A Comprehensive Review on Different Treatment Strategies of Cystic Fibrosis

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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The thesis “[Different Treatment Strategies of Cystic Fibrosis]” submitted by Ramiza Islam Radoa (20146069) has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on November 24, 2025.

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Ethics Statement

This study did not involve any human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues and field research.

Abstract

Cystic Fibrosis (CF) is a chronic autosomal recessive disease that causes absence or dysfunction of Cystic Fibrosis Transmembrane Conductance Regulator protein (CFTR) that mediates ion channels. CF is a multi-organ disease that affects the function of lung, kidney, and pancreas by causing chronic inflammation, mucus accumulation, infection, loss of function, resulting in morbidity and mortality. CF is a pediatric disease in which patients die in the first year of life but the advancement in the medical field increases the longevity of patients up to 5 decades. Even though CF doesn't have any ultimate cure or prevention method, the advanced medical technology reduces the symptoms and improves the health. Early diagnosis also helps in CF treatment. Recent development of CFTR modulators target the mechanisms of mutation and reverse it and restore function. Also, many other drugs are under clinical trials to check their safety and efficacy

Keywords: Cystic Fibrosis Transmembrane Conductance Regulator, CFTR Modulator, Ion channel, Protein

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List of Acronyms

IRT	Immunoreactive Trypsinogen
ICM	Intestinal Current Measurement
PEX	Pulmonary Exacerbation
ASL	Airway Surface Liquid
ROS	Reactive Oxygen Species
HTS	High Throughput Screening

Chapter 1

Introduction

Cystic Fibrosis is an autosomal recessive disease caused by different mutations in the CFTR gene, which encodes chloride ion channels and regulates mucociliary clearance (Esposito et al,.2023). This condition is characterized by mucus buildup in the airway and digestive tract along with difficulty in breathing, persistent infection, and chronic inflammation, which leads to irreversible tissue damage that requires a lung transplant (Loza et al,.2019). Apart from lungs it damages other organs like pancreas and intestine and obstruct their functions which causes abdominal pain, malnutrition, pancreatic insufficiency and increases the chances of acute pancreatitis in cystic fibrosis patients by further decreasing the well-being of health ((Madácsy et al., 2018) CFTR protein composed of 5 domains among them two Transmembrane domains (TMD1 & TMD2) are connected to nucleotide binding domain (NBD1 & NBD2) and R domain where serine residue is phosphorylated by cAMP dependent protein kinase to open ion channel (Jaques et al., 2020). The channel transports chloride ion and bicarbonate, which are secreted into the epithelial surface, regulating the secretion of fluid. Defective CFTR genes hinder the ion secretion, as a result, water resorption occurs and builds up thick, viscous mucus which accumulates on the surface of the lung, pancreas, liver, and intestine (Kerda et al., 2012). The growth of mucus is enhanced by dehydration of the airway surface liquid (ASL), which is composed of the periciliary layer (PCL), which is covered with the mucus layer (ML). The ASL is regulated by ENaC-mediated sodium absorption along with other ions secreted by CFTR (Moore & Tarran., 2018). Any impairment in the CFTR gene increases the production of ENaC, which causes the dehydration of ASL, which further depletes the PCL and ML volume. As a result, the layers failed to trap and prevent the penetration of inhaled particles, along with decreasing the ability of cilia to remove particles through mucociliary clearance and

developing infection (Derichs et al., 2011). In the lungs, the accumulated mucus causes infection, irritation, chronic disease, and loss of function. In the digestive tract, mucus clogs the pancreas, resulting in blockage of digestive enzymes, which causes malnutrition and vitamin deficiency also causing gall bladder stones and liver disease by clogging the ducts in biliary and liver function. The haplotype poly-thyroxine tract, TG repeats, and M470V are three polymorphisms involved in the development of Cystic Fibrosis disease. The poly-t is located at the junction of intron 8 and exon 9, which influences the transcription and reduces the amount of CFTR protein. T5, T7 or T9 affects the splicing efficiency of exon 9, like in the presence of T5 CFTR will lack exon 9 where poly-T and TG -repeats are located, which produces dysfunctional protein (Huang et al., 2008). M470V polymorphism is located on exon 10 and affects the function of CFTR (Huang et al., 2008). The first case of CF was discovered in 1938 by American physician and researcher Dorothy Hansine Andersen & the incidence of the disease is 1/2500 (Scotet et al., 2020). However, medical development makes it possible to increase the life span up to 5 decades, where previously patients died in the first year, due to Cystic Fibrosis is not a pediatric disease anymore. However, 7000 cases are reported per year globally till now (Scotet et al., 2020). Besides, recent studies show that countries that implement Newborn Screening (NBS), such as Australia since 2000, have a low incidence of 1/3000, which indicates better monitoring due to NBS improving the quality and quantity of CF patients' life (Massie et al., 2012). The high incidence is observed in European countries, especially Ireland, 1/1353 - 1/25000 due to the presence of 2 copies of the F508del mutation over 55.6% population (Kere et al., 1994). In Asia, Cystic Fibrosis is very rare and in Africa, there is not enough data to determine the prevalence. The Cystic Fibrosis Transmembrane Conductance Regulatory (CFTR) gene is a transporter responsible for regulating ion channels, which maintains pH and ionic balance of airway mucosa, which also a factor for the pathogenesis of cystic fibrosis (Moreno at al., 2021). Different mutations of the CFTR gene

lead to cystic fibrosis. More than 2000 mutations of CFTR have been discovered since 1989, that is the reason CFTR genes are categorized in six different groups based on their gene dysfunction. Class I produces no functional CFTR protein, Class II is CFTR trafficking defects, Class III is impaired gating, Class IV is decreased channel conductance, Class V is reduced production of CFTR and Class VI is decreased the stability of CFTR (Veit at al., 2016). Most common mutation is F508del which causes misfolding of protein, inhibits trafficking and leads to degradation which ultimately hinder the ion channel pathway (Bishay & Sawicki., 2016).

Mutation class	**Result**	**Example**
Class I	Premature termination of mRNA causes defective protein production.	G542X; W1282X
Class II	Irregular post translation of CFTR prevents trafficking which processes defective proteins.	F508del
Class III	Impaired ion channel activity leads to irregulation in ion secretion and mediation.	G551D
Class IV	Disrupts the channel structure which reduces ions flow causes defective conduction	R117H
Class V	Alter the stability of mRNA & mature CFTR protein which reduces the amount of functional CFTR.	2789+5G>A

Class VI	Reduce stability of CFTR protein	Phe508del

Table 1: Classification of CFTR Modulation

NewBorn Screening or (NBS) is the earliest test conducted on neonates to determine any health issues such as Cystic Fibrosis. Diagnosis of Cystic Fibrosis is a multistep process. In heel prick test, blood is collected from the heel of a baby to analyze. If a high level of Immunoreactive trypsinogen is present, then it indicates CF, even though it is required to detect a pair of faulty genes (Farrell et al., 2010). IRT level increases due to the clogging of the pancreatic duct. Then come sweat tests, which measure the amount of salt in the skin. 60-120 mmol/L of chloride and sodium is present in CF patients, which is double than the normal level (Mishra et al., 2005). There is also an antenatal test which identifies possible parents who are both carriers of the disease. During pregnancy tests like chorionic biopsy where tissue is collected from placenta as sample and amniocentesis were performed. ICM or Intestinal Current Measurement where tissue is collected from the intestine. There are also some non-invasive tests like nasal potential differences (NDP) which measure the flow rates of sodium and chloride ions. Other tests like lung function test, sinus test, chest x-ray are conducted for diagnosis of CF.

Different treatments for CF have been developed over the years and we are going to review some of them, such as antibiotics, anti-infective, mucolytics, ion channel therapy, airway clearance therapy, gene therapy and most recent CFTR modulators. Also, scientists develop many devices to clear the excess mucus and help in breathing for CF patients.

1.1 Symptomatic and Supportive Therapies

Apart from progressive damage in lung function CF patients also suffer from chronic infections and inflammation due to invasion of multiple bacterial pathogens like Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae, Stenotrophomonas maltophilia,

Burkholderia cepacian (Chmiel & Davis., 2003). The mode of action classified into two categories, one is bacteriostatic which prevents the growth of bacteria and another is bactericidal which killed the bacteria. The drugs target and inhibit bacterial cell wall, cell membrane, foliate synthesis, protein synthesis and DNA/RNA synthesis to suppress bacterial growth or kill them off. Antibiotic treatment use in CF to eradicate microbes before they evolve in chronic form, preventing pulmonary exacerbation and ensures infection free lungs (Nijland et al., 2020). Different antibiotics therapy is required for different bacterial or multi bacterial infections. However, there is difficulty in choosing correct antibiotic therapy, so many research organizations conducted various trials to eliminate the difficulty. Bacteria like gram positive, gram negative, atypical bacteria can cause infection in CF patients which require treatment of different classes antibiotics for coverage for different spectrum.

Oral antibiotics are useful in the treatment of mild CF exacerbation compared to IV however the same treatment has a chance of failure in case of severe CF exacerbation . Flucloxacillin use against Staphylococcus, amoxicillin and clavulanic combination to counter Haemophilus influenzae, Nontuberculous mycobacteria infected patient with CF are recommended to use clarithromycin, azithromycin, ethambutol, rifabutin, rifampicin for 2-4 weeks, minocycline,doxycycline antibiotics for Achromobacter xylosoxidans and Stenotrophomonas maltophilia infection, TMP-SMX drug for CF patients with Burkholderia cepacia complex infection, fusidic acid 2-3 times a day for 2-4 weeks for Staphylococcus aureus MRSA infection, CF patients with Pseudomonas aeruginosa infection are recommended to use antibiotic combination of ciprofloxacin and tobramycin (Ciuca et all.,2022).

IV antibiotics treatment is used to treat mostly Pexs. Fusidic acid and rifampicin is injected in CF patients with Staphylococcus aureus infection for 2-4 weeks and for exacerbation vancomycin or linezolid is administered 2-3 times a day in a period of 14 to 21 days. A large

number of IV drugs are available for Burkholderia cepacia complex infection like aztreonam, tobramycin, tazobactam, ceftazidime (Ciuca et all., 2022). Also, in case oral antibiotics resistance IV route is used. The Standardized Treatment of Pulmonary Exacerbation 2 is a trial study to determine the optimal duration of IV Antibiotic therapy (West et al.,2017) Another STOP360 trial study is designed to figure out whether a monotherapy of IV beta lecture is superior than the combination IV therapy of aminoglycosides and beta lactam because the monotherapy is not associated with side effects like nephrotoxicity and ototoxicity (ClinicalTrials.gov,n.d.)).

Inhaled antibiotics reduce Pexs, airway infection, lung damage and bacteria load; however, only a few products are commercially available (Maiz et al., 2013). Inhaled antibiotics are most effective due to a high concentration that is directly delivered to the lungs. Therefore it is required to maintain some essential quantities in the selection of inhaled antibiotics like stability, suitable pH,limited systemic absorption, retained activity in airway movement (Nichols et al., 2019). Tobramycin Inhalation Solution (TIS) is a bactericidal agent effective against PA infection and reduces sputum pseudomonas density (Cystic Fibrosis, n.d.). Even though it has some side effects its well tolerable by CF patients and more beneficial. Pseudomonas aeruginosa microbes from a thick biofilm which shows resistance against antibiotic treatment and prevent mucus clearance and obstruct the airway pathway. Tobramycin with CaEDTA can penetrate the biofilm . Additionally, CaEDTA can penetrate PA by inhibiting the cell wall by rupturing the lipopolysaccharide layer of outer membranes (Puvvadi et al., 2020). Aztreonam Lysine Inhalation (AZLI) inhibits the cell wall synthesis of PA and other gram-negative bacteria. The combination of TIS & AZLI is more effective in patients with respiratory decline function and also increases the time between Pexs that require antibacterial treatment (McCoy et al., 2008). Amikacin Liposome Inhalation Solution (ALIS) treat Mycobacterium aviemore by penetrating and destroying the MAC biofilm (Khan &

Chaudary., 2020). It also reduces sputum PA like TIS. Colistimethate Sodium is available in both inhalation and IV form in the treatment of PA infection for 6 year or older CF patients (Schuster et al., 2012). Vancomycin is bactericidal agent with active against Methicillin-resistant Staphylococcus aureus (MRSA) and other gram-positive bacteria by inhibiting their cell wall synthesis (Patel et al., 2023) Inhaled Fluroquinolones have high potency and broad spectrum to inhibit DNA synthesis of bacteria and reduce Pexs in CF therapy (Flume et al., 2016). Inhaled antibiotics have the capacity to achieve higher therapeutic efficacy in the treatment of Cystic Fibrosis.

Phage therapy also known as bacteriophage which uses viruses to infect a specific bacterium and replicate in the bacterial cell to lysis and ultimately kill it (Chan et al., 2021). New antibiotic drug development is facing limitations due to resistance. Phage therapy is being studied to find an applicable therapy for CF patients with PA, SA & NTM infection. Studies also report that this therapy decreases bacterial density and sputum quantity, increases median times between Pexs, improves chest imaging and lung function. Inhaled pyophage is an Inhalation preparation of five phages against PA, MSSA, Streptococcus, Proteus and E. coli which is administered in CF patient which resulted in reduce in sputum bacterial density and improve refractory respiratory symptoms and overall clinical conditions (Suh et al., 2022). Lung transplant has risk for recurrent infection in post transplantation but the use of antibiotics can be limited due to polypharmacy and drug toxicity to avoid this obstacle Phage therapy can be used with promising outcome (Aslam et al., 2019). The ADR of Phage therapy is not severe even though multiple studies are conducted to learn about safety profile, dosage regimen, stability and potential side effects to help CF patients suffering from MDR infection.

Iron or Iron dependent processes like Ferric uptake regulator (FUR) are necessary for the survival and reproduction of bacteria (Visaggio et al., 2022). The inhaled formulation can target

PA infection in cystic fibrosis and also other pathogens due to the large spectrum of gallium treatment (ClinicalTrials.gov, n.d.). There is also inhaled nitric oxide as antibiotics which can disrupt biofilm and kill bacteria. NO in high dose is well tolerable and has pulmonary vasodilator and anti-inflammatory effects . Sodium fusidate or fusidic acid inhibit protein synthesis of bacteria to prevent replication and it is available in various forms like IV, oral, topical to counter MRSA and PA infection. It is also observed to be used as monotherapy or combination with other antibiotics to treat SA infections (Fernandes.P, 2016.). Lefamulin inhibits the protein translation process of bacteria and it is also available both in oral and IV form to treat MRSA infection in CF patients along with other bacteria like MSSA, H influenzae, Mycoplasma pneumonia (Esposito et al., 2023). Inhaled Glycopolymer SNSP113 or Poly-N-glucosamine can break down biofilm and has properties to treat NTM, gram negative bacteria and SA infection as both combination or monotherapy (Narayanaswamy et al., 2022). It is safe and tolerable in CF and other patients.

There is no established optimal duration for the antibiotic treatment in CF patients. Therapy cycles which are too long results in complications and cycles that are too short leads to retreatment in next 30 days (VanDevanter et al., 2016.). The mean duration of the therapy cycle is usually 14 days but according to the Cystic Fibrosis Foundation Registry it varies from 4 to 23.5 days (VanDevanter et al., 2016.). CF care centers in United States studies and suggested that shorter duration of therapy is suitable for the treatment of Pulmonary exacerbation (Ng et al., 2020). Even though there is no fixed duration for antibiotic therapy due to lack of scientific evidence, it is said to continue the treatment until remission of symptoms restoring lung function which happens to be 14 days (Canton et al., 2015.). On the other hand, the treatment can be prolonged in case of severe lung dysfunction or multidrug Pseudomonas aeruginosa infection.

The affected CFTR gene in cystic fibrosis damages the ion channel located in the epithelial cell membrane which leads to imbalances ion transportation. The mismatch ion transportation leads to depletion of airway surface liquid which in return builds up a thick mucus layer in the airway (Puthia et al., 2020). Airway obstruction occurs due to thick mucus which leads to hypoxia. Hypoxia decreases the oxygen level in the body by blocking nasal pathways which leads to neutrophilic inflammation. Inflammation synthesis interleukin 1-alpha & 1-beta which are pro-inflammatory substances and activates dysfunctional immune response (Balazs & Mall., 2019). IL Inflammation increases the byproduct of cellular metabolism like reactive oxygen species (ROS) which inhibit some functional activities of the respiratory system. Furthermore, it causes hyperinflammation and prolongs the chances of infection in CF patients (Bossche et al., 2021). Apart from neutrophil, monocytes and macrophage are also responsible for inflammation.

Corticosteroid is the first Anti-inflammatory drug research for the treatment of Cystic Fibrosis which reduces the chronic inflammation in CF. It targets intracellular signaling, esicosanoids, and cytokines. Glucocorticosteroid is a class of steroid which inhibits synthesis of IL 1beta, IL-8, NFKB and activator proteins responsible for inflammation (Mitri et al., 2020). Inhaled and Oral CS is developed for CF treatment but only the safety profile of inhaled CS is established which is better than the oral form but the efficacy is still under studies. The ADR of CS is glucose intolerance, growth impairment which still remains an issue even after the treatment is complete, also toxicities and osteoporosis, osteopenia even decreases the function of the lung if used for a long time, even though the use of CS is limited in some cases of CF it is still an extremely significant drug to reduce exacerbation (Balfour-Lynn & Welch., 2014).

Ibuprofen is the first NSAID drug which is used in the treatment of CF; it is an alternative of GS which has notably fewer side effects or adverse effects (Konstan et al., 2003). It targets the neutrophil and prevents its activation. Neutrophil is an inflammatory cell which provides first

line defense against pathogens but in CF neutrophils cause disturbance in homeostasis of ion secretion; increase chloride and sodium ions and decrease the magnesium ion concentration (Phol et al., 2014). This phenomenon alters the function of the CFTR gene and causes inflammation but ibuprofen have inhibitory effects against neutrophil and treat lung inflammation. High doses of ibuprofen specially in children are recommended by specialists to reduce inflammation. However, there is a scope of GIT bleeding or inflammation if the treatment is prolonged which limits the use of this drug (Lands & Stanojevic., 2019). Even with these risks it cannot outweigh the benefits therefore more research is conducted on this drug.

In Cystic fibrosis inflammatory substances like neutrophil along with pro- inflammatory cytokines like IL-8, IL-6, TNF-alpha which activates neutrophils and neutrophil like other inflammatory agent which can even leads to necrosis or apoptosis (Chmiel et al., 2013). On the other hand, IL-10 is a type of cytokine which contains anti-inflammatory properties. IL-10 inhibits neutrophil production and influences the apoptosis of the agent along with reduction T cells (Bonfield et al., 1999). According to studies IL-17 also shows efficacy in treatment of CF by reducing airway neutrophil (Ferretti et al., 2003). Anakinra, an IL-1 anticytokine reduces signs and symptoms and prevents lung inflammation in CF (Balazs & Mall., 2019)

Oxygen radicals are released by neutrophil which damages local tissue and causes inflammation. CF lung is more susceptible to oxidants than normal lung due to antioxidants deficiency caused by malabsorption. Malabsorption occurs because the CFTR mutation gene produces excessive mucus which clog pancreatic ducts. Antioxidants like glutathione and N-Acetyl cysteine have the ability to reverse abnormalities in CFTR. It can inhibit H202 which in return deactivates inflammation (Tirouvanziam et al., 2006). N-Acetyl cysteine reduces neutrophils in sputum by decreasing IL-8 which is a precursor of neutrophils (Tirouvanziam et

al., 2006). Dysfunctional CFTR disrupts the homeostasis of oxidant-antioxidant which leads to airway inflammation. Aerosolized glutathione treatment can reduce inflammation but the therapy is still not well researched (Bishop et al., 2005).

Immune cells like neutrophil release protease like matrix metalloproteinase, serine and cysteine which activates protease receptor, CFTR and matrix degradation, cytokines upregulation, ENaC activation, mucus modulation (Gagger et al., 2011). CF patients have a large number of protease inhibitors still they get overwhelmed by the protease release by anti-inflammatory agents. The protease and PI imbalance increased in CF patients in time of exacerbation which ultimately causes airway inflammation and injury. The proteolytic damage can be prevented by alpha1 - antitrypsin Inhalation therapy (Watz et al., 2019). There are also other drugs like EPI-hNE4 and protease inhibitory activity. Protease antagonists work in two ways: either increase the Protease Inhibitors or inhibit the Protease production.

Even though macrolides are antibiotics they also reduce information by altering neutrophil function. It also restores chloride secretion in faulty CFTR. Dihydrofolate reductase inhibitor, eicosanoid modulators, intracellular signaling modulators are some examples of anti-inflammatory treatment of CF. CXCR2 antagonists can interfere with the inflammation in the airway.

In 85% cases Cystic Fibrosis leads to pancreatic insufficiency (PI) which causes maldigestion (Wilschanski & Durie., 1998). PI refers to inadequate absence of pancreatic enzymes due to thick layers of mucus clogging the pancreatic duct which causes malnutrition in patients. Pancreatic insufficiency is treated with FDA approved PERT capsules containing pancreatic extract which are available in three formulations: enteric coated, non-enteric coated & lipase enzyme cartridge (Freswick et al., 2022). This treatment also has a major side effect known as

Fibrosing colonopathy (Borowitz et al., 2013). There is a chance of enzyme failure because of fluctuation in dosage regimen, foods that are incompatible with the drug.

Physiotherapy by manually or instrumentally stopping defective ion secretion and preventing the buildup of thick and viscous mucus in the airway surface. Airway clearance therapy reduces the sputum and slows down infection or disease progression. Devices like flutter, acapella, cornet also help in this therapy. Short acting bronchodilator like salbutamol and long acting like salmeterol improves obstruction by preventing bronchospasm (Mogayzel et al., 2013). Also inhaled Corticosteroid, mucolytic agent, hypertonic saline are also applicable.

1.2 Ion Channel and Protein Modulators Therapies

Cystic Fibrosis Transmembrane Conductance Regulator is an AMP dependent ion channel that mediates the transport of ions across epithelial cells in airway (Riordan et al., 1989). But the mutation of CFTR interrupts the normal ion channel activity by decreasing Chloride ion secretion and increases sodium ion absorption which leads to dehydration and builds up a thick mucus layer to obstruct the function airway and other organs (Lopes-Pacheo.2016). Even though, CF is a pediatric disease but patients can live to at least upto five decades that's why initially the treatment target is limited to reduce symptoms and increase quality life. However, due to medical advancement scientists develop treatment and medication which restore the CFTR function and prevent the progress of CF. Ion channels like TMEM16A, SLC26A9 & ENaC are targeted to improve the secretion of chloride ions and reduce sodium ions (Gentzsch & Mall., 2018).

Epithelial Sodium Ion Channel (ENaC) secretes and absorbs abnormal amounts of Sodium ion in CF causes hypernatremia (Fakioglu & Altun., 2020). Hypernatremia in CF absorbs water in excess amounts causing dehydration by depleting airway surface liquid. It also decreases chloride ion secretion which further complicates the situation. ENac antagonists are designed to reverse the process. Amiloride is the first ENac inhibitor which blocks the secretion of sodium in epithelial cells but it causes acute hyperkalemia which disrupts kidney function in the first generation (Hirsh et al., 2008). However, the second generation amiloride is free of ADR like hyperkalemia. SPX-101 is another inhibitor which shows activity like short palate lung nasal epithelial clone 1 (SPLUNC1) and inhibits and decreases sodium absorption (Lenox & Myerburg., 2017). Also, PSX-101 undergoes phase I and Phase II trials which determine its safety profile due to absence of ADR like hyperkalemia (Cladwell et al., 2005). There are also antisense oligonucleotides chemically modified synthetic RNA like molecules which suppress

sodium releasing stimuli and repair CFTR dysfunction caused by deletion mutation. QUB-TL1, BI 1265162, ARO-ENaC are also inhibitors under trial (Fakioglu & Altun., 2020). ENaC Inhibitors may cause pulmonary edema due increases in water.

Calcium-activated chloride channels mediate the secretion of chloride ions apart from CFTR, so when impairment occurs due to CF the ion secretion is balanced by Calcium activated chloride channel (CaCC) (Guilbault et al., 2006). TMEM16A is a CaCC channel which activates and increases chloride secretion to increase hydration, but the deactivation of the receptor is also beneficial due to mucus reduction (Rock et al., 2009). Cinnamaldehyde is TMEM16A activator which increases intracellular calcium and matrine inhibits this protein receptor (Huang et al., 2018). Depolarizes calcium which increases chloride levels in the body. Absence of this protein dramatically reduces chloride ion and CFTR function. SLC26A9 is another protein that mediates epithelial chloride ion secretion. P2Y2 along with ATP and UTP increases Chloride ion by CaCC . Inhaled denufosol contains P2Y2 attributes to stimulate chloride secretion in CF but due to its short half-life is not significant despite being effective and tolerable (Ratjen et al., 2012). Duramycin is an antibiotic which increases calcium level to activate chloride secretion in CF (Grasemann et al., 2007).

In osmotic therapy hypertonic saline along with dry powder mannitol creates an osmotic gradient to withdraw water to lung epithelial cells (Robinson et al., 1997). This process prevents the dehydration and depletion of ASL or airway surface liquid which ensures mucociliary clearance (MCC) repair mucociliary transport to maintain ion homeostasis and prevent airway obstruction (Haq et al., 2016). 6-7% HS is used in CF treatment and studies show that mild improvement in CFTR and reduce PEx (Elkins et al., 2006) In CF treatment The dry powder mannitol is delivered to lungs in Inhalation preparation and hypertonic saline release inflammatory agent which alters the mucus in sinus to increase the hydration in ASL.

The osmotic property in mannitol improves sputum and decreases exacerbation in children and in adults it improves lung function.

Ionophore is an artificial ion transporter which transports ions like calcium, chloride, and bicarbonate across the membrane (Davis et al., 2010). In CF they potentiate chloride transportation which came into halt due to undesirable CFTR mutation. Tambjamines & prodiginnies are some of the examples of ionophores (Cossu et al., 2018) ;(Fiore et al., 2019). Amphotericin B is an FDA approved antifungal drug used as ionophore in cf treatment. AmB forms a non-selective ion channel in CFTR mutation and recovers lung function by increasing Chloride ion secretion (Muragila et al., 2019). Also, they restore antimicrobial activity and reduce ASL viscosity.

CFTR modulators composed of a group of small molecules which are designed as drugs to target the impaired proteins resulting from CFTR mutation (Clancy et al., 2019). Cystic Fibrosis damages the ion secretion flow and ion homeostasis, it decreases chloride ion and increases sodium ion absorption which causes ASL dehydration, lung infection, inflammation and functional loss. CFTR modulators mediated ion channel activity by increasing chloride transportation and reducing sodium absorption (Boeck., 2014). This drug therapy has been developed to enhance or restore the normal mechanisms of lung function and CFTR which are influenced by mutation. Four CFTR modulators are available for therapeutic use: ivacaftor, lumacaftor, tezacaftor, elexacaftor are used in combination with others or as monotherapy based on the genotype or mutation (Moreno et al., 2021). All of these drugs are designed by Vertex Pharmaceutical. These CFTR modulators are identified by High Throughput Screening

Ivacaftor is the first CFTR modulator use as potentiator which deals with class III G551D CF mutation which restricts the chloride transportation by interfering with ATP function and decreases protein folding of ion channels (Guigui et al., 2016). Recent studies show that the

binding site of ivacaftor is the interface between Transmembrane Domains (TMD) (Liu et al., 2017). It is metabolized by CYP34A and eliminated via bile so CYP34A inhibitors like azole and macrolides and food like grapefruit prevent the metabolism which can lead to toxicity. It is tolerable and safe to use for ages 6 and above. It reduces chloride in sweat, increases chloride transportation, improves lung function, and restores pancreatic function if used in the initial state. FDA approved ivacaftor for the treatment of other mutations like G1244E, G178R, S549N, S1251N, S1255P but it shows no activity against F508del, the most common mutation. Ivacaftor improved and increases the trafficking and folding of gene which in return increases transportation of chloride ions (Jaques et al., 2020)

Lumacaftor is a CFTR corrector which shows activity against F508del, a class II mutation (80). F508del hinders the protein folding which affects the ion transportation. Monotherapy of lumacaftor doesn't show a good result but a combination with potentiator ivacaftor improves protein folding and ion transportation significantly and one of these combinations is Orkambi (Elborn et al., 2016). Orkambi firstly initiates correct protein folding then modulates the ion channel. The combination consists of 200g lumacaftor and 250g ivacaftor. It also treats other mutations like A561E, W1282X (Haggie et al., 2017). Common adverse reactions like diarrhea, nausea is identified in studies and serious reaction hepatobiliary diseases are presented in 0.5% of patients (Moreno et al., 2021). A study conducted in Spanish CF patient shows that the combination therapy reduces Pexs without altering ppFEV1 & BMI. Even though the treatment efficacy is mild due ivacaftor and lumacaftor concentration compatibility. Lumacaftor induced CYP34A responsible for ivacaftor metabolism which reduces the plasma concentration of ivacaftor and on the other hand high concentration of ivacaftor declines the ability to restore F508del CFTR mutation (Boyel et al., 2014). The simultaneous administration of lumacaftor increased the restoration of CFTR which then improved the protein folding.

Tezacaftor is another CFTR modulator which facilitates the folding and trafficking of protein towards the cell surface (Jaques et al., 2020). It has moderate efficacy in F508del mutation but combination with other modulators increases the efficacy of therapy. Along with the treatment of residual function mutation which repairs CFTR protein, increases the synthesis of CFTR channel, regulates channel gating, improves channel conductance and facilitates the protein trafficking. The combination treatment of Tezacaftor and Ivacaftor approved by FDA & MHRA as a film coated tablet known as Symdeko and Symkevi (Moreno et al., 2021). The tablet comprises 100mg tezacaftor and 150mg ivacaftor. Despite having similar efficacy in CF treatment, the combination of tezacaftor and ivacaftor is better tolerated than the combination of lumacaftor and ivacaftor. In Phase III trials, this combination shows well-tolerability, improvement in ppFEV1, and reduced exacerbations.

Elexacaftor is a corrector CFTR modulator which facilitates CFTR function across epithelial cells (Ridley & Condren., 2020). It is available in triple combination therapy with tezacaftor the corrector and ivacaftor is the protentiator known as Trikafta in US and Kaftrio in EU (Moreno et al., 2021). It is recommended to CF patients where even one copy of F508del mutation is present along with other mutations which reduces CFTR processing. Patients with this condition have several respiratory symptoms, pancreatic insufficiency and increased exacerbation. This triple combination improves the symptoms, lung function, reduces Pexs and increases CFTR processing in phase III trials. The only ADR of this treatment is liver damage which should be considered before conducting the treatment. This treatment is approved for 12 year or older CF patients for 2–5-year CF patients this treatment is under studies (Jaques et al., 2021).

Read through agent is a type of CFTR modulator which interacts with ribosomes and adds amino acid to mutated sites that correspond to Premature Termination Codon (PTC) that

collapse the protein synthesis which will result in shortened translation process (Sharma et al., 2020). Read-through agents prevent this situation by suppressing stop codon and ensuring production of functional protein. PTC result in protein synthesis due different mutations like frameshift,nonsense or splicing variant. Gentamicine under aminoglycosides which are available in topical or IV form is the first read through agent that increases functional CFTR expressions and restores chloride secretion (Howard et al., 1996). High doses of this drug may cause ototoxicity and nephrotoxicity (Prayle et al., 2010). Ataluren is read through an agent screen by HTS to inhibit CFTR mutation but apart from decreasing Pexs it shows no significant promises in first clinical trials (Welch et al., 2007). ELX.02 is a modified aminoglycoside which suppresses nonsense mutation Duchenne Muscular Dystrophy (nmDMD) and it has less toxicity and more safety profile than gentamicin (Mercuri et al., 2020).

Apart from the four approved modulators, there are others which are categorized as potentiator, corrector, stabilizer and amplifier which are being assessed in clinical trials for safety and efficacy. Potentiator are agent allows CFTR dependent ion conductance; ABBV-3067 facilitates opening of Na+ channel, Deutivacaftor (VX-561), Dirocaftor (PTI-808), Icenticaftor (QBW251) but all of them are in different clinical trials to determine quality, safety and efficacy (Lopes-Pacheo et al., 2016) ;(Moreno et al., 2021). Another CFTR modulator is the corrector. Compounds that enhance protein folding, processing and tracking. Galicaftor, VX-121, Posencaftor that maintain the stability of protein conformation to prevent protein misfolding which maintains the ion secretion and transportation. Stabilizer is another modulator that maintains the stability and structure of CFTR protein in cell membranes and prevents the degradation and reduction of the gene (Fukuda & Yoneda., 2018). Cavosonstat is considered the first stabilizer to undergo clinical trials. Cavosonstat inhibits S-nitrosoglutathione to stabilize the mature CFTR protein to perform their functions. In phase I trial it shows reduction in chloride concentration for homozygous F508del in CF patients but

in phase II there is no significant result (Donaldson et al., 2017). The last one is an amplifier which will increase the expression of CFTR mRNA and biosynthesis of CFTR protein (Giuliano et al., 2018). Nesolicaftor is an example of amplifier which improves CFTR function by increasing CFTR expressions to increase protein. It is under clinical essay to determine its efficacy and safety in both monotherapy and combination therapy. These modulators are not applicable to all types of mutation, they are designed based on the genotype. So, a specific modulator will work for one or a few mutations. Individual patients will have different reactions based on factors like what type of mutation, whether they are homozygous or heterozygous, which organ is affected by CF. So, more studies and clinical trials are performed to improve the drug.

1.3 Genetic and Regenerative Therapies

Gene therapy in cystic fibrosis aims to increase CFTR expression and function by repairing the gene function or replacing the mutated gene with a corrected copy of the CFTR gene. In this therapy, genetic materials are administered to modify or manipulate gene expression. Gene transfer and gene editing is included in gene therapy. In gene transfer, a corrector CFTR gene is delivered into a preexisting cell via vectors, which are categorized into viral vectors and non-viral vectors. Additionally, in case of gene editing, a corrected copy of the CFTR gene is introduced permanently in the mutated gene to repair the synthesis and function (Egan, 2020).

Viral vector or viruses like adenovirus is the first selected approach of gene delivery for their large carrying capacity. However, insufficient transduction and repeated administration decreases efficiency by immune response (Naldini et al., 1996). Scientists chose another approach in the form of Adeno Associated Virus (AAV) which was clinically safe but it

required repeated administration due to limited transduction like adenovirus. It has low binding to the target site that's why frequent administration is needed (Zabner et al., 2000). But serotypes AAV2 and AAV6 have high transduction effects. In case of lentiviral vectors, they display low immunogenicity and have high packaging capacity that prove lentiviral can integrate and transduce into the genome of both dividing and non-dividing cells (Castellania & Cones, 2010). It ensures persistent expressions of the CFTR gene. Viral vectors are chosen because of their invasive attributes. Even though viral vectors show high transduction efficacy, strong binding property to airway target site, safe delivery and long and stable efficiency other non-viral vectors are under studies and trials to overcome barriers like strong immunogenicity, insertional mutagenesis and be cost effective.

Viral Vectors	**Adenovirus**	**Adeno Associated Virus**	**Lentivirus**
Genome	dsDNA	ssDNA	ssRNA
Packaging capacity	7.5kb	5kb	8kb
Integration into genome	No	No	Yes
Targeted Cells	Non-dividing	Non-dividing	Both
Duration of expressions	Transient	Long	Long

Table 2: Characteristics of Viral Vector

Non-viral vectors are less effective, especially the unmodified ones. They have low transduction efficiency and also have low accumulation in target sites (Takahashi et al., 2013; Symth et al., 2015). Exosome is a nanoscale extracellular vesicle which transports materials to near or distant recipient cells because of this they consider as vehicles to transport genetic materials in vivo therapy. They are used as vectors to transport CFTR mature glycoprotein and CFTR RNA to membrane bound CFTR to correct genetic defects in CF cells (Gonzalez et al., 2012). Another non- viral vector is artificially created vessels with aqueous core and lipid by layer (Akbarzadeh et al., 2013). The mixture of cationic lipid mixture and liposome shows Studies state that the mixture has the ability to improve lung function without any ADR (Alton et al., 2015). Lipid nanoparticles (LNP) are encapsulated and deliver therapeutic RNA formulation to the lungs. The successful delivery of vectors decreases due to thick mucus which prevents the penetration of drug in the target site (Suk et al., 2011). LNP increases the penetration of thick mucus so the drug can reach the target site (Nafee et al., 2018) ;(Wan et al., 2018). It increased CFTR function but the inefficient delivery of these non-viral vectors is a limitation so it required both in vivo and in vitro tests (Sahay et al., 2013).

The Gene editing mechanism replaces the mutated gene with a corrected copy of the same gene. In CF this technique applies into the CFTR protein of epithelial cells in the airway. This technique operates by creating double DNA strands that penetrate the host chromosome at the mutation site, introduce the correct functional protein initially absent and restore the function (Esposito et al., 2023). Clustered Regulatory Interspersed Palindromic Repeats (CRISPR) or CRISPR-associated nuclease 9 (CAS9), zinc finger nuclease (ZFNs), transportation activators like effector nuclease (TALENS), triplex forming PNA/DNA are some technologies applied under gene editing. CAS9 is a natural nuclease used for accurate DNA editing. CAS9 forms a complex with gRNA selective to the target site and introduces DNA strands break in host DNA. Then activates the nonhomologous end-joining (NHEJ) process which sticks the end of broken

DNA strands and homology- directed repair (HDR) which uses donor DNA to repair the damaged DNA in precise mechanism (Hille & Charpentier, 2016). This technology induced pluripotent stem cells acquired from lung cells of CF patients, corrected the mutation and inserted it back into environmental niches (Park & Bae., 2018). Cpfl is a tool used in this technique to easily generate gRNA (Ledford, 2015). It can alter the Phe508del mutation by introducing correct Phe508del DNA. This technique can use vectors for delivery but transfection issues will arise.

ZFNs constructed endonuclease facilitate the correction of induced pluripotent stem cells acquired from CF airway cells and repair Phe508del mutation and function (Crane et al., 2015). The advantage of this technology is, without integrating any sequence into the genome they repair the genetic sequence. However, it is prone to error, lacks desired flexibility and shows high immunogenicity as side effects. In triplex forming PAN, peptide nucleic acids are synthetic DNA with peptide backbone. PAN and correct DNA delivered to the mutation site where PAN binds the DNA and the endogenous system repairs the mutation and restores the function. TALEN technology also repairs the Phe508del mutation and restores CFTR expressions. Even though, with all this benefit, this technology is still in the clinical stage to ensure safety and precision.

Drugs partially or fully comprising RNA nucleotide use in RNA Therapy that over expressed or downregulate any gene. For example, mRNA therapy expresses a mutated protein in patients like mutated CFTR protein in Cystic Fibrosis patients and opposite happens in siRNA (Sahin et al., 2014). Modified nucleotide is incorporated in vitro mRNA to chemically modify it due to this they have low immunogenicity, improved expression capacity, higher safety profile and greater stability. mRNA, tRNA and short RNA molecules called Oligonucleotides are some RNA molecules which are used as therapeutic tools in CF (Lueck et al., 2019).

MRT5005 is an mRNA product candidate in clinical stage design and developed to treat all genetic disorders regardless of any mutation. MRT5005 delivered correct copies of protein encoded mRNA via nebulizer in lungs to restore CFTR function. A clinical trial conducted in 12 CF patients among 11 of them carry one copy of F508del mutation. They were grouped into four groups and MRT5005 administered in different doses to each group for eight days. There is no significant improvement in 8mg but in 16 mg which is almost double the ppFEV1 from baseline to 15.7%. In 24mg one patient ppFEV1 increases upto 21.4% while other two patients didn't show any impairment. MRT5005 in moderate dose with CFTR modulator is beneficial for patients (). Then, antisense oligonucleotides interfere with protein transcription. Eluforsen is an ASO repair mRNA encoding CFTR with F508del mutation. The treatment is beneficial for CF patients carrying only one copy of F508del mutation.

Nonsense mutations further translate stop codon and alter the whole sequence of CFTR protein which in return permanently stop synthesis of CFTR protein. t-RNA is designed to inhibit nonsense mutation. Human bronchial epithelial cells with G42X/F508del genotype treated with ECT101 a stimulus which increases chloride ion secretion to restore CFTR function (Egan, 2021).

Cell based therapy replaces damaged lung cells by hematopoietic stem cell transplantation instead of restoring them (Gratwohl & Niedewieser., 2012). A cell is correcting a CF patient's damaged cells by applying gene editing techniques ex vivo and transplanting it after removing the affected cells which are unable to carry CFTR protein. Exogenous cells like bone marrow cells, mesenchymal stromal cells (MSC) are selected as regenerative lung cells for this therapy. Pluripotent stem cells also insert a therapeutic unit. Although the therapy is more effective than some others, the physiology of the lung made it difficult to have easy access to perform the surgery.

Chapter 2

Methodology

This narrative review aims to provide an idea of current treatment strategies of Cystic Fibrosis by focusing on different drug therapy, gene therapy, ion channel therapy, cell based therapy and all. This review shows different success rates of this treatment on Cystic Fibrosis patients. A search conducted on relevant studies from 2000-2024 to obtain related knowledge. Biomedical and Clinical databases like PubMed and Cochrane library and journals like ATS and mdpi are included in this review. The search terms or keywords for this review are cystic fibrosis, cystic fibrosis transmembrane conductance regulator, CFTR modulators, therapy, mucociliary clearance, ion channel etc. Articles are in the English language used for this review. The criteria for selecting articles are review on Cystic Fibrosis treatment and improvement or positive result on patients.

Chapter 3

Discussion

The ongoing established treatment of Cystic Fibrosis has mostly provided a positive outcome but still there are slight disadvantages like severe side effects, ADR,decreases in lung function and immunity is present. In severe CF exacerbation oral antibiotic treatment can fail on the other hand inhaled antibiotics are highly effective but only a number of commercial products are available. IV route is much better in case of oral resistance and availability of marketed products. Anti-inflammatory drugs like corticosteroids are limited in treatment due to decreasing the lungs function, toxicity growth impairment and glucose tolerance. Also prolonged use of NSAID drugs like ibuprofen can lead to GIT bleeding. But it treats lung infection by inhibiting neutrophil which outright damages the damage. In ion channel therapy the first generation of ENaC Inhibitors leads to hyperkalemia and disrupts kidney function but the second generation of these Inhibitors pass the safety profile. In CFTR modulators monotherapy doesn't show as good results as combination therapy. The triple combination therapy of tezacaftor, Ivacaftor and elexacaftor is most suitable for CF treatment because it passes the phase III trial and it increases lung function, CFTR processing and decreases Pexs. In gene therapy, a corrector CFTR is delivered via vector to modify the faulty genes. Lentiviral vectors have low immunogenicity and long duration of expressions. mRNA therapy design and developed to work for all CFTR mutations in lungs by restoring CFTR functions. Although, the treatment strategies of Cystic Fibrosis are showing satisfactory results but further studies are needed to ensure long term safety and efficacy.

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Chapter 4

Conclusion

The treatment strategies prolonged the lifespan and improved the health of Cystic Fibrosis patients. Many different treatments have been discovered, some achieve complete efficacy, some show mild or moderate improvement and some are unapproved because their benefit outweighs the risk. Antibiotic, anti-inflammatory, CFTR modulators, gene therapy and some other treatment approaches have been reviewed along with their safety and ADR reaction and some other shortcomings. In antibacterial treatment high dose is required due to increase in the hepatic and renal clearance which decreases the half-life of antibiotics. Anti-inflammatory treatment inhibits the activation of neutrophils and some other inflammatory agent which may comprise the immune system and body becomes susceptible to other diseases. Many different classes of medication are in clinical trials along with genetic and RNA therapy. They show potential but further studies are required for it. In 1989 CFTR gene was discovered which gives the treatment a new and improved dynamic. This treatment approaches prevent infection, inflammation, reverse mutation, increases CFTR expressions, increases chloride secretion and decreases sodium absorption, decrease mucus from lungs, pancreas and some other organs and restores their functions. The recent discovery of CFTR modulators shifted the treatment target from reducing symptoms to reform function of the airway and other organs.

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