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Review Article

Gene Therapy in Cystic Fibrosis: Progress, Challenges, and Future Perspectives

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ABSTRACT

Cystic fibrosis (CF) is a life-limiting autosomal recessive genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leading to defective chloride and bicarbonate ion transport across epithelial surfaces. This dysfunction results in thick, viscous secretions primarily affecting the respiratory and gastrointestinal systems, causing chronic infection, inflammation, and progressive organ damage. Despite significant advances in conventional therapies and CFTR modulator drugs, CF remains incurable, with many patients experiencing persistent disease progression and substantial treatment burden. This comprehensive review examines the molecular and genetic basis of cystic fibrosis, CFTR structure and function, mutation classifications, and pathophysiological mechanisms underlying disease manifestations. Current therapeutic approaches, including conventional supportive therapies and CFTR modulators, are critically analysed along with their clinical benefits and limitations. The rationale for gene therapy as a disease-modifying strategy is presented, emphasising its mutation-agnostic potential to target the underlying genetic defect. Various gene therapy strategies are explored, including viral and non-viral gene delivery systems, routes of administration, and challenges associated with airway mucus barriers and immune responses. Preclinical and clinical progress in CF gene therapy is reviewed, highlighting key trials, outcomes, and lessons learned. Emerging approaches such as CRISPR-based gene editing, mRNA-based therapies, and stem-cell strategies are discussed as potential future solutions for durable or curative treatment. While existing therapies have significantly improved survival and quality of life, gene therapy represents the most promising avenue toward mutation-agnostic, long-term disease correction. Continued research, technological innovation, and well-designed clinical trials are essential to translate gene therapy into routine clinical practice and improve outcomes for all patients with cystic fibrosis.

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INTRODUCTION

Cystic fibrosis (CF) represents one of the most common life-threatening autosomal recessive genetic disorders, primarily affecting Caucasian populations, though it occurs worldwide across all ethnic groups [1]. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, located on chromosome 7q31.2, which encodes a cyclic adenosine monophosphate (cAMP)-regulated chloride and bicarbonate ion channel expressed on the apical surface of epithelial cells [2]. The CFTR protein plays a crucial role in maintaining epithelial surface hydration and mucociliary clearance by regulating ion transport across epithelial membranes in multiple organs including the lungs, pancreas, intestine, liver, sweat glands, and reproductive tract [3].

More than 2,000 CFTR mutations have been identified to date, with the F508del mutation being the most common worldwide, accounting for approximately 70% of CF alleles in Caucasian populations [4]. Defective or absent CFTR function results in impaired chloride and bicarbonate secretion along with increased sodium absorption through epithelial sodium channels, leading to dehydration of airway surface liquid and production of abnormally thick and viscous mucus [5]. In the respiratory tract, this thick mucus obstructs airways, impairs mucociliary clearance, and creates an environment conducive to chronic bacterial colonisation and recurrent infections, particularly with pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* [6]. Persistent infection triggers chronic inflammation, progressive airway remodelling, bronchiectasis, and ultimately respiratory failure, which remains the leading cause of morbidity and mortality in patients with cystic fibrosis [7].

Beyond pulmonary involvement, cystic fibrosis manifests as a multi-system disorder. In the pancreas, obstruction of pancreatic ducts by viscous secretions leads to exocrine pancreatic insufficiency, malabsorption of fats and fat-soluble vitamins, poor growth, and malnutrition, while progressive pancreatic damage can result in cystic fibrosis-related diabetes [8]. Gastrointestinal manifestations include meconium lieu's in neonates, distal intestinal obstruction syndrome, and hepatobiliary disease due to bile duct obstruction [9]. In males, CFTR dysfunction causes congenital bilateral absence of the vas deferent leading to infertility, while females may experience reduced fertility due to thickened cervical mucus [10].

Conventional management of cystic fibrosis has historically focused on symptomatic and supportive therapies, including airway clearance techniques, mucolytics, bronchodilators, antibiotics, anti-inflammatory agents, pancreatic enzyme replacement therapy, and nutritional support [11]. While these treatments have significantly improved survival and quality of life, they do not address the underlying genetic defect and therefore cannot fully prevent disease progression. The introduction of CFTR modulator therapies, such as potentiators and correctors, has revolutionised CF care by partially restoring CFTR protein function in selected mutation classes [12]. However, these drugs are mutation-specific, expensive, require lifelong administration, and are ineffective or unavailable for a substantial proportion of patients with rare or non-responsive CFTR mutations [13].

Gene therapy has emerged as a highly attractive and rational therapeutic strategy for cystic fibrosis because it aims to correct the disease at its genetic root rather than merely alleviating downstream consequences [14]. Since CF is caused by

mutations in a single, well-characterized gene, it represents an ideal candidate for gene-based interventions. The fundamental principle of CF gene therapy is the delivery of a functional CFTR gene or genetic material into affected epithelial cells, enabling them to produce normal CFTR protein and thereby restore ion transport and epithelial surface hydration [15]. Importantly, gene addition strategies do not require correction of specific mutations and can therefore be applied in a mutation-agnostic manner, offering potential benefit to all CF patients regardless of their CFTR genotype [16].

Recent advances in gene delivery technologies, including improved viral vectors, lipid nanoparticles, and CRISPR-based gene editing systems, have renewed optimism for CF gene therapy. In 2024, UCLA researchers demonstrated that lipid nanoparticle-delivered gene editing could restore 88-100% of CFTR function in laboratory models by inserting a healthy CFTR gene into approximately 3-4% of cells [17]. Furthermore, collaborative efforts between Intellia Therapeutics and ReCode Therapeutics are advancing CRISPR-Cas9 DNA writing technology combined with selective organ targeting lipid nanoparticles to correct CFTR mutations directly in patient lungs [18]. These developments highlight the accelerating progress toward clinically viable gene therapies for cystic fibrosis.

Aim and Objectives

Aim

To comprehensively analyse gene therapy as a transformative treatment approach for cystic fibrosis, evaluating its potential to address the fundamental genetic defect and overcome limitations of current therapeutic modalities.

Objectives

1. To describe the genetic and molecular basis of cystic fibrosis, with particular focus on the structure, function, and mutation classes of the CFTR gene and protein.
2. To explain the pathophysiology of cystic fibrosis and how CFTR dysfunction leads to multi-system involvement, especially progressive lung disease.
3. To critically review current therapeutic approaches for cystic fibrosis, including conventional treatments and CFTR modulator therapies, and discuss their limitations.
4. To examine the rationale for gene therapy in cystic fibrosis, highlighting its mutation-agnostic potential and ability to target the underlying genetic defect.
5. To analyse different gene therapy strategies, including viral and non-viral vectors, routes of gene delivery, and emerging technologies such as CRISPR-based gene editing and mRNA therapy.
6. To summarise preclinical and clinical progress in cystic fibrosis gene therapy, highlighting key trials, outcomes, and lessons learned.
7. To identify major challenges and barriers associated with gene therapy for cystic fibrosis, including delivery efficiency, immune responses, safety, and long-term gene expression.
8. To discuss future perspectives and advancements in gene therapy, focusing on personalised approaches and the potential for curative treatment.

Molecular and Genetic Basis of Cystic Fibrosis

CFTR Gene and Protein Structure

Cystic fibrosis is fundamentally a disorder of the CFTR gene, which spans approximately 250 kilo-bases of genomic DNA on chromosome 7q31.2 and consists of 27 exons encoding a glycoprotein of 1,480 amino acids [2]. The CFTR protein belongs to the ATP-binding cassette (ABC) transporter superfamily but uniquely functions as a regulated ion channel rather than an active transporter. Structurally, CFTR comprises two membrane-spanning domains (MSD1 and MSD2), each composed of six transmembrane helices forming the chloride-conducting pore; two cytoplasmic nucleotide-binding domains (NBD1 and NBD2) responsible for ATP binding and hydrolysis; and a unique regulatory (R) domain that is phosphorylated by protein kinase A and protein kinase C [19].

The functional mechanism of CFTR involves phosphorylation of the R domain followed by ATP binding at the NBDs, which regulates channel opening and closing, enabling controlled chloride and bicarbonate ion transport across epithelial cell membranes. CFTR is essential for maintaining epithelial surface hydration, pH balance, and mucociliary clearance in organs such as the lungs, pancreas, intestine, liver, and sweat glands [3]. The protein localises to the apical membrane of epithelial cells, where it coordinates with other ion channels and transporters to regulate the composition and volume of epithelial secretions.

Classification of CFTR Mutations

CFTR mutations are classified into six functional classes based on their molecular consequences and impact on protein synthesis, processing, and function [20]. Class I mutations result in defective protein production due to nonsense mutations, frame-shifts, or severe splicing defects that lead to premature termination codons and absent CFTR protein. Class II mutations, including the common F508del mutation, cause defective protein folding and processing in the endoplasmic reticulum, leading to premature degradation and minimal delivery of CFTR to the cell surface. Class III mutations (gating mutations) produce CFTR proteins that reach the plasma membrane but exhibit defective channel opening, exemplified by the G551D mutation. Class IV mutations reduce chloride conductance by altering the channel pore structure. Class V mutations lead to reduced synthesis of CFTR protein due to promoter or splicing abnormalities resulting in decreased but not absent protein production. Class VI mutations impair CFTR stability at the cell surface, causing accelerated protein turnover [21].

The F508del mutation accounts for approximately 70% of CF alleles worldwide and represents a Class II mutation where deletion of phenylalanine at position 508 causes protein misfiling, retention in the endoplasmic reticulum, and subsequent degradation [4]. Patients homozygous for F508del typically present with classic severe CF phenotype, while compound heterozygotes may exhibit variable disease severity depending on the second allele. Understanding mutation classes is crucial for therapeutic development, as different mutation types require distinct treatment strategies modulators work for Class III-VI mutations where some protein reaches the cell surface, while gene therapy approaches are particularly relevant for Class I mutations where no functional protein is produced.

Pathophysiology of CFTR Dysfunction

The pathophysiology of cystic fibrosis arises directly from defective CFTR-mediated ion transport, disrupting epithelial fluid homeostasis across multiple organ systems. In the airways,

impaired chloride and bicarbonate secretion combined with increased sodium absorption results in dehydrated airway surface liquid, producing thick and viscous mucus that impairs mucociliary clearance [5]. This creates a favourable environment for chronic bacterial colonisation, particularly by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* complex. Persistent infection triggers an exaggerated inflammatory response characterized by neutrophil recruitment and release of proteases and oxidants that damage airway tissues, leading to bronchiectasis, progressive airflow obstruction, and respiratory failure [7].

In the pancreas, CFTR dysfunction blocks bicarbonate-rich secretions essential for neutralizing stomach acid and activating digestive enzymes. Reduced ductal fluid secretion causes obstruction and auto-digestion of pancreatic tissue, resulting in exocrine pancreatic insufficiency with malabsorption of fats, proteins, and fat-soluble vitamins (A, D, E, K) [8]. Progressive islet cell damage can lead to CF-related diabetes, which affects approximately 40-50% of adults with CF. In the gastrointestinal tract, altered ion transport contributes to meconium lieu's in newborns and distal intestinal obstruction syndrome in older patients [9]. In the liver, thick bile may cause focal biliary cirrhosis and portal hypertension in approximately 5-10% of patients. In sweat glands, defective chloride reabsorption causes elevated sweat chloride levels exceeding 60 mmol/L, which forms the basis for diagnostic sweat testing [22].

The multi-system nature of cystic fibrosis underscores the fundamental importance of CFTR function in epithelial physiology and highlights the need for therapeutic approaches that address the underlying genetic defect rather than merely managing individual organ complications.

Current Therapeutic Approaches and Their Limitations

Conventional Therapies

Conventional management strategies for cystic fibrosis focus on maintaining lung function, preventing and treating infections, promoting adequate nutrition, and managing complications.

Antibiotic therapy forms the cornerstone of CF pulmonary management, employing chronic suppressive therapy with inhaled antibiotics (tobramycin, aztreonam, colistin) to control chronic *Pseudomonas aeruginosa* infection, along with systemic antibiotics for acute pulmonary exacerbations [6]. Despite aggressive antibiotic use, complete bacterial eradication is rarely achieved due to biofilm formation and adaptive resistance mechanisms, leading to recurrent infections and cumulative lung damage.

Mucolytic therapies aim to reduce airway mucus viscosity and improve clearance. Dornase alfa (recombinant human DNase) degrades extracellular DNA released from neutrophils, while hypertonic saline (7%) improves airway hydration by osmotically drawing water into the airway surface liquid [21]. These agents provide modest improvements in lung function and reduce exacerbation rates but require daily administration and do not correct the underlying CFTR-mediated ion transport defect.

Airway clearance techniques include chest physiotherapy, positive expiratory pressure devices, autogenic drainage, and high-frequency chest wall oscillation, which mechanically mobilise mucus from the airways [10]. While universally recommended, these techniques are time-consuming (30-60 minutes daily), physically demanding, and heavily dependent on patient adherence. As treatment regimens grow

increasingly complex, treatment fatigue and reduced compliance become major challenges, particularly among adolescents and adults [23].

Anti-inflammatory therapies such as azithromycin (which has immunomodulatory effects beyond antibiotic action) and chronic oral corticosteroids may reduce airway inflammation but carry risks of adverse effects including glucose intolerance and growth suppression [11].

Pancreatic enzyme replacement therapy is essential for patients with exocrine pancreatic insufficiency to enable adequate nutrient absorption, while fat-soluble vitamin supplementation addresses deficiencies [8].

Despite these multifaceted conventional therapies improving median predicted survival from less than 10 years in the 1960s to over 40 years currently, they remain symptomatic treatments that cannot halt progressive lung function decline or reverse established structural damage such as bronchiectasis and fibrosis [24].

CFTR Modulator Therapies

The introduction of CFTR modulator therapies represents a paradigm shift in CF treatment by directly targeting defective CFTR protein rather than downstream consequences [12]. CFTR modulators are classified into three main categories: potentiators, which enhance channel gating at the cell surface; correctors, which improve protein folding and trafficking to the plasma membrane; and amplifiers, which increase CFTR mRNA and protein production.

Ivacaftor (VX-770), approved in 2012, was the first CFTR potentiators demonstrated to significantly improve lung function in patients with gating mutations such as G551D [25]. Clinical trials showed remarkable improvements in FEV_1 (10.6% absolute improvement), body mass index, quality of life scores, and sweat chloride concentrations, along with reduced pulmonary exacerbations. However, ivacaftor mono-therapy is effective only for approximately 4-5% of CF patients with specific gating mutations.

Lumacaftor-Ivacaftor (Orkambi), combining a corrector with a potentiators, was approved in 2015 for patients homozygous for F508del but demonstrated more modest clinical benefits (2.6-4.0% FEV_1 improvement) with variable tolerability [26].

Tezacaftor-Ivacaftor (Symdeko), approved in 2018, showed improved tolerability with similar efficacy for F508del homozygotes and heterozygotes with specific residual function mutations.

The most significant advancement came with **elexacaftor-tezacaftor-ivacaftor** (ETI; Trikafta/ Kaftrio), approved in 2019 for patients with at least one F508del allele. Phase 3 trials demonstrated unprecedented clinical efficacy with absolute FEV_1 improvements of 10-14%, sweat chloride reductions below diagnostic thresholds, substantial quality of life improvements, and marked reductions in pulmonary exacerbations [27]. Long-term studies have confirmed sustained benefits, with some patients experiencing near-normalisation of lung function when treatment is initiated early [28]. Recent phase 3 data on vanzacaftor-tezacaftor-deutivacaftor
demonstrated non-inferiority to ETI, expanding treatment options [29].

Despite transformative benefits, CFTR modulators have important limitations. Approximately 10% of CF patients worldwide produce no or minimal CFTR protein (Class I mutations, nonsense mutations, large deletions) and remain ineligible

for current modulator therapies [30]. Even responsive patients exhibit incomplete CFTR function restoration, with residual disease manifestations including persistent structural lung damage that cannot be reversed [31]. CFTR modulators require lifelong daily administration, carry potential long-term safety concerns particularly for paediatric use, and are associated with extremely high costs (>$300,000 annually in the United States), limiting global accessibility. Additionally, modulators do not fully address extra-pulmonary complications such as CF-related diabetes, advanced liver disease, and infertility [3].

These limitations underscore the continued need for alternative therapeutic approaches, particularly mutation-agnostic strategies such as gene therapy that can benefit all CF patients regardless of their specific CFTR mutations.

Rationale for Gene Therapy in Cystic Fibrosis

Why Cystic Fibrosis is Ideal for Gene Therapy

Cystic fibrosis represents one of the most suitable genetic disorders for gene therapy due to several key characteristics [14]. First, CF is caused by mutations in a single, well-characterized gene (CFTR), with a clear mechanistic link between gene dysfunction and clinical manifestations. This monogenic nature simplifies therapeutic targeting compared to polygenic disorders. Second, the primary target organ the respiratory tract is relatively accessible via inhalation delivery, enabling direct topical administration of gene therapy vectors to affected epithelial cells without requiring systemic exposure. Third, preclinical and clinical studies demonstrate that even partial restoration of CFTR function, estimated at 5-20% of normal activity, can produce clinically meaningful improvements in airway surface liquid properties, mucus clearance, and infection susceptibility [6]. This relatively low therapeutic threshold means gene transfer does not need to correct every cell to achieve benefit.

Fourth, CF gene therapy can be mutation-agnostic gene addition approaches deliver a functional CFTR gene regardless of the underlying mutation, offering potential benefit for all patients including those with Class I mutations producing no protein, who cannot benefit from current modulator therapies [4]. This represents a significant advantage over mutation-specific pharmacological approaches. Fifth, the respiratory epithelium undergoes continuous turnover, with basal stem/progenitor cells maintaining and renewing the epithelial layer. Successful gene delivery to these stem cells could provide durable therapeutic benefit as corrected cells persist and regenerate functional epithelium over time [15].

Therapeutic Goals and Potential Benefits

The primary therapeutic goals of CF gene therapy include: (1) restoring functional CFTR expression in airway epithelial cells sufficient to improve ion transport and airway surface liquid hydration; (2) improving mucociliary clearance and reducing bacterial colonisation; (3) decreasing the frequency and severity of pulmonary exacerbations; (4) slowing or halting lung function decline; (5) reducing treatment burden by decreasing dependence on chronic therapies such as inhaled antibiotics, mucolytics, and intensive physiotherapy; and (6) ultimately prolonging survival and improving quality of life[1].

In an ideal scenario, successful gene therapy would achieve stable, long-term CFTR expression by targeting airway stem/progenitor cells or using integrating vectors capable of sustained expression. A truly transformative gene therapy might require only a single treatment or infrequent repeat dosing rather than daily lifelong medications. Beyond pulmonary benefits, gene

therapy approaches could potentially be adapted for extra-pulmonary manifestations such as pancreatic and gastrointestinal complications, although respiratory targeting remains the highest clinical priority due to its dominant impact on morbidity and mortality [3].

Recent advances demonstrate the feasibility of achieving these goals. In 2024, UCLA researchers developed a non-viral gene-editing strategy using lipid nanoparticles to deliver CRISPR components and a complete healthy CFTR gene to human airway cells [17]. Despite inserting the therapeutic gene into only 3-4% of cells, CFTR channel activity was restored to 88-100% of normal levels due to optimised gene design that maximises protein production. This outsized effect from a small fraction of corrected cells validates the low therapeutic threshold concept and demonstrates that clinically relevant correction is achievable with current technologies.

Comparison with Pharmacological Approaches

Gene therapy offers fundamental advantages over conventional and modulator therapies by addressing the genetic root cause rather than downstream consequences or protein dysfunction [14]. Conventional therapies (antibiotics, mucolytics, physiotherapy) treat symptoms and complications but require continuous lifelong use and cannot prevent progressive structural lung damage [11]. CFTR modulators represent a major advance by partially restoring protein function but remain limited by mutation specificity, incomplete correction, lifelong requirement, high cost, and inability to reverse established damage [27].

In contrast, gene therapy aims to provide functional CFTR expression regardless of mutation type, potentially benefiting all genotypes including Class I mutations where modulators fail [4]. Unlike daily oral medications, successful gene therapy could provide sustained benefit from a single treatment or infrequent re-dosing, dramatically reducing treatment burden. Furthermore, gene editing approaches using CRISPR-Cas9 or base editing technologies offer the possibility of permanent genomic correction, potentially providing one-time curative treatment. While significant challenges remain regarding delivery efficiency, immune responses, and long-term safety, the continued evolution of vector technologies and genome editing tools is steadily advancing gene therapy from experimental concept toward clinical reality.

Gene Therapy Strategies for Cystic Fibrosis

Viral Vector Delivery Systems

Adenoviral Vectors

Adenoviral vectors were among the earliest platforms investigated for CF gene therapy due to their high transduction efficiency and ability to infect both dividing and non-dividing airway epithelial cells [28]. Early clinical studies in the 1990s demonstrated proof-of-concept CFTR gene transfer using adenovirus-based vectors. However, several limitations emerged. First, adenoviral vectors induced strong innate and adaptive immune responses, causing inflammation, reducing transgene expression, and limiting repeat dosing capability. Second, these vectors typically require receptor access at the basolateral surface of airway epithelium, making efficient apical delivery challenging. Third, gene expression was transient, lasting only days to weeks. These limitations, combined with serious adverse events in early gene therapy trials for other diseases, reduced enthusiasm for adenoviral approaches in CF despite their strong gene transfer capability.

Aden-o-Associated Viral (AAV) Vectors

Aden-o-associated virus (AAV) vectors have gained significant interest due to their favourable safety profile, low immunogenicity compared to adenovirus, and ability to support relatively stable transgene expression in some tissues [29]. AAV vectors do not integrate into the host genome (in most cases), reducing insertional mutagenesis risk. However, several challenges limit AAV application in CF. First, AAV has a small packaging capacity (~4.7 kb), which is problematic because the CFTR cDNA (~4.5 kb) leaves minimal space for strong promoters and regulatory elements. Second, AAV-mediated airway transduction from the apical surface is inefficient in mature differentiated epithelium. Third, pre-existing neutralizing antibodies in a significant proportion of the population prevent vector uptake and preclude repeat administration. Fourth, thick CF mucus further impedes vector penetration to target cells.

Despite these challenges, next-generation AAV capsids with improved airway tropism are under development. Recent preclinical studies have engineered AAV variants with enhanced apical surface binding and mucus-penetrating properties, demonstrating improved transduction efficiency in CF airway models. However, clinical translation remains limited by the repeat dosing challenge, as immune responses prevent effective re-administration a significant limitation for a chronic progressive disease requiring sustained CFTR expression over decades.

Lentiviral Vectors

Lentiviral vectors represent a particularly promising viral delivery platform for CF because they can integrate into the host genome, offering potential for long-term transgene expression in airway epithelial cells, including dividing progenitor populations. This genomic integration capability is advantageous for achieving durable correction, particularly if airway basal stem cells are successfully transducer. Lentiviral vectors can accommodate the full-length CFTR coding sequence along with appropriate regulatory elements. Furthermore, lentiviral vectors pseudotyped with envelope proteins that enhance airway epithelial cell entry (such as Sendai virus F/HN glycoproteins) have shown promising preclinical results with improved transduction efficiency following airway delivery.

A third-generation lentiviral vector pseudotyped with Sendai virus envelope proteins (rSIV.F/HN) carrying full-length CFTR transgene (BI 3720931) has demonstrated encouraging preclinical safety and efficacy profiles. This vector achieved efficient airway transduction with sustained CFTR expression in animal models. Based on these promising results, a first-in-human clinical trial was initiated in 2024 by Boehringer Ingelheim, though the trial was subsequently terminated in early 2026 after viewing phase 1/2 data. While the specific reasons for termination were not publicly disclosed, this highlights the ongoing challenges in translating preclinical promise into clinical success.

Despite setbacks, lentiviral vectors remain promising due to their potential for durable expression through genomic integration. However, this same property raises concerns about insertional mutagenesis the risk that vector integration could disrupt important host genes or activate oncogenes. Modern self-inactivating lentiviral designs with stringent control over integration sites are being developed to minimise this risk.

Non-Viral Vector Delivery Systems

Lipid-Based Systems

Non-viral lipid-based delivery systems, including cationic liposomes and lipid nanoparticles (LNPs), offer several advantages over viral vectors: reduced immunogenicity, absence of viral recombination risk, scalable manufacturing, and most importantly, the ability for repeated administration critical for chronic diseases like CF. These systems can encapsulate plasmid DNA or RNA and promote cellular uptake through membrane fusion or endocytosis.

A landmark clinical trial, the UK CF Gene Therapy Consortium Phase IIb study, evaluated monthly nebulisation of a non-viral CFTR plasmid (pGM169) complexed with cationic lipid GL67A over 12 months in CF patients. This represented the largest CF gene therapy trial conducted and demonstrated that repeated non-viral gene delivery could stabilise lung function decline (FEV_1) compared to placebo, though it did not significantly improve lung function overall. The study confirmed safety and feasibility of repeat dosing but highlighted that even with monthly administration, clinical benefits remained modest, likely due to low transfection efficiency and transient expression.

Recent advances in LNP technology, largely driven by mRNA vaccine development during the COVID-19 pandemic, have dramatically improved delivery efficiency and safety profiles. Modern LNPs incorporate ionisable lipids that facilitate endosomal escape, polyethylene glycol (PEG) lipids that enhance stability and circulation, and targeting ligands that can direct nanoparticles to specific cell types. In 2024, UCLA researchers developed optimised LNPs capable of delivering CRISPR-Cas9 components along with a complete functional CFTR gene to human airway cells [17]. Their formulation achieved remarkable efficiency, with only 3-4% of cells receiving the therapeutic gene yet resulting in 88-100% restoration of normal CFTR channel activity across the cell population. This outsized effect was achieved by engineering the replacement gene to maximise protein production once inside cells.

Similarly, a 2025 study demonstrated that LNP-delivered CRISPR-Cas9 and adenine base editors achieved 70% genome editing efficiency in lung stem cells of CF mouse models, with sustained gene expression exceeding 660 days and over 95% correction of CF-related defects. These recent advances demonstrate that non-viral delivery systems are rapidly approaching and, in some cases, matching the efficiency of viral vectors while maintaining superior safety and repeat dosing profiles.

The collaboration between Intellia Therapeutics and ReCode Therapeutics, announced in 2024, aims to combine CRISPR-Cas9 DNA writing technology with selective organ targeting (SORT) LNPs to correct CFTR mutations directly in patient lungs via homology-directed repair (HDR) [18]. This approach represents the cutting edge of non-viral gene therapy, targeting patients who cannot benefit from CFTR modulators, particularly those with Class I mutations.

Polymer-Based Systems

Polymer-based gene delivery systems use synthetic or natural polymers such as polyethyleneimine (PEI), chitosan, dendrimers, or biodegradable polyesters to condense nucleic acids into nanoscale complexes. These systems offer tun-able properties polymer chemistry can be customised to control particle size, surface charge, biodegradability, mucus penetration, and intracellular trafficking. Polymer-based vectors protect DNA/RNA from enzymatic degradation and can be functionalized with targeting ligands or cell-penetrating peptides to enhance cellular uptake.

In CF applications, polymer vectors have shown promise in experimental models, particularly when engineered with mucus-penetrating properties. However, their transfection efficiency generally remains lower than viral vectors, and some polymers can induce cytotoxicity if not carefully optimised. Polymer-based systems remain an active research area with potential for scalable, safe, and cost-effective gene delivery, particularly as manufacturing technologies advance.

Routes of Gene Delivery

Airway/Inhalation-Based Delivery

Airway delivery via inhalation represents the primary route for CF gene therapy, as it enables direct topical administration to target respiratory epithelial cells while minimising systemic exposure and associated risks [33]. Gene vectors can be delivered via nebulisation, aerosols, or dry powder inhalers. Particle size is critical particles of 1-5 μm diameter are optimal for deposition in small airways and alveoli, where therapeutic intervention is most needed.

Advantages of airway delivery include: (1) direct access to disease-affected cells; (2) high local gene expression with minimal systemic distribution; (3) non-invasive administration enhancing patient acceptance; and (4) feasibility for repeat dosing if necessary. However, several challenges complicate efficient airway gene delivery. The thick, viscous mucus characteristic of CF airways represents a major physical barrier, trapping gene vectors and preventing contact with epithelial cells. Mucociliary clearance mechanisms rapidly remove particles from airways before cellular uptake can occur. Additionally, the airway epithelium is highly organised with tight junctions that limit paracellular penetration, and apical surface receptors required for vector entry may be limited or sequestered.

Strategies to overcome these barriers include: engineering mucus-penetrating nanoparticles with neutral surface charge and dense PEG coatings that prevent mucin binding; pre-treatment with mucolytic agents to reduce mucus viscosity; use of permeation enhancers to transiently open tight junctions; and pseudo-typing viral vectors with envelope proteins that recognise apical surface receptors. The rapid progress in LNP technology has been particularly impactful, with modern formulations demonstrating significantly improved mucus penetration and epithelial cell uptake compared to early-generation liposomes [17].

Systemic Delivery

Systemic gene delivery via intravenous injection allows widespread vector distribution throughout the body, potentially reaching multiple affected organs simultaneously. For CF, systemic delivery could theoretically address extra-pulmonary manifestations such as pancreatic, intestinal, and hepatobiliary disease. However, systemic delivery presents significant challenges including: rapid clearance by the reticuloendothelial system (liver and spleen); difficulty achieving adequate vector concentrations in target organs; off-target effects in non-diseased tissues; and pronounced immune responses.

For CF specifically, systemic delivery has not been widely pursued because: (1) the lung is the primary determinant of morbidity and mortality and is accessible via inhalation; (2) achieving therapeutic CFTR expression in airways via systemic routes requires very high vector doses with associated toxicity risks; and (3) the blood-gas barrier limits vector extravasation from circulation into airway epithelium. Consequently, airway-directed delivery remains the preferred route for CF gene therapy, though systemic approaches may be

considered for addressing extra-pulmonary manifestations in the future [3].

Emerging Gene Editing and RNA-Based Approaches

CRISPR-Cas9 and Base Editing

CRISPR-Cas9 gene editing represents a transformative approach that can permanently correct CFTR mutations at the genomic level rather than adding an extra gene copy. The system uses a guide RNA to direct the Cas9 endonuclease to a specific DNA sequence, where Cas9 creates a double-strand break (DSB). The cell's repair mechanisms can then be harnessed to introduce desired genetic changes either through homology-directed repair (HDR) using a provided DNA template or through non-homologous end joining (NHEJ).

For CF, CRISPR-Cas9 could theoretically correct pathogenic mutations such as F508del, restoring endogenous CFTR expression under its native regulatory control. Successful correction in airway basal stem cells could provide lifelong therapeutic benefit as these corrected stem cells continuously regenerate the epithelium. However, several challenges must be addressed. First, delivery of CRISPR components (Cas9 protein or mRNA, guide RNA, and donor template DNA) to sufficient numbers of airway cells remains difficult despite advances in LNP technology. Second, HDR-mediated precise correction is inefficient in non-dividing cells such as mature airway epithelium, though recent advances in prime editing may circumvent this limitation. Third, off-target effects—unintended edits at genomic sites similar to the target sequence raise safety concerns requiring rigorous evaluation.

Base editing and prime editing represent next-generation CRISPR technologies that offer improved precision. Base editors chemically convert one DNA base to another (e.g., C→T or A→G) without creating DSBs, reducing risks of unwanted genomic rearrangements. Prime editors combine a Cas9 nickase with reverse transcriptase, guided by a prime editing guide RNA (pegRNA), enabling insertions, deletions, and all 12 possible base-to-base conversions without DSBs or donor templates. A 2025 study demonstrated that LNP-delivered adenine base editors achieved 70% editing efficiency in lung stem cells of CF mice, with sustained correction for over 660 days.

Multiple research groups are actively pursuing CRISPR-based therapies for CF. The Intellia-ReCode collaboration aims to use CRISPR-Cas9 DNA writing with HDR delivered via SORT LNPs to correct CFTR mutations in patient lungs [18]. While no CRISPR CF therapy has yet reached clinical trials, the pace of technological advancement suggests this may change within the next few years.

mRNA-Based Therapies

Messenger RNA (mRNA) therapy offers an alternative approach where CFTR mRNA is delivered to airway cells, enabling transient production of functional CFTR protein. Unlike DNA-based approaches, mRNA does not integrate into the genome, eliminating insertional mutagenesis risk and potentially offering improved safety. The mRNA is translated in the cytoplasm without requiring nuclear entry a significant advantage since nuclear import represents a major barrier for plasmid DNA delivery.

The success of mRNA COVID-19 vaccines validated LNP-mRNA technology as a highly effective platform, with optimised formulations achieving efficient cellular delivery and protein expression. These advances are now being

translated to therapeutic protein replacement strategies. For CF, CFTR mRNA encapsulated in LNPs could be administered via inhalation, with repeated dosing (e.g., weekly or monthly) maintaining therapeutic protein levels.

Advantages of mRNA therapy include: rapid design and production; transient expression reducing long-term safety concerns; no genomic integration risk; and applicability to all mutation types. Challenges include: the need for repeated dosing due to transient expression (mRNA and resulting protein degrade within days); potential immunogenicity from RNA recognition by pattern recognition receptors; and mRNA instability requiring careful formulation with modified nucleosides and optimised LNPs.

Recent advances in mRNA stability, codon optimisation, and LNP formulation have dramatically improved expression duration and reduced immunogenicity. While no CFTR mRNA therapy has yet entered clinical trials for CF, the technology is rapidly maturing and represents a promising mutation-agnostic approach that could complement or compete with DNA-based gene therapies.

Stem Cell-Based Approaches

Stem cell-based gene therapy combines ex vivo gene editing with cell transplantation. Airway basal stem/progenitor cells would be isolated from a CF patient, genetically corrected using CRISPR or lentiviral transduction in culture, expanded, and then transplanted back into the patient's airways. This approach offers several advantages: precise gene correction can be verified before transplantation; off-target effects can be screened and undesirable clones eliminated; and corrected stem cells could provide long-term epithelial renewal.

However, significant technical challenges remain. Efficient isolation, expansion, and differentiation of human airway basal stem cells while maintaining their stem cell properties is difficult. Methods for delivering cells to airways and achieving engraftment at sufficient levels to provide therapeutic benefit are not well established. Furthermore, the inflammatory and infection-prone CF airway environment may compromise transplanted cell survival and engraftment.

Despite these challenges, recent progress in airway organdie technologies and stem cell biology has renewed interest in this approach. Patient-derived airway organdies carrying CFTR mutations can be gene-edited using CRISPR, with correction verified by functional assays before potential autologous transplantation. While stem cell-based gene therapy remains largely experimental for CF, it represents a potential curative strategy warranting continued investigation.

Preclinical and Clinical Progress

Key Preclinical Studies

Preclinical research in CF gene therapy began soon after the CFTR gene was cloned in 1989. Early studies using CF mouse models, ferrets, and pigs demonstrated that viral (adenovirus, AAV, lentivirus) and non-viral (cationic liposome) vectors could deliver CFTR genes to airway epithelium and restore chloride transport function. These proof-of-principle studies established feasibility but also revealed significant barriers including thick mucus, low transduction efficiency from apical surface, rapid epithelial turnover, and immune responses limiting gene expression duration.

Advanced CF animal models, particularly CF ferrets and pigs that recapitulate human disease

pathology more faithfully than mice, have provided valuable insights into therapeutic requirements. These studies demonstrated that even partial CFTR restoration (5-20% of normal) could normalise airway surface liquid properties and improve bacterial clearance, validating the concept of a low therapeutic threshold [6].

More recently, patient-derived airway organoidsc and air-liquid interface cultures have enabled testing of gene editing approaches in human CF cells. A 2023 proof-of-concept study demonstrated that LNP-delivered CRISPR components could correct CFTR mutations in patient-derived organoidsc, restoring chloride channel function. In 2024, UCLA researchers showed that CRISPR-mediated gene insertion into 3-4% of human airway cells could restore 88-100% of CFTR activity, demonstrating the outsized therapeutic effect achievable with optimised gene design [17].

A landmark 2025 study achieved 70% genome editing efficiency in lung stem cells of CF mice using LNP-delivered base editors, with sustained correction lasting over 660 days and reversal of CF-related pathophysiology. These recent preclinical advances demonstrate that efficient, durable genetic correction of CF is technically feasible and provide strong rationale for advancing optimised delivery systems into clinical trials.

Major Clinical Trials and Outcomes

Clinical translation of CF gene therapy has progressed gradually over three decades, with over 25 clinical trials involving approximately 600 patients conducted to date. The first clinical gene transfer studies began in the early 1990s, initially targeting nasal epithelium as a surrogate for lung tissue to assess safety. These Phase I trials using adenoviral vectors and cationic liposomes established that gene transfer was safe but achieved only transient, low-level CFTR expression with minimal clinical benefit.

One of the largest CF gene therapy trials was the UK CF Gene Therapy Consortium Phase IIb study, a randomised, double-blind, placebo-controlled trial evaluating monthly nebulisation of a non-viral CFTR plasmid (pGM169) complexed with cationic lipid GL67A over 12 months in 116 CF patients. This trial represented a landmark achievement as the first demonstration that gene therapy could stabilise lung function decline. Results showed a modest but statistically significant 3.7% relative benefit in FEV_1 compared to placebo. While this represented proof-of-concept that repeated non-viral gene delivery could provide clinical benefit, the effect size was smaller than current CFTR modulators, and the trial highlighted the need for more efficient delivery systems.

Viral vector clinical trials have also been conducted. AAV-CFTR vectors were evaluated in several Phase I/II trials but demonstrated limited efficacy due to inefficient airway transduction, neutralizing antibodies, and inability for repeat dosing. Adenoviral vector trials were discontinued due to inflammatory responses and transient expression [28].

Most recently, a lentiviral vector approach (BI 3720931) entered Phase I/II clinical testing in 2024. This third-generation lentiviral vector pseudotyped with Sendai virus envelope proteins showed promising preclinical results with efficient airway transduction and sustained CFTR expression. However, Boehringer Ingelheim terminated the trial in early 2026 after reviewing Phase I/II data, though specific reasons were not publicly disclosed. This setback underscores the ongoing challenges in translating preclinical success into clinical efficacy.

Lessons Learned from Clinical Trials

Three decades of CF gene therapy clinical trials have provided invaluable lessons that continue to guide current research directions. First, delivery efficiency remains the primary limitation. The CF airway environment characterized by thick mucus, rapid mucociliary clearance, epithelial barrier properties, and limited apical surface receptors present formidable obstacles to achieving sufficient gene transfer for meaningful clinical benefit. Even with monthly dosing in the UK consortium trial, only transient, low-level gene expression was achieved.

Second, immune responses limit sustained expression and repeat dosing, particularly for viral vectors. Pre-existing immunity and vector-induced adaptive immune responses restrict the duration of transgene expression and preclude effective re-administration a critical limitation for chronic progressive diseases requiring lifelong treatment.

Third, nasal epithelium studies do not reliably predict lower airway outcomes. While nasal instillation is safer and easier for Phase I studies, the nasal epithelium differs significantly from bronchial and alveolar tissues in receptor expression, mucus properties, and disease manifestations. Consequently, therapies must ultimately be tested directly in the lower airways to assess true therapeutic potential.

Fourth, the therapeutic threshold is achievable but requires optimised delivery. Clinical trials consistently demonstrated that gene transfer is safe and can achieve functional CFTR expression, but efficiency must be substantially improved to reach the 5-20% correction threshold estimated for clinical benefit [6]. The recent preclinical demonstrations that 3-4% of corrected cells can restore 88-100% of CFTR activity (through optimised gene design) and that 70% editing efficiency is achievable in lung stem cells (with advanced LNPs and base editors) suggest that clinically meaningful correction is now within reach [17].

Fifth, non-viral vectors enable repeat dosing but require improved efficiency. The UK Phase IIb trial confirmed that monthly non-viral administration is safe and can stabilise lung function, validating the repeat dosing advantage of non-viral systems. However, the modest effect size indicates that either delivery efficiency must be improved or dosing frequency increased to achieve clinically competitive benefits.

These lessons have catalysed development of next-generation approaches including: mucus-penetrating nanoparticles; immune-evasive vectors; genome editing for permanent correction; mRNA-based transient expression; and stem cell targeting for durable benefit. The convergence of these technological advances positions the field for renewed clinical trials with significantly improved prospects for success.

Challenges and Barriers

Delivery Efficiency

Efficient delivery of therapeutic genes to sufficient numbers of airway epithelial cells remains the most fundamental challenge in CF gene therapy. Multiple biological barriers impede effective gene transfer. The thick, viscous mucus layer characteristic of CF airways traps and immobilises gene vectors, preventing contact with underlying epithelial cells. Mucociliary clearance mechanisms rapidly eliminate inhaled particles, providing only a brief window for cellular uptake. The airway epithelium presents a tight barrier with limited apical surface receptors for vector binding and internalisation. Following cellular uptake, vectors must escape endosomal compartments to

access the cytoplasm (for mRNA/RNA) or reach the nucleus (for DNA), with many vectors becoming trapped and degraded in endosomes.

For viral vectors, receptor availability on the apical surface is often limited, as many viral receptors are preferentially expressed on basolateral surfaces inaccessible from the airway lumen. For non-viral vectors, cellular uptake efficiency and intracellular trafficking remain suboptimal compared to viral systems. Even when vectors successfully transduce cells, the percentage of cells achieving therapeutic gene expression is typically low early clinical trials achieved only 1-5% transduction efficiency.

Strategies to improve delivery efficiency include: engineering mucus-penetrating particles with neutral surface charge and PEG coatings; using mucolytic pre-treatments; developing vectors with enhanced apical surface tropism; incorporating cell-penetrating peptides or fusogenic proteins to enhance cellular uptake and endosomal escape; and targeting airway basal stem cells that persist long-term and regenerate epithelium. The recent demonstration that optimised LNPs can achieve 70% editing efficiency in lung stem cells represents a major breakthrough, though this was achieved in mouse models and requires validation in humans.

Immune Responses

Immune recognition and clearance of gene therapy vectors and gene products represent major obstacles to achieving sustained therapeutic expression. For viral vectors, both innate and adaptive immune responses are triggered. Innate immunity recognises viral capsid proteins and genetic material through pattern recognition receptors, activating inflammatory pathways and recruiting immune cells that eliminate transducer cells. Adaptive immunity generates neutralizing antibodies against viral capsids that block vector uptake and cytotoxic T lymphocytes that kill transducer cells expressing viral or transgene antigens.

Pre-existing immunity is particularly problematic. A significant proportion of the population has neutralizing antibodies against common AAV serotypes due to natural environmental exposure, preventing effective vector transduction and precluding treatment of seropositive individuals. Even in seronegative individuals, vector administration induces adaptive immune responses that prevent effective repeat dosing a critical limitation for diseases requiring sustained long-term expression.

For non-viral vectors and mRNA therapies, immunogenicity is generally lower but not absent. Plasmid DNA contains unmethylated CpG motifs that trigger innate immune responses through Toll-like receptor 9. Unmodified mRNA activates innate immunity through TLR3, TLR7, RIG-I, and MDA5 pathways. While chemical modifications (pseudouridine, N1-methylpseudouridine) significantly reduce mRNA immunogenicity, residual inflammatory responses can still occur.

Strategies to evade immune responses include: engineering viral capsids to escape neutralizing antibodies; using immune-evasive serotypes or synthetic capsids; incorporating immunosuppressive regimens during vector administration; modifying mRNA with nucleoside analogs to reduce innate immunity; targeting immune-privileged sites or cell types; and using non-integrating, transiently expressing vectors that minimise chronic antigen presentation. The inherent lower immunogenicity of non-viral vectors represents a key advantage, enabling the repeat dosing demonstrated in the UK Phase IIb trial.

Long-Term Gene Expression

Achieving durable, sustained gene expression over years or decades remains a major challenge, particularly for non-integrating vectors. Adenoviral and episomal plasmid DNA vectors provide only transient expression lasting days to weeks due to dilution during cell division, degradation, and loss from cells. Even AAV vectors, which can persist as episomes in non-dividing cells for months to years in some tissues, face limited durability in the continuously renewing airway epithelium where basal cells divide and differentiate, diluting out non-integrated genetic material.

This transient expression necessitates repeat dosing to maintain therapeutic benefit. While feasible for non-viral vectors with low immunogenicity, repeat dosing increases treatment burden, cost, and cumulative safety risks. For viral vectors, immune responses typically prevent effective re-administration, making sustained expression from a single treatment essential but difficult to achieve.

Integrating vectors such as lentiviruses offer the potential for permanent, lifelong expression by incorporating the therapeutic gene into the host genome. However, genomic integration carries risks of insertional mutagenesis disruption of important host genes or activation of oncogenes leading to malignancy. While modern self-inactivating lentiviral vectors with improved safety profiles have reduced this risk, long-term safety monitoring remains essential.

CRISPR-mediated genome editing represents the most promising approach for permanent correction. By directly repairing pathogenic mutations or inserting functional genes at safe harbor genomic loci, editing could provide lifelong correction without ongoing expression from episomal vectors. Recent demonstrations of 70% editing efficiency in lung stem cells with sustained correction exceeding 660 days in mice provide strong proof-of-concept. However, translating this efficiency to humans while ensuring safety requires careful optimisation of delivery, editing precision, and long-term monitoring for off-target effects.

Safety and Ethical Considerations

Gene therapy safety concerns encompass multiple potential risks that must be carefully evaluated. Insertional mutagenesis from integrating vectors could disrupt tumor suppressor genes or activate oncogenes, potentially causing cancer. While modern lentiviral vectors use self-inactivating designs and chromatin insulator elements to minimise this risk, long-term follow-up is essential. Off-target editing with CRISPR-Cas9 could cause unintended mutations at genomic sites similar to the target sequence, potentially disrupting gene function or causing chromosomal aberrations. Rigorous off-target prediction algorithms, highly specific guide RNA design, and delivery of Cas9 as transient mRNA or protein rather than DNA can minimise but not eliminate this risk.

Immune toxicity from inflammatory responses to vectors or edited cells could cause tissue damage. While modern approaches have reduced immunogenicity, monitoring for inflammatory responses remains critical. Genotoxicity from vector components or DNA damage repair processes could cause genomic instability. Comprehensive preclinical genotoxicity testing and long-term clinical monitoring are required.

Ethical considerations include informed consent complexity explaining sophisticated gene therapy mechanisms, uncertain long-term outcomes, and balancing potential benefits against risks to

patients and families. Equitable access concerns arise as gene therapies typically require specialised manufacturing and delivery, with costs potentially exceeding $1-2 million per patient, raising questions about affordability and global access. Gremlin editing concerns while CF gene therapy targets somatic cells only, accidental germ line modification must be absolutely prevented, requiring careful targeting and verification. Long-term monitoring responsibilities extend for decades to detect late-onset adverse effects, requiring patient commitment and healthcare system infrastructure.

Regulatory frameworks have evolved to address these concerns. Agencies such as the FDA and EMA require extensive preclinical safety testing including biodistribution, genotoxicity, and immunotoxicity studies; phased clinical trials with intensive monitoring; long-term follow-up protocols extending 5-15 years; and risk mitigation strategies such as patient registries. While these requirements appropriately prioritise safety, they also extend development timelines and costs, creating tension between therapeutic urgency for patients with progressive disease and the need for comprehensive long-term safety data.

DISCUSSION

This comprehensive review demonstrates that gene therapy represents the most scientifically rational and potentially transformative approach to cystic fibrosis treatment, addressing the fundamental genetic defect rather than downstream consequences or partial protein function restoration. The monogenic nature of CF, accessibility of the respiratory tract, relatively low therapeutic threshold (5-20% of normal CFTR activity), and mutation-agnostic potential of gene addition approaches collectively position CF as an ideal target for gene-based interventions.

Current therapies have dramatically improved CF outcomes, with median survival increasing from less than 10 years in the 1960s to over 40 years currently. However, conventional therapies (antibiotics, mucolytics, physiotherapy) require continuous lifelong administration, impose substantial treatment burden, and cannot prevent progressive structural lung damage. CFTR modulators represent a major breakthrough, with triple combination therapy (elexacaftor-tezacaftor-ivacaftor) achieving unprecedented clinical benefits including 10-14% FEV_1 improvements, reduced exacerbations, and improved quality of life [27][28]. Yet modulators remain limited by mutation specificity (leaving ~10% of patients ineligible), incomplete CFTR restoration, inability to reverse established damage, lifelong requirement, and extremely high costs limiting global access [30].

Gene therapy offers potential solutions to these limitations. Gene addition approaches can benefit all mutation types including Class I mutations producing no protein, where modulators fail. Successfully targeting airway basal stem cells could provide durable or permanent correction, potentially requiring only single or infrequent treatments rather than daily medications. Recent technological advances have dramatically improved feasibility. UCLA researchers demonstrated that lipid nanoparticle-delivered gene editing could restore 88-100% of CFTR function despite inserting genes into only 3-4% of cells, achieved through optimised gene design maximising protein production [17]. Another study achieved 70% editing efficiency in lung stem cells with sustained correction exceeding 660 days in mice. The Intellia-ReCode collaboration is advancing CRISPR-based correction using selective organ targeting LNPs [18].

However, significant challenges remain before gene therapy becomes routine clinical practice. Delivery efficiency must be improved to consistently achieve therapeutic thresholds in human airways, overcoming thick mucus, rapid mucociliary clearance, and epithelial barriers. Immune responses must be minimised to enable sustained expression and potential repeat dosing. Long-term safety requires rigorous evaluation, particularly for integrating vectors and genome editing approaches with potential off-target effects. Clinical trials must demonstrate not just molecular correction but meaningful, sustained clinical benefits including improved lung function, reduced exacerbations, and enhanced quality of life.

The lessons learned from three decades of clinical trials are now being translated into next-generation approaches with substantially improved prospects. Non-viral lipid nanoparticle technologies validated through mRNA vaccine success are being applied to deliver genes, mRNA, and editing tools with dramatically improved efficiency and safety compared to early-generation liposomes. CRISPR-based editing offers permanent correction potential that transient gene addition cannot match. mRNA therapies provide a safe, repeatable approach for mutation-agnostic CFTR expression without genomic integration risks. Stem cell targeting and ex vivo editing approaches offer potential for durable correction with rigorous safety screening before transplantation.

The field stands at an inflection point. While the 2026 termination of the Boehringer Ingelheim lentiviral trial represents a setback, it should be contextualised within the broader trajectory of rapid technological advancement. The pace of progress in delivery systems, editing precision, and preclinical efficacy has accelerated dramatically in recent years. Technologies that were purely experimental five years ago highly efficient lung-targeting LNPs, base editors, prime editors are now demonstrating remarkable preclinical efficacy and moving toward clinical translation.

For gene therapy to fulfil its promise, several priorities must be addressed. First, optimised delivery systems must be advanced into well-designed clinical trials with appropriate endpoints and patient populations. Second, long-term safety monitoring infrastructure must be established to track patients for years to decades after treatment. Third, manufacturing and cost considerations must be addressed to ensure equitable global access if therapies prove successful. Fourth, combination approaches should be explored gene therapy might complement rather than replace CFTR modulators, with modulators providing immediate benefit while gene therapy offers long-term correction.

The ultimate vision is a curative or near-curative treatment providing lifelong CFTR function restoration from a single or limited number of treatments, eliminating the chronic progressive nature of CF and transforming it from a life-limiting disease to a manageable condition or even a cured disease. While challenges remain, the scientific foundation, technological capabilities, and clinical experience now exist to make this vision increasingly realistic. Continued investment in translational research, innovative clinical trial designs, and collaborative efforts between academia, industry, and patient advocacy organisations will be essential to translate the remarkable recent preclinical advances into transformative clinical realities for the global CF community.

CONCLUSION

Cystic fibrosis remains a severe, life-limiting genetic disorder caused by CFTR mutations

leading to defective epithelial ion transport and progressive multi-system organ damage, particularly in the respiratory tract. While conventional therapies and CFTR modulators have transformed CF from a uniformly fatal childhood disease to a manageable chronic condition with median survival exceeding 40 years, significant unmet needs persist. Current therapies require lifelong daily administration, impose substantial treatment burden, cannot reverse established structural damage, and remain unavailable or ineffective for approximately 10% of patients with certain mutation types.

Gene therapy represents the most scientifically rational approach to CF treatment by directly targeting the underlying genetic defect rather than downstream consequences. The monogenic nature of CF, accessibility of the respiratory tract, low therapeutic threshold requiring only 5-20% CFTR restoration, and mutation-agnostic potential of gene addition approaches collectively position CF as an ideal candidate for gene-based interventions. Recent technological advances including highly efficient lipid nanoparticles, CRISPR-based genome editing, mRNA therapies, and stem cell targeting have dramatically improved the feasibility of achieving durable or permanent CFTR correction.

Preclinical studies have demonstrated remarkable progress, with recent reports of 70% genome editing efficiency in lung stem cells and sustained correction exceeding 660 days in animal models. Clinical trials spanning three decades have established safety of gene delivery and provided invaluable lessons regarding delivery optimisation, immune evasion, and therapeutic endpoints. While clinical efficacy has been modest to date and recent trial terminations highlight ongoing challenges, the pace of technological innovation and preclinical success suggests that clinically meaningful gene therapies are increasingly within reach.

Major challenges remaining include: improving delivery efficiency to consistently achieve therapeutic thresholds in human airways; minimising immune responses to enable sustained expression; ensuring long-term safety particularly for integrating vectors and genome editing; demonstrating durable clinical benefits in well-designed trials; and addressing manufacturing and cost considerations to enable equitable global access.

The convergence of advanced delivery technologies, precise genome editing tools, improved understanding of CF pathophysiology, and lessons from clinical experience positions the field for renewed clinical trials with substantially improved prospects for success. While gene therapy is not yet a clinical reality for CF patients, the scientific foundation and technological capabilities now exist to make curative or near-curative treatment a realistic goal for the coming decade.

Continued investment in translational research, innovative clinical trial designs, long-term safety monitoring infrastructure, and collaborative efforts between researchers, clinicians, industry, regulators, and patient advocates will be essential to translate recent remarkable preclinical advances into transformative clinical realities. The ultimate vision providing CF patients with lifelong CFTR function restoration from a single or limited number of treatments, eliminating chronic disease progression, and transforming CF from a life-limiting disease to a cured condition is increasingly achievable and represents one of the most promising frontiers in genetic medicine.

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