-mediated destabilisation, cold-chain storage requirements for RNA-LNP formulations, and the technical challenge of achieving efficient endosomal escape, which remains the principal bottleneck limiting overall transfection efficiency across all liposomal platforms.

Parameter	Viral Vectors	eric Non-viral Vectors	Liposomal / LNP Systems
Transfection efficiency	Very high	Low–moderate	Moderate–high
Immunogenicity	Moderate–high	Low	Low
Insertional mutagenesis risk	Present (integrating vectors)	Absent	Absent
Cargo capacity	Limited (4.7–36 kb)	Large / flexible	Large / flexible
Manufacturing complexity	High	Low–moderate	Moderate
Scalability	Limited	High	High
Representative clinical example	Zolgensma (AAV)	Investigational PEI/DNA vaccines	Patisiran, mRNA-LNP vaccines

Comparative Summary of Viral, Polymeric Non-viral, and Liposomal Gene Expression Systems

Clinical Applications of Nucleic Acid-Based Gene Therapy

The convergence of improved vector engineering, refined patient selection, and regulatory experience has enabled nucleic acid-based gene therapy to progress from experimental protocols to approved, reimbursed therapeutic products across multiple disease areas. As of the most recent regulatory tallies, more than a dozen gene and cell therapy products have received marketing authorisation from the US FDA and/or the European Medicines Agency, with several hundred additional candidates in active clinical development.

Primary Immunodeficiency
Ex-vivo lentiviral gene therapy correcting ADA-SCID and X-linked SCID by transduction of autologous CD34+ haematopoietic stem cells has produced durable immune reconstitution in the majority of treated patients, effectively curing a condition that was previously uniformly fatal in infancy without bone-marrow transplantation.

Oncology
CD19-directed CAR-T cell therapies (tisagenlecleucel, axicabtagene ciloleucel) have achieved complete remission rates of 40–90% in relapsed/refractory B-cell acute lymphoblastic leukaemia and diffuse large B-cell lymphoma, while oncolytic viral therapy (talimogene laherparepvec) and suicide-gene strategies extend the gene-therapy armamentarium to solid tumours, including advanced melanoma.

Ophthalmology
Subretinal delivery of voretigene neparvovec restores functional RPE65 protein in patients with biallelic RPE65-mediated inherited retinal dystrophy, producing sustained improvement in functional vision and demonstrating the feasibility of local, low-dose AAV administration to an immune-privileged site.

Neuromuscular and CNS Disorders
A single intravenous infusion of onasemnogene abeparvovec delivers a functional SMN1 transgene across the blood–brain barrier in infants with spinal muscular atrophy, halting disease progression when administered pre-symptomatically. Investigational AAV-microdystrophin constructs for Duchenne muscular dystrophy and AAV-based CNS gene therapies for Batten disease and Parkinson's disease are in active clinical development.

Infectious Disease
The rapid clinical development of ionizable lipid nanoparticle-encapsulated mRNA vaccines against SARS-CoV-2 demonstrated, at unprecedented scale, that nucleic acid-based gene expression systems can be manufactured, distributed, and administered safely to hundreds of millions of individuals, establishing a durable platform now being extended to influenza, respiratory syncytial virus, and personalised cancer neoantigen vaccines.

Emerging and Investigational Applications:
Beyond the established indications above, nucleic acid-based delivery systems are under active clinical investigation for haemophilia A and B (AAV-mediated Factor VIII/IX gene transfer, with valoctocogene roxaparvovec and etranacogene dezaparvovec now approved), inherited metabolic liver disorders such as ornithine transcarbamylase deficiency and Crigler–Najjar syndrome, lysosomal storage disorders (Gaucher, Fabry, and Pompe disease) via enzyme-replacement gene constructs, cardiovascular disease through VEGF and SERCA2a gene transfer aimed at therapeutic angiogenesis and improved cardiomyocyte calcium handling in heart failure, and chronic pain and neurodegenerative disease via AAV-mediated delivery of pain-modulating or neurotrophic transgenes directly to the central or peripheral nervous system. Collectively, these emerging applications illustrate the continued broadening of the therapeutic scope achievable with modern viral, non-viral, and liposomal gene expression systems.

Evaluation Of Nucleic Acid Delivery Systems
Rigorous in-vitro and in-vivo characterisation is essential to establish the physicochemical quality, biological performance, and safety of gene expression systems prior to clinical translation.

- **Particle Size, Polydispersity Index, and Zeta Potential:** determined by dynamic light scattering (DLS), governing biodistribution, cellular uptake, and colloidal stability.
- **Encapsulation / Complexation Efficiency:** quantified by gel retardation (electrophoretic mobility shift) assays, fluorescent dye-exclusion assays (e.g., PicoGreen/RiboGreen), or ultracentrifugation separation of free versus encapsulated nucleic acid.
- **Transfection / Transduction Efficiency:** assessed in-vitro using reporter genes (luciferase, green fluorescent protein) and flow cytometry, and in-vivo by quantitative PCR for vector genome copy number and by measurement of the encoded protein or its downstream biological effect.
- **Vector Copy Number and Integration Site Analysis:** for integrating viral vectors, essential for assessing the risk of insertional mutagenesis and clonal dominance.
- **Immunogenicity Testing:** measurement of pre-existing and treatment-induced neutralising antibodies, complement activation, and innate cytokine (interferon) responses.
- **Biodistribution and Pharmacokinetics:** whole-body imaging using bioluminescence, fluorescence, or radiolabelled vector tracking, together with quantitative PCR of vector DNA/RNA in target and non-target tissues.

Regulatory guidance issued by the International Council for Harmonisation and national agencies further mandates long-term follow-up studies (typically 5–15 years for integrating vectors), replication-competent virus testing for viral vector lots, endotoxin and residual host-cell-protein/DNA testing for liposomal and viral products, and stability studies under defined storage conditions (including accelerated and real-time cold-chain stability for RNA-lipid nanoparticle formulations) to support product shelf-life claims.

CHALLENGES AND FUTURE DIRECTIONS
Despite substantial clinical progress, nucleic acid-based

delivery systems continue to face several translational challenges: immunogenicity and pre-existing neutralising antibodies that limit repeat AAV dosing; insertional mutagenesis risk associated with integrating retroviral/lentiviral vectors; limited packaging capacity constraining delivery of large genes (e.g., dystrophin) within AAV capsids; inefficient endosomal escape limiting the cytosolic bioavailability of non-viral and liposomal carriers; high manufacturing cost and complexity, particularly for viral vector production at commercial scale; and the requirement for cold-chain logistics for RNA-lipid nanoparticle products. Future directions include the engineering of capsid- and lipid-variant libraries with improved tissue tropism and reduced immunogenicity; the integration of CRISPR/Cas9, base-editing, and prime-editing technologies delivered via viral or lipid-nanoparticle vehicles for precise, permanent gene correction; the development of tissue-targeted ligand-decorated liposomes and polymer–lipid hybrid nanoparticles; self-amplifying and circular RNA constructs to reduce dosing frequency; and the application of artificial-intelligence-guided lipid and capsid design to accelerate the discovery of next-generation gene expression systems with improved safety, efficacy, and manufacturability.

Regulatory, Manufacturing, and Ethical Considerations

Regulatory agencies, including the US FDA's Center for Biologics Evaluation and Research and the European Medicines Agency, classify gene therapy products as advanced therapy medicinal products, requiring long-term (often 15-year) patient follow-up to monitor for delayed adverse events such as secondary malignancy. The extremely high acquisition cost of approved gene therapies — several approach or exceed one million US dollars per treatment course — has prompted the development of outcomes-based reimbursement and instalment payment models. Ethical considerations specific to the field include the current restriction of clinical application to somatic (non-heritable) cells, with germline gene editing remaining prohibited or under moratorium in most jurisdictions; equitable global access to curative but costly therapies; and the long-term psychosocial implications of predictive genetic information generated during patient screening and post-treatment monitoring.

CONCLUSION

Nucleic acid-based therapeutic delivery systems have evolved from a conceptually promising but clinically fraught technology into a validated pillar of modern medicine, offering curative or disease-modifying potential across inherited monogenic disorders and cancer. The rational choice between ex-vivo and in-vivo gene therapy strategies, and between viral and non-viral gene expression systems, must be guided by the target disease pathophysiology, the accessibility of the target tissue, the required durability of expression, and the acceptable safety margin for the patient population. Liposomal gene delivery systems, culminating in the clinically validated ionizable lipid nanoparticle platform, have emerged as a particularly versatile and scalable non-viral alternative to viral vectors, as demonstrated by patisiran and the mRNA-LNP COVID-19 vaccines. Continued interdisciplinary advances in vector engineering, formulation science, and gene-editing technology will be essential to expand the therapeutic reach, safety, and accessibility of nucleic acid-based medicines in the coming decade.

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