Review Article

Current and future therapeutic approaches of CFTR and airway dysbiosis in an era of personalized medicine

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Abstract

Cystic fibrosis (CF) is a life-threatening genetic disorder caused by mutations in the CFTR gene. This leads to a defective protein that impairs chloride transport, resulting in thick mucus buildup and chronic inflammation in the airways. The review discusses current and future therapeutic approaches for CFTR dysfunction and airway dysbiosis in the era of personalized medicine. Personalized medicine has revolutionized CF treatment with the advent of CFTR modulator therapies that target specific genetic mutations. These therapies have significantly improved patient outcomes, slowing disease progression, and enhancing quality of life. It also highlights the growing recognition of the airway microbiome's role in CF pathogenesis and discusses strategies to modulate the microbiome to further improve patient outcomes. This review discusses various therapeutic approaches for cystic fibrosis (CFTR) mutations, including adenovirus gene treatments, nonviral vectors, CRISPR/cas9 methods, RNA replacement, antisense-oligonucleotide-mediated DNA-based therapies, and cell-based therapies. It also introduces airway dysbiosis with CF and how microbes influence the lungs. The review highlights the importance of understanding the cellular and molecular causes of CF and the development of personalized medicine to improve quality of life and health outcomes.

Keywords: Airway dysbiosis, CFTR therapeutic approaches, personalized medicine

Introduction

Approximately 90,000 people worldwide are affected by the CFTR, a life-threatening genetic disorder.[1] Both CF gene alleles must have mutations for the disorder to exist, which is autosomal recessive.[2] Among the hereditary conditions that Caucasians are most likely to develop and die due to mutation on the long arm of chromosome 7 (1q. 31.2). CFTR, the gene for cystic fibrosis transmembrane conductance, was discovered in 1989. It is made up of 27 exons that collectively account for approximately 215 kb of the genetic sequence.[3,4] Lung function is the most serious issue, as the CFTR gene generates an ion channel that transfers bicarbonate and chloride across epithelial cells, impacting various organs.[5] Numerous investigations, however, have revealed that CFTR can also be produced in cells other than epithelial cells.[6] Moreover, the central, peripheral, and enteric nervous systems all express CFTR.[7,8] Although both epithelial and nonepithelial cell types, including many others, express CFTR. There are more than 2000 mutations, each with a different functional impact.[9] In consideration of the fundamental outcomes of the CFTR protein and its mutations [Figure 1].[10]

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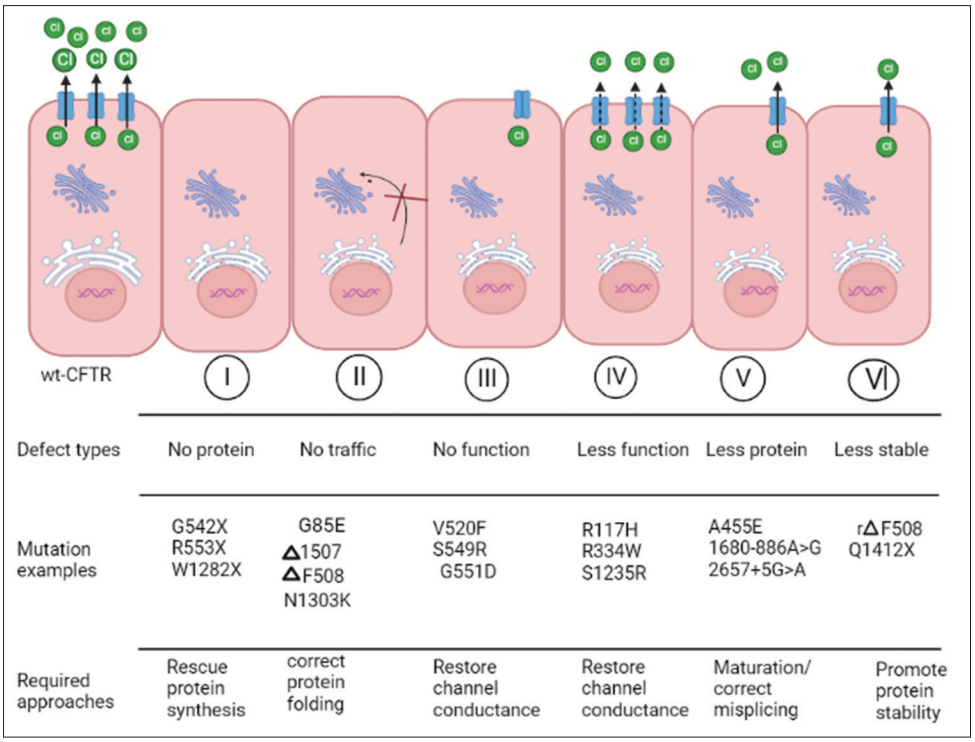

Figure 1: Types of CFTR Mutations: Distribution of CFTR Mutations into Six Functional Types Based on the Basic Molecular Defect Class I mutations do not lead to protein synthesis because the presence of premature stop codons (class Ia) or frameshifts for deletions or insertions (class Ib) precludes the translation of full-length CFTR. Class II mutants are flawed trafficking proteins because CFTR is unable to attain full folding and is degraded by the ER-associated degradation (ERAD) mechanism. Class III mutants have defective channel gating because CFTR reaches the cell surface but does not exhibit channel gating due to decreased ATP binding and hydrolysis. Class IV mutants are less functional proteins because, although the number of channels that reach the plasma membrane may be comparable to that of wt-CFTR, they exhibit decreased chloride conductance. This is true even though the number of channels that reach the plasma membrane may be similar to that of wt-CFTR. Because fewer CFTR channels are present and fewer Class V mutant proteins reach the cell surface, there is less protein maturation caused by amino acid substitution or alternative splicing. As a result, chloride transport is lost. Class VI mutations are less stable proteins because CFTR is present in the plasma

Background

The CFTR, an ion channel in epithelial cells, regulates salt and fluid balance in various exocrine organs through a transmembrane chloride and bicarbonate channel.[11] CFTR, a protein chloride channel and a member of the ATP-binding cassette (ABC) transporter family, is a protein that is produced by the CF gene. It consists of a functional regulatory region (R) with several phosphorylation consensus sites, two membrane-spanning domains (MSD1, MSD2), and two nucleotide-binding domains (NBD1, NBD2). CFTR is physiologically expressed outside of airway epithelial cells and controls the chloride ion channel [Figure 2].[12] Mutations in the CFTR cause less efficient transport of chloride and bicarbonate ions, leading to mucus formation and airway blockage, inflammation, infection persistence, and lung damage. The CFTR maintains healthy airway surface fluids through sodium ion absorption and sodium ions leaving the cell through the basolateral Na + K + pump. The CFTR protein, acting as a chloride channel, removes chloride ions from the cell. Calcium-activated channels may partially clear the cells of chloride. The CFTR's physiological expression and functionality are crucial in endocrine tissues for proper electrochemical exchanges and regulatory routes.[13]

Methodology

Searching and appraising the literature

Following a predetermined methodology, we used a procedure consistent with the 2009 Preferred Reporting Items for Systematic Reviews (PRISMA) standards to systematically review, published research. Human studies published between November 1985 and January 2023 databases from PubMed, Embase, and Cochrane database were searched. Additional searches were made for Krystal biotech, clinical trial.gov, and cystic fibrosis foundation. Clinical trial registries maintained by the European Medicines Agency, the National Institutes of Health in the United States, and the World Health Organization were also searched for comprehensiveness. To find CF therapeutic trials we employed the following key phrases: CFTR or CF and drug treatment or clinical trial on search engines.

Data extraction and screening

The review methodology utilized in this investigation was created in compliance with the PRISMA statement. Two researchers independently assessed the eligibility of the literature review and abstracts. The degree of consent in the articles chosen for full-text review and then for inclusion in the review by the two

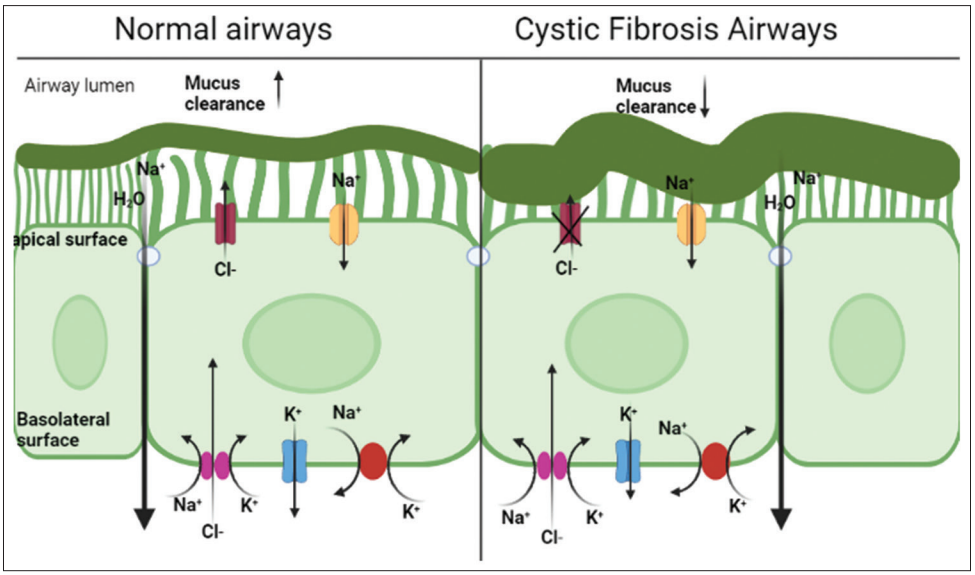

Figure 2: The cystic fibrosis defect, ASL dehydration, and impaired mucus clearance are caused by a mutation in the CFTR gene, which inhibits Cl- from being released and leads to unrestricted Na+ absorption

investigators was recorded, and disagreements were settled by the chief investigator.

Various treatment methods

According to each class of CFTR mutation, specific therapies are designed for Class I mutations, read-through therapies, and NMD inhibitors are used; for Class II mutations, correct CFTR folding and trafficking to the apical plasma membrane (PM) are used; and for Class III mutations and any mutant with residual function at the PM, boost CFTR channel function potentiators are used. Some treatments in preclinical studies are not mutation-specific, such as stem cell therapy, which is implied to cure airway tissue damage, and gene therapy to change the genome.[14]

Biological therapies

The CFTR protein can be produced through genome editing to repair the CF transmembrane conductance regulation gene, but natural obstacles like mucus, adaptive immunological responses, and intracellular restrictions hinder gene flow into the lungs.[15] Finally, gene therapies require repeated dosages since the airway epithelium is constantly regenerating. Choosing the right distribution strategy is crucial as a result. The most often used agents in CF gene therapy are adenoviruses, adeno-associated viruses (AAVs), and lentiviruses, coupled with nonviral lipoplexes and peptide nanoparticles.[14]

Viral vectors—adenoviruses

For CF gene therapy, lentiviral (LV) and AAV vectors and several intriguing vectors have been developed. In April 2022, 4D moleculartherapies were utilized to improve AAV capsids for pulmonary gene therapy.[16] The individual AAV vector 4D-710 had been administer to the patient.[17] To improve pulmonary gene therapy, SP-101, the primary potential AAV vector from Spirovant, was created using the capsid protein's site-directed mutagenesis.[18] Clinical testing is underway for a new vaccine with truncations in CFTR cDNA, compared to AAV's 4.7 kb packaging capacity, which allows for repeated use without compromising efficacy.[19] LV vectors, with their unique characteristics, are particularly beneficial for treating diseases like CF, resulting in consistent and long-lasting gene expression in animal models, and being pseudotyped in multiple laboratories.[20] To accelerate the creation of BI 3720931 as a viable long-term treatment option for patients with CF (PwCF), Boehringer–Ingelheim licensed. In October 2021, the GTC licensed the rights to the development of a lentiviral vector pseudotyped with the HN and F proteins of the Sendai virus.[21] Krystal Biotech has received approval for a Phase 1 clinical trial in Australia using a nonintegrating KB407 HSV-1-derived vector to enter PwCF's airways with full-length human CFTR.[22,23] Bandara *et al.* suggest that HD-Ad, a large and low-immunogenic adenovirus, could be a promising avenue for CF gene therapy.[24]

Non-Viral Vectors

Nonviral vector solutions are being explored due to concerns over immunogenic reactions, transgenic miss-insertions, packing large nucleic acids, and bulk production issues.[25] Liposomal vector advancements have demonstrated the safe and practical delivery of large DNA molecules.[26] In the United Kingdom, the Phase 2b trial showed a slight increase in FEV1 compared to a placebo after a year, suggesting lung function stabilization in treated patients.[27] The conclusion is speculative due to the decline in the placebo group, but no proof exists that respiratory cells expressed WT-CFTR.[28]

Polymers made of reversible poly (b-amino esters) (PBAEs) can be used to protect DNA nanoparticles from irritation and bodily harm during breathing.[29] Additionally, endosomal ejection of plasmid genetic material and stability were observed in GL67A38, which was cationic lipid-tagged.[30] Nanoparticles can transport

circular DNA fragments retaining transgenes and regulatory elements, potentially reducing immunogenicity, and enhancing integration in preclinical research.[26]

CRISPR/Cas9 Approach

In 2013, the CRISPR-associated protein 9 enzyme and clustered regularly interspaced short palindromic repeats (CRISPR), a revolutionary gene-editing technique, was published (Cas9).[31] CRISPR-Cas9 is a popular gene-editing technique for modifying mammalian cell genomes in culture due to its precise editing capabilities, versatility in silencing genes, affordability, and simplicity.[32] The guide RNA (gRNA) and the Cas9 protein enzyme are the two main components of the CRISPR-Cas9 system. The Cas9 protein makes a dsDNA break when the gRNA recognizes a certain sequence pattern at the target region, double-stranded breaks (DSB). The damaged DNA is then repaired by the cell's own DNA repair processes via non-homologous end joining (NHEJ) and homology-directed repair (HDR).[33,34] NHEJ immediately joins the broken ends and can insert or delete genes, which results in mutants.[34] The HDR reaction, which allows homologous recombination with a repair template, is effective for adding desirable genes or wild-type variations, but it occurs infrequently.[35]

Base editing: Base editing was developed to address the inefficiency of the CRIPSR-Cas9 system, which relies on DSBs for frequent insertions or deletions at DNA cleavage sites.[36] This increases the effectiveness of the CRISPR-Cas9 system. While cytosine base editors (CBEs) allow the irreversible conversion of the adenine base from C-G to T-A base pairs moderators (ABEs) catalyze the enzymatic conversion of A-T base pairs into base pairs consisting of G-C.[35]

Initial editing: Advanced CRISPR editing has become a promising tool for changing variable-length DNA sequences at specific locations. Combining a reverse transcriptase with a Cas9 protein with catalytic impairment allows for the editing of the target area using a main editing guide RNA.[37] Prime editing is a promising gene replacement therapy for CF, successfully repairing the CFTR mutation in patient-derived intestinal organoids, but its targeting efficacy varies and off-target changes occur.[38]

RNA replacement

Full-length CFTR mRNA substitution is a promising therapeutic strategy for CF variations, avoiding nucleus entry and transcriptional cellular machinery, and offering additional advantages. Therefore, mRNA substitution will need to be repeated frequently as a therapy. Arcturus therapeutics is developing one such preclinical method called ARCT-032, which is a LUNAR® lipid LNP containing a complete CFTR mRNA that can be inhaled as an aerosol. The LUNAR® formulation enables the synthesis of a fully functioning CFTR protein, which makes it easier to transport mRNA to epithelial cells.[39]

Antisense-oligonucleotide-mediated therapy

It is still difficult to distribute oligonucleotides to extrahepatic tumors effectively. To be successful in clinical settings, ASO-based medications must also overcome inadequate biological activity and off-target side effects.[40] Nebulized ASOs like SPL84 and SPL23 show potential therapeutic benefits in treating human nasal epithelial (HNE) cells in healthy individuals, while inhaled ASOs offer stability, acceptable tolerability, and minimal systemic exposure.[41,42] Crosby *et al.*'s[43] study supports the idea that inhaled ASO-based medicines can effectively decrease target mRNA in mouse lungs, supporting the successful treatment of CF patients. Sequence alignment analysis confirms the aerosol route delivery safety of ASOs like SPL84, with off-target hybridization risks. Splice-flipping ASOs control splicing, reducing hybridization risks.

Gene therapy and gene editing are two DNA-based treatments

Gene therapy uses CFTR cDNA to produce both conventional and aberrant CFTR proteins, benefiting all CF patients. However, efficient delivery methods are needed to transport the cDNA to airway epithelial cells for transcription and translation.[44,45] Cellular DNA repair is a method used in gene editing to correct mutations in the CFTR gene. It involves inserting the correct CFTR DNA sequence and nuclease into target cells, facilitated by nucleases like CRISPR/CRISPR-associated nuclease 9 (Cas9), transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs). CRISPR/Cas9 is a leading tool in CF gene-modification research due to its affordability and minimal chance of missing target breaking.[46,47] A proof-of-concept study that showed how gene editing may treat gastrointestinal organs with the p.Phe508del mutations was described in 2013.[48]

Therapies based on cells

Induced pluripotent stem cells (iPSCs) are a unique therapeutic approach that uses *ex vivo* gene editing to mimic embryonic stem cells. These fully mature cells, like fibroblasts or cutaneous cells, undergo reprogramming to differentiate into specific lineages, such as the airway epithelium, with unlimited growth potential.[44,49,50] Future CF treatments may use *ex vivo* gene editing using iPSCs. According to a study, the p.Phe508del mutation in fibroblast-derived iPSCs that transformed into airway epithelial cells was genetically fixed using the CRISPR/Cas system and TALENs.[51] Cells from the patient are taken out and reprogrammed in the laboratory to produce iPSCs, and then they are repaired for the CFTR gene mutation. After that, the altered iPSCs are transformed into basal airway stem cells, which might transform into any variety of pseudo-stratified bronchial epithelium.[52] The patient's airway epithelium's basement membrane is transplanted with corrected basal cells, creating an autologous graft that entirely corrects CFTR and repopulates the airways.[53] Engrafting iPSCs into human airways requires a pure sample of airway epithelial cells, potentially requiring up to 60 million regenerated cells for CF patients [Figure 3].[54]

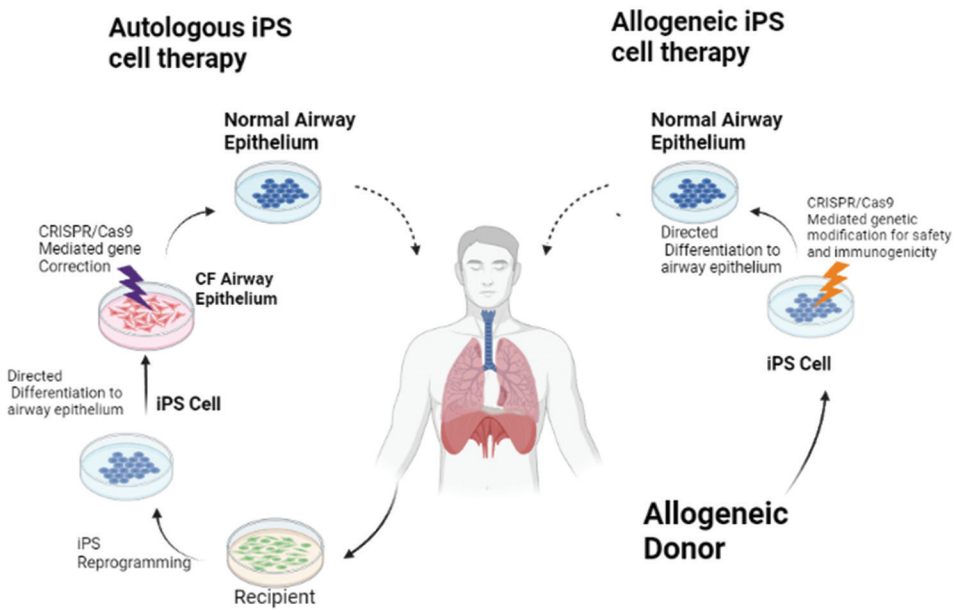

Figure 3: Using induced pluripotent stem cells, a schematic picture of cell-based therapy strategies is shown. In autologous iPS cell therapy, somatic cells from the CF patient are removed, multiplied, and reprogrammed to create induced pluripotent cells that are unique to that patient. Following differentiation into proximal airway epithelium and genetic correction to produce normal airway epithelium, these cells are transplanted back into the patient. In allogeneic iPS cell therapy, proximal airway epithelium is generated and transplanted into CF patients using a universal iPS line (from a healthy donor) that has been genetically engineered for safety and immunogenicity

Other novel approaches

A number of the initial stages pharmaceutical companies are also building cutting-edge capabilities and platforms to help with the creation of gene and RNA-based therapies. With their novel Gene Coding TM technology, CFTR's genomic locus is the target of SalioGen Therapeutics' effort to splice in a substantial "super exon" for the gene. Omega therapeutics is developing LNPs with a focus on the lungs Tessera Therapeutics and Carbon Biosciences are developing Gene Writing and CGT-001 technologies for genetic alterations, intending to deliver larger DNA treatment payloads with minimal neutralizing immunity.[40]

Airway dysbiosis with cystic fibrosis patent

The relationship between airway dysbiosis, airway inflammation, and impaired lung function in people with chronic obstructive pulmonary disease (PwCF) remains unclear.[55]

Its diverse anatomical structure, including the trachea, bronchi, bronchioles, and alveolar sacs, is consistent with pulmonary biogeography.[56,57] Bacteroidetes and Firmicutes, as well as Proteobacteria and Actinobacteria to a lesser extent, are the two major phyla of lung bacteria.[56,58] High-throughput sequencing of 16S rRNA genes has identified a "core microbiome" comprising species typical of most humans.[60] *Streptococcus, Prevotella, Fusobacterium, Veillonella, Porphyromonas, Haemophilus*, and *Neisseria* are the primary pathogens in healthy individuals.[59] The CFTR protein's absence or nonfunction significantly affects the rheology of CF mucus due to the presence of anaerobic bacteria in the oxygenation-focused organ.[57] CF airways cause dysbiosis, polymicrobial proliferation, and hyper viscosity in the respiratory system, with over 1000 species identified through shotgun metagenome sequencing and differences in nasal, nasopharyngeal, oral, and lung samples.[61-63] The CF pulmonary microbiome, comprising over 99% of the airway population, is complex and requires detailed NGS understanding, including Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Streptococcus, Prevotella, Veillonella, Rothia, Actinomyces, Gemella, Granulicatella, Fusobacterium, Neisseria, Atopobium, Porphyromonas.[64,65] The microbiome perspective enhances understanding of multidrug-resistance gene determinants by anticipating the entire "resistome" including all genes associated with antibiotic resistance in pathogenic and nonpathogenic bacteria.[66]

Virome: The mucosal environment and weakened immunity significantly impact the CF lung virus, with mutual lung viruses present in 60% of PwCF and associated with higher morbidity. Their presence causes inflammation, exacerbation, and reduced lung function due to disruption of IFN and NF-kappaB pathways.[67,68]

Mycobiome: Opportunistic pathogens seen in CF patients' sputum include fungi, such as *Aspergillus fumigatus*.[61] Nonetheless, the majority of CF airway fungi are transitory and belong to the Candida or Malassezia groups. Some fungi may interact with bacteria and/or viruses and play a role in immune response and inflammation.[62] Archaic bacteria, single-celled prokaryotic bacteria found in anaerobic environments, including humans, exhibit diversity in anatomical niches, with CF lung containing 0.1% of all archaeal phyla.[69]

Personalized care for patients with cystic fibrosis

Personalized medicine allows for the selection of effective medications based on dosage, routine, and supervision. However, optimizing CF care programs is challenging due to the lack of practical efficiency data, the addition of new drugs without understanding their effects, and the physiologic interactions between existing therapies and highly effective CFTR modulator drugs.[70] Therapeutic drug monitoring and pharmacogenomics offer a valuable method for managing PwCF, but their use is underutilized. Pharmacokinetic-pharmacodynamic (PK/PD) modeling is needed for each new medication, and ongoing research could uncover new uses for controlling side effects and enhancing therapy.

Pharmaceutical precision dosing is crucial for clinical treatment but requires specialized resources for fast drug measurement, genotyping, and pharmacologic interpretation. Therapeutic drug monitoring (TDM) can improve personalized therapy despite medication interactions by directing patient dosage procedures [Figure 4].[71]

Personalized medicine is crucial for optimizing CF care programs, but it faces challenges due to a lack of long-term data, the gradual introduction of new drugs with inadequate knowledge of their effects on existing therapies, and the physiological impacts of highly efficient CFTR modulator drugs when combined with current therapies. Novel care regimens for CF medications are progressively created, approved, and advised without sufficient data on how they may influence widely used existing medications. For example, drugs like lumacaftor, lumacaftor, and ivacaftor (ETI) have been shown to improve lung function, reduce CF symptoms, and lower the likelihood of sudden pulmonary flare-ups.[72-75] The health outcomes of CF treatments focusing on the lungs are similar, highlighting the challenge of customizing care regimens. ETI has been proven to increase sputum viscosity and mucociliary clearance, raising questions about whether other strenuous inhalation therapy is significantly aiding airway clearance and pulmonary function in patients with CF receiving ETI. Inhaled alfa-dornase and hypertonic saline promote lung health by enhancing secretion removal.[76,77] Modulators can significantly reduce bacterial airway infections, raising questions about the effectiveness of long-term suppressive inhalation antibiotics on microbiology and clinical status.[78] Chronic azithromycin treatment has been found to significantly reduce acute pulmonary exacerbations in controlled trials, despite a decrease in prevalence among most ETI users.[79-81] The increased exocrine pancreatic function seen in limited studies of high electron mobility transistors (HEMT) that were started at a young age allows some people to forgo the requirement for the replacement of pancreatic enzymes.[82,83]

Outlook and Conclusion

Since the CFTR gene was discovered 30 years ago, some significant milestones have been reached in CF experimental and clinical research. The creation of treatment approaches that target the underlying dysfunctions brought on by CF mutations has been made possible by our increased understanding of the cellular and molecular

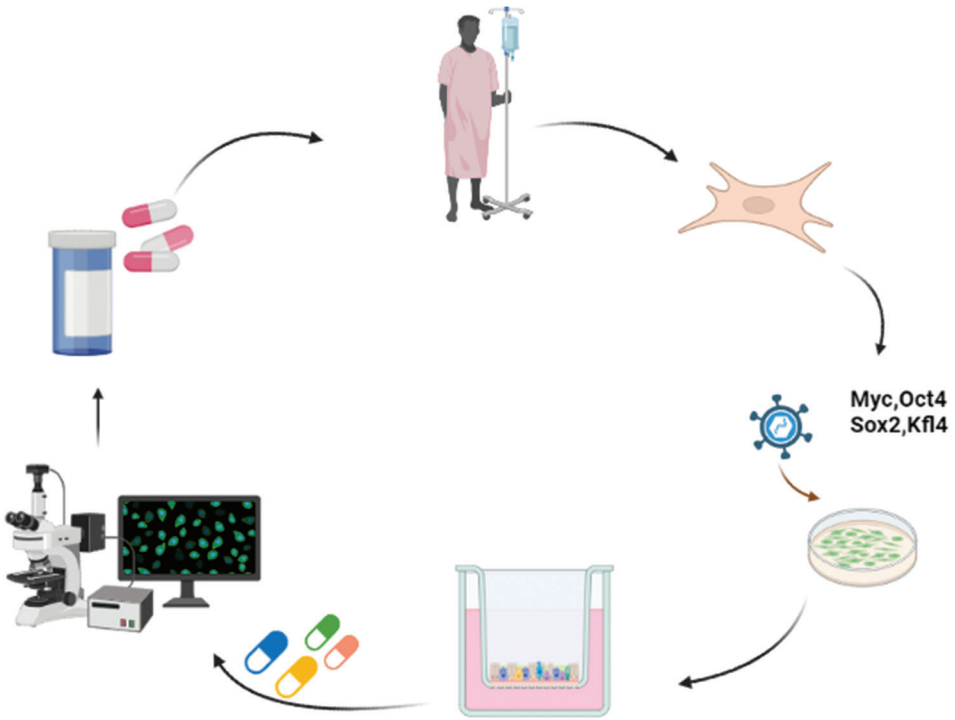

Figure 4: By introducing the reprogramming genes into adult somatic cells (such as skin fibroblasts and peripheral blood mononuclear cells), iPSCs can be produced (c-Myc, Oct4, Sox 2, and Kfl4). To test small compounds to restore the CFTR's decreased activity, iPSCs could be employed to create iPSC-derived lung, cholangiocytes, or intestinal epithelial cells

causes of the disease. Therapeutic modalities have evolved to provide personalized information and are now being used in clinics. These developments will continue to improve health outcomes, fostering a collaborative effort to enhance quality of life and health outcomes. CFTR mutations that probably call for novel therapeutic strategies, these challenging variants can be treated with the help of RNA, ASO, and gene treatments, and gene and RNA therapies can treat all CF-causing mutations. However, treatments based on genes, RNA, and ASO that are used to treat human illness are still in their infancy. Many open questions still need to be answered, such as what type of vector is best for delivering genes and how the mucous membrane works. This article explores the impact of microbiome science on cystic fibrosis (CF) lung and the influence of various microbes on its transduction and transfection effectiveness.

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Conflicts of interest

There are no conflicts of interest.

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