# Crispr-Based Gene Editing a Novel Pharmacological Approach for Cystic Fibrosis Treatment

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***Abstract*—Cystic fibrosis (CF) remains a serious life-threatening genetic disorder despite advances in CFTR modulator therapies. Approximately 10% of CF patients lack effective pharmacological options due to rare or nonsense mutations. CRISPR-based gene editing technologies offer transformative potential by directly correcting CFTR mutations at the genomic level, providing durable or curative therapeutic alternatives. This comprehensive review synthesizes current knowledge on CRISPR/Cas9, prime editing, and base editing approaches for CF, emphasizing recent preclinical advances in delivery systems, editing eGciency, and functional validation in patient-derived models. Particularly promising are Lung Selective Organ Targeting (SORT) lipid nanoparticles (LNPs) achieving ~60% lung basal cell transfixion and restoring CFTR function in patient-derived bronchial epithelial cells with 16% gene correction eGciency. Editing modifications, such as 4-FEN (FEN1 nuclease fusion to PEmax), demonstrate enhanced specificity with minimal off-target effects. This review discusses current clinical translation challenges, safety considerations, and the potential for combining CRISPR gene editing with existing CFTR modulators. We also examine emerging strategies including base editing, pegRNA optimization, and non-viral delivery technologies that collectively represent the next frontier in CF therapeutics, potentially enabling the first disease-modifying or curative treatments for all CF genotypes by the early 2030s.**

***Index Terms*—CRISPR/Cas9, gene editing, cystic fibrosis, CFTR, prime editing, lipid nanoparticles, delivery, gene therapy.**

## I. INTRODUCTION

Cystic fibrosis, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, remains a debilitating genetic disorder affecting over 70,000 individuals worldwide, Over 2,000 disease-causing mutations have been identified, classified into six functional categories based on the type of CFTR protein defect produced. The most common mutation, deltaF508 (ΔF508), accounts for approximately 70% of CF cases globally and results from a three-nucleotide deletion causing abnormal protein folding and proteasomal degradation [1,2].

While recent approval of CFTR modulator therapies—particularly triple-combination agents like elexacaftor/tezacaftor/invocator (Trikafta)—has revolutionized CF care for responsive genotypes, these therapies remain inaccessible to approximately 10% of CF patients, particularly those with nonsense (Class I) or rare mutations [2][3]. Furthermore, CFTR modulators provide symptomatic management rather than permanent disease correction, necessitating lifelong administration at significant cost (~$300,000 annually in the United States). [1,2,3].

CRISPR-based gene editing technologies represent a paradigm shift toward potentially curative CF therapeutics by enabling direct correction of mutated CFTR genes at the genomic level. [2] Since the discovery of CRISPR/Cas9 in 2012, multiple editing platforms have been developed, each with distinct advantages: classical CRISPR/Cas9 with homology-directed repair (HDR) for large insertions/deletions; prime editing (PE) for precise small insertions/deletions without double-strand breaks; and base editing for single-nucleotide variants[3,4,5].

This comprehensive review synthesizes recent preclinical advances in CRISPR gene editing for CF, emphasizing delivery innovations, editing technologies, and functional validation in relevant models. We examine the current landscape of CRISPR-based CF therapies, identify key translation challenges, and discuss future directions toward clinical implementation.

Cystic Fibrosis: Unmet Medical Need and Limitations of Current Therapies Disease Burden and CFTR Mutations

Cystic fibrosis is an autosomal recessive disorder with an incidence of approximately 1 in 3,500 live births in persons of Northern European descent [1]. The disease is caused by loss or dysfunction of the CFTR protein, a cAMP-regulated chloride channel essential for regulating ion and water balance across epithelial surfaces. Impaired chloride secretion results in thick, viscous secretions that obstruct airways, pancreatic ducts, and other organ systems, leading to chronic infections, progressive organ damage, and multi-system complications [1,2].

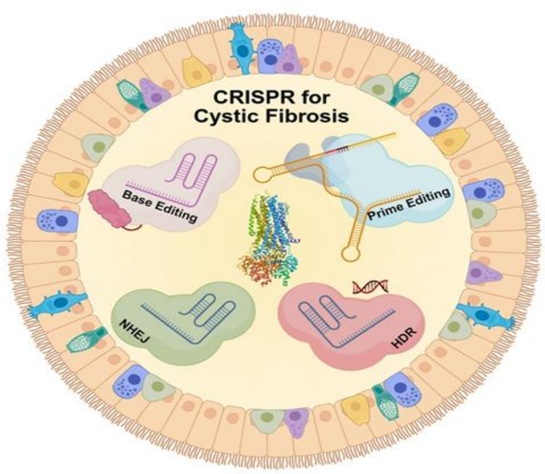


Figure: CRISPR Cystic fibrosis

CFTR mutations are classified functionally into six categories[1]:

Class I: Defective protein synthesis (nonsense, frameshift, splice-site mutations; 2-5% of cases)

Class II: Defective traGcking (ΔF508, most common; ~70% of cases in North America and Europe)

Class III: Disordered gating (e.g., G551D)

Class IV: Reduced chloride conductance

Class V: Reduced CFTR quantity

Class VI: Increased protein degradation

## II. CURRENT CFTR MODULATOR THERAPIES AND THEIR LIMITATIONS

The introduction of CFTR modulators has significantly improved the management of cystic fibrosis by targeting the underlying defects in CFTR protein function. Ivacaftor (Kalydeco), a CFTR potentiator, was the first modulator approved for patients with Class III gating mutations such as G551D and has been shown to improve lung function by approximately 10% in terms of predicted $FEV_1$. Subsequently, Lumacaftor/Ivacaftor (Orkambi), which combines a CFTR corrector with a potentiator, was developed for patients carrying Class II mutations and provides modest improvements in lung function of approximately 3–4%. Tezacaftor/Ivacaftor (Symdeko) further improved treatment outcomes by offering comparable efficacy with a more favorable safety profile. The most significant advancement came with the approval of the triple-combination therapy Elexacaftor/Tezacaftor/Ivacaftor (Trikafta) in 2019, which has demonstrated clinical benefits in nearly 90% of individuals with cystic fibrosis.

Despite these remarkable achievements, several important limitations remain. Approximately 10% of patients carrying rare or uncommon CFTR mutations still lack effective treatment options. Individuals with Class I nonsense mutations, which introduce premature stop codons into the CFTR gene, derive little or no benefit from currently available modulators. In addition, the extremely high cost of these therapies, often exceeding $300,000 per year, creates substantial barriers to access and affordability worldwide. Another major drawback is that CFTR modulators require lifelong administration because they do not permanently correct the underlying genetic defect. Furthermore, even in responsive patients, these therapies generally restore only partial CFTR function, and many individuals continue to experience progressive pulmonary decline and disease-related complications over time.

Collectively, these limitations highlight the need for more effective disease-modifying and potentially curative treatment strategies. As a result, gene-editing and gene-therapy approaches, particularly CRISPR-based technologies, have emerged as promising alternatives capable of directly correcting the genetic mutations responsible for cystic fibrosis.

CRISPR Gene Editing Technologies for CFTR Correction

Classical CRISPR/Cas9 with Homology-Directed Repair (HDR)

Mechanism: CRISPR/Cas9 creates a double-strand break (DSB) at the target site guided by a single guide RNA (sgRNA), which is then repaired via homology-directed repair (HDR) using exogenous donor DNA templates[4, 5].

For CFTR:
ΔF508 insertion: Requires 3-bp insertion into deletion site
Nonsense mutations (e.g., G542X): Requires precise point correction via HDR Other indels: Direct in-frame correction possible [4, 5]

Advantages:
Precise correction of large indels and point mutations
Proven eGcacy in multiple disease models

Limitations:
Low HDR eGciency in non-dividing cells (1-20% typical).
High indel formation via non-homologous end joining (NHEJ) Off-target activity due to sequence similarity, DSB-related genotoxicity concerns

## III. PRECLINICAL CF RESULTS

Patient-derived airway basal stem cells: ~10-20% correction of ΔF508. [4,5]
Ferret CF models: Successful CFTR gene targeting with corrected ion transport in organoid derivatives[2]
In vivo mouse lungs (SORT LNP delivery): Up to 2.34% G542X correction in whole lung tissue, with restoration of CFTR function in patient-derived intestinal organoids (~20% swelling with forskolkin activation).
Prime Editing (PE): Advanced "Search-and-Replace" Editing Mechanism: Prime editing uses a catalytically dead Cas9 nickase fused to reverse transcriptase (RT) and a prime editing guide RNA (pegRNA) containing the desired edit sequence. The nCas9 creates a single nick, the pegRNA primer hybridizes and serves as an RT template to install the edit, followed by enzymatic removal of the flap and mismatch repair. [4, 5, 6]
Advantages:
No DSBs → minimal indels and off-target effects
Compatible with any PAM sequence (depending on variant)
Scarless editing without foreign DNA insertion
Works in non-dividing cells
Programmable for precise small indels (including ΔF508). [4,5,6]

Limitations:
Moderate eGciency at specific genomic loci
AT-rich regions (including CFTR F508del site) present particular challenges
Transient RT expression may limit editing window

Larger construct size complicates delivery[4,6]

- PE Variants:
- PE2: Improved Cas9 nickase and RT
- PE3: PE2 + secondary sgRNA nicking non-edited strand; improved eGciency
- PE4: PE2 + MLH1dn (mismatch repair inhibitor); enhanced eGciency but potential safety concerns.
- PEmax: Codon-optimized RT + nuclear localization signals; eGcient but without extra factors.
- PE7: LA protein fusion for enhanced stability.

Preclinical CF Results:
4-FEN modification (FEN1 nuclease fusion to PEmax): Moscow Research Centre for Medical Genetics, 2025Patient-derived airway basal cells (F508del/F508del)
Editing eGciency: 2.13% (2.13-fold improvement over standard PEmax at 1.0%)
Minimal off-target mutations at predicted sensitive loci.
γH2AX foci assay: No increased DNA damage compared to controls Challenge: AT-rich CFTR region remains diGcult; <2.13% eGciency below estimated 10% clinical threshold
PE2-NG with optimized pegRNAs: Parallel Moscow study
Up to 24% F508del correction in basal airway cells with optimized pegRNA design.
Higher eGciency than 4-FEN but requires extensive pegRNA screening. [6]
Base Editing: Direct Nucleotide Conversion
Mechanism: Base editors fuse deaminase enzymes (adenine base editors [ABEs] or cytosine base editors [CBEs]) to catalytically dead Cas9, enabling direct

A•T↔G•C or C•G↔A•T conversions without DSBs. [4,5].

For CFTR:
Point mutations (e.g., R785*, G542X nonsense mutations): Direct A-to-G conversion EGciency: 67-98% in optimized protospacers[4,5]

Advantages:
No DNA breaks; ultra-low off-target activity
Very high eGciency for point mutations
Reversible editing for safety studies
Rapid implementation for new targets

Limitations:
Only corrects single-nucleotide variants
Cannot correct ΔF508 (3-bp deletion)
PAM site requirements; limited targeting options for some mutations.
Bystander editing (off-target nucleotides within editing window). [4]

Preclinical CF Results:
Patient organoid models: 87-98% correction of Class I nonsense mutations in optimized spacers.
Published eGciency data from systematic reviews
Comparative Summary of CRISPR Approaches

Delivery Systems: Addressing the Critical Bottleneck
Delivery Challenges in Cystic Fibrosis
The lungs of CF patients present unique barriers to CRISPR component delivery[2,3,4,5]:

Anatomical complexity: Extensive alveolar surface (~70 m²), vascularization, multiple cell types (basal cells, ciliated cells, Club cells, immune cells).
Mucus barrier: Thick, viscous secretions caused by CFTR dysfunction obstruct local delivery

Epithelial permeability: Limited transcellular transport; tight junctions restrict paracellular passage.
Immunogenicity: Viral vectors risk triggering innate immune responses, preventing repeat dosing [3, 4]
Integration risk: Viral vectors (AAV, lentivirus) carry oncogenic integration potential [3,5]
Viral Vector Approaches (Historical Context)

## IV. ADENO-ASSOCIATED VIRUSES (AAV)

Advantages: Small,size,low immunogenicity
Limitations: Packaging capacity limits options (cannot fit large Cas9 + RT constructs); limited repeat dosing due to neutralizing antibodies [3,4]
CF application: Zhou et al. (2019) demonstrated helper-dependent adenoviral vectors delivering CRISPR/Cas9 to pig airway epithelium with functional restoration [2,3]
Adenoviruses:

Advantages: Large packaging capacity, high transduction eGciency
Limitations: Significant immunogenicity, safety concerns; limited repeat dosing [3,4]
Current status: Viral vectors face clinical translation barriers, motivating non-viral alternatives.
Lung Selective Organ Targeting (SORT) Lipid Nanoparticles: Breakthrough Delivery Platform
Technology: SORT LNPs are engineered non-viral nano particles incorporating ionizable amino lipids, phospholipids, cholesterol, PEG-lipids, and permanently cationic lipids (SORT molecules) that enable systemic intravenous delivery with tissue selectivity [5]

Optimization for CF:
Wei et al. (Nature Communications, 2023) developed improved Lung SORT LNPs:

- DOTAP10 LNPs: Optimized for in vitro cell studies; 5-30% DOTAP incorporated
- DOTAP40 LNPs: Optimized for in vivo lung targeting; 40% DOTAP incorporated

Composition ofDOTAP40 SORT LNPs (optimized molar ratio): 5A2-SC8 (ionizable amino lipid): 36 mol% DOPE (phospholipid): 20 mol% Cholesterol: 40 mol% PEG-DMG: 4 mol%
DOTAP (permanently cationic, SORT molecule): 40% incorporation [5]

Delivery Capability & Tropism:
In vivo lung transfection: ~60% of lung basal cells achieved after single IV injection (0.3 mg/kg mRNA)[5] Cell-type specificity: Effective targeting of lung epithelial cells, including basal cell progenitors critical for long-term epithelium restoration[5]Multi-cargo delivery: Successfully encapsulates and delivers Cas9 mRNA, sgRNA, and

ssDNA HDR templates simultaneously[5] Safety profile: Minimal liver toxicity vs. DDAB LNPs; repeated dosing (up to 3 weekly administrations) maintains eGcacy without immune tolerance[5]

Advantages of SORT Lipid Nanoparticles over Viral Vectors:
SORT lipid nanoparticles (LNPs) offer several advantages compared with conventional viral delivery systems. They exhibit low immunogenicity, allowing repeated administration without inducing strong immune responses. Unlike viral vectors, SORT LNPs do not integrate into the host genome, thereby minimizing the risk of insertional mutagenesis. Their manufacturing process is rapid, scalable, and independent of species-specific constraints. Furthermore, systemic intravenous administration enables efficient delivery of therapeutic cargo while circumventing the thick mucus barrier characteristic of cystic fibrosis lungs.

## V. PRECLINICAL IN VIVO EVALUATION OF SORT LNPS

In Vivo CRISPR/Cas9-Mediated HDR Correction in Cystic Fibrosis Models:
The therapeutic potential of SORT LNP-mediated CRISPR/Cas9 gene editing has been demonstrated in several preclinical cystic fibrosis models. In a G542X cystic fibrosis mouse model carrying a nonsense mutation, animals received three weekly intravenous administrations of DOTAP40 LNPs encapsulating Cas9 mRNA, sgRNA, and a single-stranded DNA HDR template targeting the mutant CFTR gene. This treatment achieved up to 2.34% correction of the CFTR G542X mutation throughout lung tissue. Importantly, next-generation sequencing analysis did not reveal any detectable off-target editing events. Functional assessment using intestinal organoids derived from treated mice demonstrated approximately 20% forskolin-induced swelling, indicating successful restoration of CFTR-dependent chloride transport compared with untreated controls. [6, 7,8 ]

Studies in Patient-Derived F508del Human Bronchial Epithelial Cells:
Ex vivo studies were conducted using patient-derived F508del human bronchial epithelial (HBE) cells. In undifferentiated cells, treatment with DOTAP10-HDR LNPs containing Cas9 mRNA, sgRNA, and an HDR repair template resulted in approximately 16% correction of the ΔF508 mutation. Functional analysis revealed complete restoration of CFTR-mediated chloride transport, reaching levels comparable to wild-type cells. Western blot analysis further confirmed the expression of mature CFTR protein following gene correction, whereas untreated cells lacked detectable protein expression.
In fully differentiated airway epithelial cultures maintained at an air–liquid interface, editing efficiency was reduced to approximately 4%. This lower efficiency is likely attributable to reduced cellular proliferation and the presence of a thick mucus layer, which more closely mimics the physiological environment of CF airways. Nevertheless, measurable improvement in chloride transport was observed, and co-administration of the CFTR modulator VX-809 significantly enhanced functional recovery. [6, 7,8 ]

Synergistic Effects of Combination Therapy:
Combination therapy involving gene correction and pharmacological modulation produced superior outcomes compared with either treatment alone. DOTAP10-HDR treatment restored CFTR activity to approximately 110% of wild-type levels, whereas VX-809 alone achieved about 53% restoration. Notably, combining DOTAP10-HDR with VX-809 increased CFTR function to nearly 126% of wild-type activity. These findings suggest a synergistic interaction between genome editing and CFTR modulators, highlighting a potential therapeutic strategy for patients with partial gene correction. [6, 7,8 ]

## VI. SAFETY CONSIDERATIONS AND OFF-TARGET ASSESSMENT

Genomic Safety of CRISPR-Based Editing:
The safety profile of CRISPR technologies remains a major consideration for clinical translation. Conventional CRISPR/Cas9 systems may exhibit off-target editing rates ranging from 10–40% due to unintended double-strand breaks at homologous genomic sites. In contrast, prime editing generally demonstrates lower off-target activity, typically between 5–10%, while base editing shows the highest

precision, with off-target rates often below 1%.[6, 7,8]

Safety Evaluation of Prime Editing:
A 2025 study evaluating the 4-FEN prime editing system performed deep sequencing analysis of six predicted off-target loci associated with tumor-related genes, including ARID4A, BTLA, IL1RAP, TOX2, WNK2, and GYPE. No statistically significant increase in insertions, deletions, or single-nucleotide variants was detected at these sites when compared with control samples. Examination of the target CFTR locus also revealed no reproducible insertion or deletion events, with only two low-frequency single-nucleotide variants occurring at frequencies below 0.5%.
Similarly, studies utilizing SORT LNP-mediated Cas9 delivery in G542X cystic fibrosis mice reported no detectable off-target genome modifications, further supporting the safety of this delivery platform. [6, 7,8 ]

Genotoxicity Assessment:
The genotoxic potential of the 4-FEN prime editing system was evaluated using γH2AX foci analysis, a widely accepted marker of DNA double-strand breaks. Cells treated with 4-FEN PEmax displayed a median of 1.26 γH2AX foci per nucleus, comparable to untreated control cells (1.64 foci per nucleus) and substantially lower than irradiated positive controls (7.89 foci per nucleus). These results indicate that prime editing does not significantly increase DNA damage and maintains a favorable safety profile. [6, 7,8 ]

Clinical Translation Considerations:
Several critical questions remain regarding the clinical implementation of CRISPR-based therapies for cystic fibrosis. Long-term persistence of genomic corrections in airway basal stem cells must be established to ensure durable therapeutic benefit. The potential immunogenicity associated with repeated administration of SORT LNPs also requires further investigation. Additional concerns include the possibility of off-target editing in non-target tissues following systemic delivery, the long-term risk of tumorigenesis, and the compatibility of corrected cells with ongoing CFTR modulator therapy.
Current projections suggest that Investigational New Drug (IND) applications for CRISPR-based CF therapies may be submitted within the next few years. Early-phase clinical trials are expected to focus primarily on safety, dose optimization, and comprehensive monitoring of off-target effects. Initial studies will likely target patients with nonsense mutations such as G542X or those who are ineligible for existing CFTR modulator therapies. [6, 7,8 ]

Remaining Scientific Challenges and Future Directions:
Although significant progress has been made, several challenges continue to limit the clinical application of CRISPR technologies in cystic fibrosis. HDR-mediated editing remains inefficient in slowly dividing airway cells, necessitating further optimization of alternative approaches such as prime and base editing. The AT-rich nature of the CFTR F508del region complicates pegRNA design and editing efficiency. Additionally, the inflammatory environment of CF lungs may affect both delivery efficiency and the long-term persistence of edited cells.
Future research is expected to focus on next-generation editing systems with enhanced precision, improved pegRNA design strategies, advanced delivery technologies, and combination therapies integrating gene editing with CFTR modulators. These innovations may ultimately facilitate the development of safe, durable, and broadly applicable curative treatments for cystic fibrosis. [6, 7,8 ]

## VII. FUTURE DIRECTIONS AND EMERGING STRATEGIES

Advances in Next-Generation Gene Editing Technologies:
Continuous improvements in base editing technologies are expected to enhance therapeutic outcomes by minimizing unintended bystander edits through the development of more precise deaminase enzymes. Efforts are also focused on improving the delivery efficiency of dual adenine base editor (ABE) and cytosine base editor (CBE) systems, thereby expanding their applicability to a broader range of cystic fibrosis-causing mutations, particularly nonsense variants.

Prime editing technologies are likewise undergoing significant refinement. Future strategies include the development of optimized pegRNA libraries tailored to specific genomic targets, incorporation of mismatch repair inhibitory factors such as MLH1dn to increase editing efficiency, and the integration of prime editing with base editing approaches to address complex mutations. Furthermore, advanced prime editor variants, including PE7 and PE8, are being designed with enhanced reverse transcriptase stability to improve editing performance and durability.

Alternative genome-editing approaches are also under investigation. Peptide nucleic acid (PNA)-based triplex-forming oligonucleotides have emerged as a potential alternative to Cas9-mediated editing and have demonstrated encouraging results in preclinical cystic fibrosis lung models. In addition, transcriptional modulation strategies employing small molecules or epigenetic editing tools may increase the expression of residual CFTR protein or activate compensatory ion transport pathways. Another promising approach involves the insertion of a complete CFTR complementary DNA (cDNA) sequence to achieve full genetic correction, although further optimization is required before clinical application.

Combination Therapeutic Strategies:

Combining CRISPR-based gene editing with existing CFTR modulator therapies represents a promising therapeutic strategy. Studies using patient-derived human bronchial epithelial cells have demonstrated synergistic effects, suggesting that pharmacological modulators may enhance CFTR function even when gene-editing efficiency is incomplete.

Another emerging approach involves the combination of CRISPR technologies with immune-modulating therapies. Anti-inflammatory agents may help create a more favorable airway environment for gene editing, improve the survival of edited cells, and potentially reduce the frequency of pulmonary exacerbations following treatment.

Multiplex genome editing is also being explored as a means of simultaneously correcting multiple disease-causing mutations within the same patient. This strategy could address secondary defects such as splice-site mutations while enabling the introduction of additional beneficial genetic modifications, including enhanced airway hydration and mucus clearance mechanisms. [9,10,11]

Expanding the Target Patient Population:

Future CRISPR-based therapies have the potential to benefit patient populations that currently have limited treatment options. Individuals carrying Class I nonsense mutations, including G542X, W1282X, and R553X, represent particularly attractive candidates for base-editing approaches. These mutations are largely unresponsive to currently available CFTR modulators and therefore constitute important targets for early clinical trials.

In addition, more than 1,700 rare CFTR genotypes have been identified, each occurring at very low frequencies. The growing understanding of these mutations is driving the development of personalized, mutation-specific gene-editing therapies tailored to individual patient needs.

The application of gene editing may also extend beyond pulmonary disease manifestations. Potential future indications include correction of mutations in pancreatic tissue to address cystic fibrosis-related diabetes, editing of hepatocytes for the treatment of CF-associated liver disease, and genetic interventions aimed at restoring fertility in affected individuals. [9,10,11]

Manufacturing and Clinical Scale-Up:

The successful clinical translation of CRISPR therapies will require large-scale and cost-effective manufacturing processes. Production of SORT lipid nanoparticles is currently based on ethanol-dilution methods, with efforts underway to establish scalable manufacturing systems suitable for clinical use. Regulatory development is progressing through the preparation of Chemistry, Manufacturing, and Controls (CMC) documentation required for clinical approval.

Manufacturing processes for CRISPR components are also becoming increasingly standardized. Cas9 messenger RNA can be produced through established in vitro transcription methods under Good Manufacturing Practice (GMP) conditions. Similarly, sgRNAs are synthesized using conventional oligonucleotide manufacturing technologies, while synthetic biology platforms facilitate rapid generation of customized single-stranded DNA HDR templates. Current estimates suggest that treatment costs may range from approximately $50,000 to $100,000 per patient for limited-dose regimens. [9,10,11]

## VIII. COMPARISON WITH OTHER CURATIVE APPROACHES

Lung Transplantation:
Lung transplantation remains the only established curative option for patients with advanced cystic fibrosis. However, its clinical utility is constrained by a severe shortage of donor organs, limited long-term survival, and the requirement for lifelong immunosuppressive therapy, which increases the risk of infections and malignancies. CRISPR-based therapies could potentially eliminate the need for transplantation by providing durable correction of the underlying genetic defect and offering long-term therapeutic benefits that may exceed transplant survival outcomes. [9,10,11]

Viral Gene Therapy:
Several gene therapy approaches employing adeno-associated viral (AAV) and lentiviral vectors are currently under investigation. Although these strategies have demonstrated partial restoration of CFTR expression, their effectiveness remains limited by restricted vector packaging capacity, immune responses that hinder repeat dosing, and relatively short durations of transgene expression.
In contrast, CRISPR-mediated genome editing offers the possibility of permanent genetic correction. Because the modification occurs directly within the patient's genome, therapeutic benefits may persist long after treatment, potentially requiring only a single administration or a small number of doses. [9,10,11]

CFTR Modulator Therapy
CFTR modulators currently represent the standard of care for many individuals with cystic fibrosis. These agents improve the function of defective CFTR proteins but do not address the underlying genetic mutation and therefore require lifelong administration.
CRISPR-based therapies offer several advantages over conventional modulators, including the possibility of permanent correction of disease-causing mutations, applicability to previously untreatable genotypes, and potential reductions in long-term healthcare costs associated with chronic pharmacological treatment. As genome-editing technologies continue to advance, they may ultimately provide a more comprehensive and durable therapeutic solution for cystic fibrosis. [9,10,11]

## IX. CONCLUSION AND FUTURE OUTLOOK

Cystic fibrosis remains one of the most challenging inherited genetic disorders despite remarkable advances in CFTR modulator therapy. Although currently available treatments have significantly improved life expectancy and quality of life for many patients, they are not curative and remain ineffective for a substantial proportion of individuals carrying rare or nonsense CFTR mutations. Consequently, there is a pressing need for therapeutic strategies that can directly address the root genetic cause of the disease and provide long-term or permanent correction of CFTR dysfunction.
CRISPR-based gene editing technologies represent a revolutionary advancement in precision medicine and have emerged as promising candidates for achieving this goal. The development of CRISPR/Cas9, prime editing, and base editing has expanded the possibilities for correcting a wide range of CFTR mutations with increasing levels of accuracy and safety. Traditional CRISPR/Cas9-mediated homology-directed repair offers the ability to correct large deletions and insertions, while prime editing provides versatile "search-and-replace" capabilities without generating double-strand DNA breaks. Base editing further enhances therapeutic potential by enabling highly efficient correction of single-nucleotide mutations, particularly nonsense mutations that currently lack effective treatment options.
Recent preclinical studies have provided compelling evidence supporting the feasibility of CRISPR-based interventions in cystic fibrosis. Patient-derived airway epithelial cells, organoids, and animal models have demonstrated successful mutation correction and restoration of functional CFTR protein activity. Notably, the emergence of Lung Selective Organ Targeting (SORT) lipid nanoparticles has addressed one of the most significant obstacles in gene editing—the efficient delivery of therapeutic components to the lungs. These non-viral delivery systems have shown the capacity to target airway

basal cells, achieve meaningful editing efficiencies, support repeated administration, and minimize the risks associated with viral vectors. The successful correction of CFTR mutations in patient-derived bronchial epithelial cells and the restoration of chloride transport function highlight the substantial therapeutic promise of this approach.

Another major advantage of modern CRISPR technologies is the continued improvement in genomic safety. Prime editing and base editing significantly reduce the occurrence of unwanted insertions, deletions, and off-target modifications compared with conventional gene-editing methods. Studies evaluating DNA damage responses and off-target mutation profiles have reported encouraging safety outcomes, supporting further progression toward clinical development. Nevertheless, rigorous long-term evaluation remains essential to ensure genomic stability, assess tumorigenic potential, and determine the durability of therapeutic benefits following treatment.

Despite these advances, several important challenges must be addressed before widespread clinical implementation becomes a reality. Editing efficiency in slowly dividing or non-dividing airway stem cells remains a critical limitation, particularly for homology-directed repair-based strategies. Additional work is needed to optimize pegRNA design, enhance editing machinery performance, improve tissue-specific delivery, and overcome the biological barriers created by the thick mucus environment characteristic of cystic fibrosis lungs. Furthermore, large-scale manufacturing, regulatory approval pathways, long-term patient monitoring, and treatment affordability will play crucial roles in determining the accessibility and success of future gene-editing therapies.

The integration of CRISPR-based therapeutics with existing treatment modalities may offer additional benefits. Combination approaches involving gene editing and CFTR modulators have already demonstrated synergistic restoration of CFTR function in preclinical studies. Such strategies could maximize clinical outcomes while gene-editing technologies continue to evolve toward higher correction efficiencies and broader applicability across diverse CFTR mutations. Future developments may also include multiplex editing, epigenetic modulation, targeted gene insertion, and personalized mutation-specific therapies tailored to individual patients.

In summary, CRISPR-mediated gene editing represents a transformative and potentially curative approach for cystic fibrosis. Rapid progress in genome-editing platforms, delivery technologies, and safety optimization has brought the field closer than ever to clinical translation. Although important scientific, regulatory, and economic challenges remain, current evidence strongly suggests that CRISPR-based therapies could fundamentally change the treatment paradigm for cystic fibrosis, moving beyond symptom management toward permanent genetic correction. With continued research, collaborative innovation, and carefully designed clinical trials, gene editing has the potential to provide effective, durable, and universally applicable treatments for patients with cystic fibrosis, ultimately improving survival, quality of life, and long-term health outcomes worldwide.

## REFERENCES

[1] Cystic Fibrosis - StatPearls. NCBI Bookshelf. 2024 December 10. https://www.ncbi.nlm.nih.

[2] Nicosia, L., & Harrison, P. T. (2025). CRISPR for cystic fibrosis: Advances and insights from a systematic review. Molecular Therapy, 33(11), 5901–5907. https://doi.org/10.1016/j.ymthe.2025.09.046

[3] Wei, T., et al. (2023). Lung SORT LNPs enable precise homology-directed repair mediated CRISPR/Cas genome correction in cystic fibrosis models. Nature Communications, 14, 7610. https://doi.org/10.1038/s41467-023-42948-2

[4] Porteus, M. H., et al. (2020). Correcting deltaF508-CFTR through CRISPR-Cas9 ribonucleoprotein-mediated genome editing in primary human airway basal stem cells. Science Translational Medicine (referenced in multiple reviews; consult primary literature for complete citation).

[5] Volodina, O. V., et al. (2025). Prime Editing Modification with FEN1 Improves F508del Variant Editing in the CFTR Gene in Airway Basal Cells. International Journal ofMolecular Sciences, 26(16), 7943. https://doi.org/10.3390/ijms26167943

[6] Geurts, M. H., et al. (2024). Functional rescue of F508del-CFTR through revertant mutations. Bio.Rxiv preprint. https://www.biorxiv.org/content/10.1101/2024.08.28.610115v1
[7] Ensinck, A., et al. (2021). On the Corner of Models and Cure: Gene Editing in Cystic Fibrosis. Frontiers in Pharmacology, 12, 662110. https://doi.org/10.3389/fphar.2021.662110
[8] Hart, S. L., et al. (2021). CRISPR/Cas9 Gene Editing Therapies for Cystic Fibrosis. Expert Opinion on Biological Therapy, 21(2), 183-194. (Reference from systematic review; consult primary literature for exact DOI).
[9] Cystic Fibrosis Foundation. (2022). Gene Editing for Cystic Fibrosis. https://www.cff.org/re search-clinical-trials/gene-editing-cystic-fibrosis
[10] Konstan, M. W., et al. (2021). Epidemiologic Study of Cystic Fibrosis (ESCF): 25 Years of Observational Research. Pediatric Pulmonology, 56(5), 823-836. https://doi.org/10.1002/ppul.25248
[11] Davis PB. Cystic fibrosis since 1938. Am J Respir Crit Care Med. 2006 Mar 01;173(5)