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A Review on Gene Therapy for Cystic Fibrosis

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Abstract:

Cystic fibrosis is a chronic autosomal recessive disease, and it is caused by the mutations in a single gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Even though cystic fibrosis affects multiple organ systems, the majority of CF patients pass away from progressive lung illness, which starts in childhood and is typified by persistent inflammation and bacterial infection. The ΔF508 mutation is present in at least one copy in about 90% of CF patients, but there are hundreds of CFTR mutations that cause various degrees of disease severity. When the CFTR protein isn't working properly, a thick coating of mucus builds up and blocks the intestines, airways, and pancreatic ducts main cause of death. How to treat cystic fibrosis was mostly dealing with the symptoms in order to get over the disease's complications. From the early 2010s onward, the creation of a real treatment has advanced to an advance, such as modulators of tiny molecules and Genetic medicine. The first clinical trials involving CF patients were carried out in 1993 and involved the use of both viral and non-viral gene transfer agents in the bronchial and nasal airway epithelium. Even though it has steadily increased over the past few decades, people with cystic fibrosis still only live to be about 40 years old. The focus of current treatment guidelines should be on managing symptoms and complications. This article examines describe the current state of the science and recent advancements in CF gene therapy and screening.

Keywords: Cystic fibrosis, Gene therapy, Cystic fibrosis transmembrane conductance regulator (CFTR), Viral Vectors, Treatment, Disease, Small molecule modulators.

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Introduction:

Cystic fibrosis (CF) is an autosomal recessive, progressive illness that causes significant harm to the organs that secrete sweat, mucus, and digestive secretions, including as the pancreas, lungs, and sweat glands, and now impacts over 30,000 people in the United States. With a 900–1000 new births and in 3400 live births in the US, there are CF diagnoses every year. Though CF is prevalent among individuals of every race and ethnicity, it is most diagnosed in one out of every 29 Caucasians.[1] People who have the CF gene mutation. A mutation in the CF transmembrane regulator (CFTR) gene causes the hereditary disease known as cystic fibrosis (CF), which impacts several organ systems. The CFTR protein is synthesized under the guidance of the CFTR gene and forms a transport channel found in cells that produce saliva, sweat, mucus, and digestive enzymes. The CFTR protein channel serves a variety of purposes, but its primary in charge of appropriately controlling the movement of chloride and other ions across the cells' membrane discussed before. The appropriate purpose of this leads to the production of protective, watery mucus that can be cleaned by the organs cilia.[2] Mucus development results from mutations in this gene. That is prone to infection, thick, and challenging to remove from the airways.

Multiorgan illness is caused by the expression of the CFTR gene in the epithelium of numerous organs, including the liver, pancreas, gastrointestinal and reproductive tracts, kidney, and lungs. The leading cause of illness and death in the developed world is progressive pulmonary disease. Usually, the lungs experience increased inflammation, repeated infections, and surface area reduction from mucus blockage. [2] As a result, lung function declines and permanent bronchiectasis. Effects on the digestive system and gastrointestinal tract include bowel blockages, endocrine and exocrine dysfunction in the pancreas, and

overall discomfort. Numerous CF children that are not detected during a prenatal or neonatal screening, are diagnosed with cystic fibrosis (CF)following malnourishment and not succeeding. [3] Pancreatic insufficiency—a lack of absorption—is the cause of these lipids, proteins, and some vitamins—being among the initial signs of cystic fibrosis to appear in a newborn. Therefore, CF symptoms may begin to impact a child's long-term development before the illness is even recognized. Actually, it has been demonstrated that early dietary supplementation enhances lung function later in life.[3]

The most prevalent hereditary illness among white people is cystic fibrosis (CF). In the US and Europe, some 80,000 people have received a CF diagnosis. There are currently 10,000 CF patients in the UK, with over 57% of them being adults. Nonetheless, a growing number of CF patients are being found in other sizable populations, such as China and India. [4] Although CF was initially recognized as a disease in 1938, it wasn't until 1989 that the CFTR gene was discovered, a significant achievement that paved the way for the creation of CF gene therapy. More than 1990 mutations have been found in the CFTR gene to date, however not all of them can be categorically classified as disease-causing. Six groups of mutations can be distinguished based on the cellular phenotype that results. [3]

Since the discovery of CF in 1938, the main goal of treatment has been to alleviate the symptoms brought on by the faulty CFTR gene, such as repeated antibiotic infections. As a result, there has been progress in life expectancy from around five months when the illness was identified to approximately 40 years old now. An estimated 33,000 people are involved. Americans who suffer from the illness, and for the first time in 2014, a more important accomplishment was that there were more people with CF alive than children in terms of how long people with the illness live. [5]

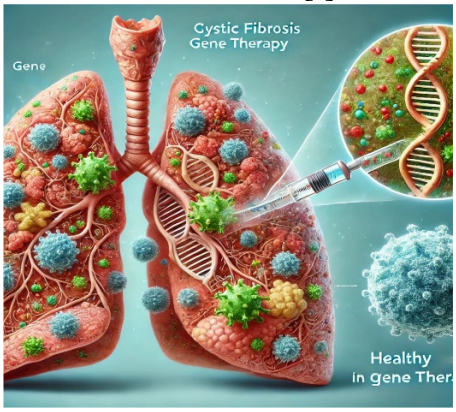

Fig:- 1

Classes of CFTR Mutations:

Since the CFTR gene was discovered in 1989, over 2000 mutations have been found to impact it, resulting in a variety of symptoms. [6] They fall into six primary categories based on to their ensuing flaw in the manufacturing of CFTR. [6]

Class I Mutations, (Defective Synthesis):

This family of mutations causes shortened or unstable mRNA, which impairs CFTR gene expression and, in turn, CFTR protein production. [7] This will cause the protein to completely disappear from the cell. surface, affecting approximately 7% of CF patients globally. The most prevalent examples of class I mutations are W1282X. as well as G542X. [7]

Classes II Mutations, (Defective Processing):

Defective protein processing is the outcome of this type of mutation. At the endoplasmic reticulum, the misfolded CFTR protein will remain after formation, resulting in inhibiting the protein's trafficking to the plasma membrane and causing premature breakdown. [6] It affecting ~ 90% of CF patients global. Examples of this class that are most frequently seen are F508del and N1303K. [6]

Classes III Mutations, (Defective Gating):
Defective gating will affect the CFTR protein that has been successfully placed at the epithelial membrane in this kind of mutation. [8] This will result in the channel being closed or CFTR channel's decreased open probability and reduced chloride ion outflow as a result. It affecting~ 5%of CF patients globally. This class's typical examples are G551D and S549R**.** [8]

Classes IV Mutations, (Defective Conductance):
This type of mutation results in channel pore flaws that reduce the open probability to around one-third that of the protein in its normal state. This will thus decrease in chloride conductance and penetration via way of the CFTR channel. It primarily affects MSD. about the CFTR protein. The most frequent instances of the R117H and R334W classes. [7]

Classes V Mutations, (Defective mRNA Stability):
This type of mutation causes splicing mistakes, which compromise the stability of mRNA. Consequently, there is less CFTR protein present in the plasma membrane overall, which lowers the release of chloride ions. [8] The two most common instances of this class are c.1680-886A>G and A455E.

Classes VI Mutations, (Defective CFTR Stability):
This type of mutation will result in instability of the CFTR channel, which is already present at the plasma membrane, which will speed up the cell's turnover rate. surface. Examples of this class that occur most frequently are 4279insA and 4326delTC are the classes I, II, and III that most serious kinds since they are connected to few or no the plasma membrane contains the CFTR protein. [10] As for classes IV, V and VI suffer from less severe illnesses since they entail merely a slight reduction in CFTR function activity. [10]

History Of Gene Therapy for Cystic Fibrosis:

Since its beginning, gene therapy for cystic fibrosis (CF) has experienced both tremendous progress and difficulties. The CFTR gene abnormalities that cause CF, a hereditary condition that affects the lungs and digestive system, result in thick, sticky mucus and a higher risk of infection. In order to restore appropriate chloride ion transport, CF gene therapy aims to insert a functional CFTR gene. [11] Important benchmarks consist of:

1. **Early Concepts and Discovery of The CF Gene (1980s):**
    - **1989**: In 1989 saw the discovery of the CFTR gene, which causes CF. By fixing the faulty gene, this discovery signaled a paradigm shift and gave researchers reason to think about gene therapy as a possible cure. [11]
2. **First Gene Therapy Trails (1990s):**
    - **1993:** Although adenoviral vectors were employed to deliver CFTR to nasal cells, the first CF gene therapy trial was unsuccessful due to immunological responses. [11]
    - **1995–1996**: Trials using liposomal vectors avoided immunological problems, but efficacy and targeting concerns hindered successful delivery to CF target cells. [11]
3. **Improved Vectors and Viral Vector Setbacks (2000s):**
    - **2000s**: Researchers switched to safer AAV vectors; however mucus accumulation made it difficult to deliver the virus to CF lung cells, greatly reducing the efficacy of gene transfer.
    - Researchers have been unable to find effective and secure delivery methods despite continuous efforts. The main obstacles in CF lungs were mucus and immunological barriers, which prevented vectors from reaching target cells. [12]
4. **Advances In Gene Therapy Techniques (2010s):**
    - **2015:** The possibility of gene therapy was demonstrated in 2015 when a UK trial using liposome-delivered CFTR gene therapy revealed small improvements in lung function in

CF patients. At the same time, CRISPR-Cas9 was developed, providing accurate CFTR mutant repair. Despite having comparable delivery issues, CRISPR created new opportunities for gene editing as opposed to merely adding it**.** [13]

5. **Recent Innovations and Ongoing Trails (2020s):**
   - **2020s**: To improve lung tissue penetration and successfully elude immune responses, next-generation vectors were created, such as lentiviruses and nanoparticles.
   - **2022–2024**: CRISPR-based gene editing is being used in promising preclinical studies to fix CFTR mutations at the DNA level, perhaps leading to long-lasting repair. An alternative to conventional gene therapy is being investigated: messenger RNA (mRNA) therapies, which use messenger RNA to create functional CFTR protein inside cells without changing DNA. [13]

Types Of Vector System:

1. **Viral Vectors:**
   Using viral vectors, a normal CFTR gene is introduced into lung epithelial cells as part of gene therapy for cystic fibrosis. Unlike lentivirus, which offers permanency but necessitates better targeting to address genomic integration difficulties, adenovirus and adeno-associated virus vectors are not integrated. [14]
   - **Adenovirus:**
     Adenovirus vectors were used in early cystic fibrosis gene therapy experiments; however, these trials were hampered by low DNA insertion efficiency, a robust immunological response, and limited genetic capability. In order to maintain sufficient CFTR expression in epithelial cells, this led to transient gene expression that need recurrent vector delivery. [14]
   - **Helper Dependent Adenoviral Vector (HDAd's):**
     The removal of viral sequences using helper-dependent adenoviral vectors (HDAd) increases DNA capacity and permits long-term expression without immunogenic consequences. A limited immune response and persistent expression are promising features of cystic fibrosis gene therapy, as demonstrated by the successful transport of the CFTR gene to the airway basal cells in mice and pigs.
   - **Adeno-Associated Virus (AAV):**
     Adeno-associated viral (AAV) vectors provide good safety and extended transgene expression for gene therapy for cystic fibrosis (CF). The rAAV5 vector's larger promoters and different capsid maximized the production of the CFTR gene, while the rAAV2 vector showed promise. The discovery of corrected CFTR abnormalities in mice and monkeys opened the door for clinical studies in CF patients, which could provide long-term treatment. [13]
   - **Lentivirus:**
     Since the CFTR gene is expressed over an extended period of time and may be administered again without causing immunity, lentivirus, a retrovirus, presents a viable treatment approach for cystic fibrosis. Feline immunodeficiency virus studies in pigs showed partial anion channel activity was recovered. Further research and trials are required as a result of a 2017 study that suggested rSIV.F/HN lentivirus for a first-in-man clinical trial, validating primate and non-primate vectors as promising gene therapy possibilities. [14]

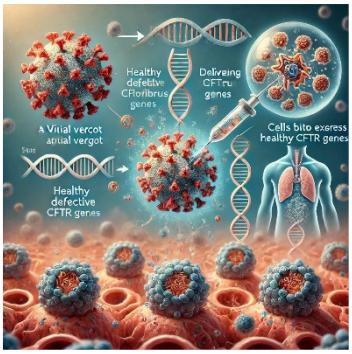

Fig:- 2

2. Non-Viral Vectors:

As an alternative to gene therapy for cystic fibrosis, researchers are investigating non-viral vectors created from synthetic chemicals and nanotechnology. Anion transport abnormalities are largely corrected by non-viral vectors that transmit functional CFTR genes to airway epithelia using lipid-based or polymer-based methods. They have advantages in terms of availability, affordability, and low immunogenicity, despite their decreased efficiency. Because of modifications like PEGylation that enhance distribution and lessen toxicity, non-viral vectors are a viable long-term option for CF gene therapy. Their effectiveness and safety are supported by clinical research. [15]

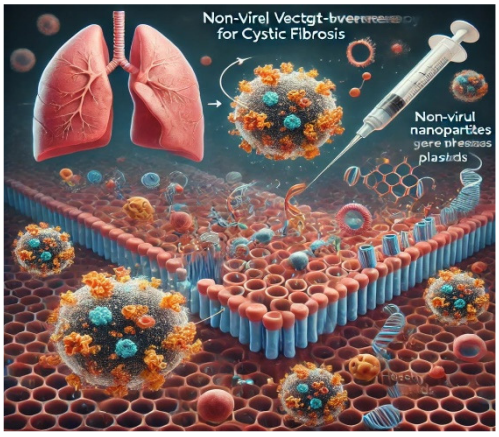

Fig:-3

Materials and Methods:

Clinical symptoms plus a positive sweat chloride test are used to diagnose cystic fibrosis (CF). CFTR genes are identified by genetic investigations and classified as either disease-causing or non-disease-causing. Chronic lung infections and pancreatic insufficiency are brought on by thick, erratic secretions caused by mutations in the CFTR gene. Infertility, pancreatic insufficiency, and respiratory infections are among the symptoms of cystic fibrosis (CF), which mostly affects the lungs and pancreas. People of Northern European ancestry are disproportionately affected by the disease; however other populations are also somewhat affected. Although there are still many unanswered questions regarding the pathophysiology of cystic fibrosis, research is still revealing the intricate pathways that underlie this hereditary condition. [16]

Symptoms of cystic fibrosis (CF) include pancreatic insufficiency, musculoskeletal issues, respiratory tract infections, and male infertility. For diagnosis, both clinical and analytical criteria are required: Typical signals for a system of one organ Positive outcomes from a neonatal screening or family history: A sweat chloride test result of ≥60 mmol/L or two CFTR mutations that result in disease. [16] The major objectives of treatment are to improve chest function through physical therapy, exercise, and airway clearance, and to lower infection rates through vaccinations and prophylactic measures. CFTR modulators

have been shown to improve lung function and quality of life. In the US, universal newborn screening guarantees prompt and effective care, allowing for early intervention that enhances patient outcomes. [16]

Symptoms of Gene Therapy for Cystic Fibrosis:-

Gene therapy for cystic fibrosis (CF) frequently causes symptoms or side effects because of how the body reacts to the treatment and how it is administered. These are the primary signs and consequences. [17]

1. **Immune Response Symptoms:-**
   Viral vectors used in gene therapy can set off immunological reactions, which can result in fever, chills, exhaustion, muscle pains, and nausea as the body defends against alleged invaders**.**
2. **Respiratory Symptoms**:-
   Coughing, wheezing, chest tightness, and shortness of breath are respiratory adverse effects of CF gene therapy that may result in a temporary loss in lung function.
3. **Inflammation Symptoms**:-
   At delivery locations, inflammation may be brought on by viral vectors or inserted genes, resulting in discomfort, redness, or swelling.
4. **Liver Toxicity**:
   Abdominal pain, jaundice, toxicity, and increased liver enzymes in blood tests can all result from gene therapy vector accumulation in the liver.
5. **Allergic Reactions**:
   Gene therapy has the potential to cause allergic reactions, which can include redness, itching, and in rare cases, severe anaphylactic reactions.
6. **Fatigue And General Malaise**:
   The body's reaction to treatment and the immune system may induce weariness and malaise during gene therapy, but these side effects will eventually go away.
7. **Potential Off – Target Effects**:
   Unintentionally changing non-target cells during gene therapy poses a rare risk of consequences that are constantly watched during studies.
8. **Nausea And Gastrointestinal Issues**:
   Due to immunity, gene therapy may result in diarrhea, vomiting, and nausea.
9. **Temporary Decline In Lung Function**:
   Gene therapy may temporarily impair lung function before progressively restoring it. [18]

Pathophysiology in Gene Therapy for Cystic Fibrosis:

CF Disease

⇩

Mutated CFTR Gene

⇩

Defective Trafficking

⇩

Truncated ,immature , partially or totally defective ,

instable CFTR protein

⇩

Dehydrated Secretion

⇩

Decreased Mucociliary Clearance

⇩

Enhanced Mucus Viscosity and Enhanced Hypersecretion

⇩

Chronic Bacterial colonization, Airway inflammation , Airway destruction

⇩

Respiratory Failure

⇩

Death

Diagnosis :

Treatment for cystic fibrosis (CF) has traditionally concentrated on managing symptoms and enhancing quality of life without addressing the underlying genetic cause. Treatments for symptoms included anti-inflammatory drugs (NSAIDs, steroids) to lessen airway inflammation, mucolytics (dornase alfa) to break down mucus, and inhaled and oral antibiotics (azithromycin, tobramycin, and ciprofloxacin, amoxicillin) to fight bacterial infections. Vitamins and pancreatic enzymes aided in nourishment and digestion. New therapies that target the genetic flaw have been developed as a result of recent developments in our understanding of the CFTR protein. The goal of gene therapy and small molecule modulators is to fix CFTR defects and stop the disease's progression. New treatments aim to correct the underlying genetic flaw, providing hope for better results. While cutting-edge methods target the underlying cause of CF, conventional symptomatic therapies are still crucial. [19]

CF patients now have more treatment options because to ongoing research and development, which aims to slow the disease's progression and enhance quality of life. There is hope for better patient outcomes as new medicines are being developed to address the primary cause of cystic fibrosis.

Cystic fibrosis (CF) therapy aims to correct altered CFTR protein abnormalities by using modulators. Key modulators including ivacaftor, lumacaftor, elexacaftor, and tezacaftor are available either alone or in combination to treat structural and functional issues.

- **Ivacaftor :**

  Ivacaftor, which was approved by the FDA in 2012, improves chloride transport by lengthening the duration of channel opening in CF patients aged ≥6 years who had a G551D mutation. However, only 2.3% of CF patients with this uncommon mutation benefit from treatment, and accessibility is hampered by its expensive cost.

- **Lumacaftor** :
  Lumacaftor and ivacaftor (Orkambi) work together to improve and restore CFTR protein function. Ivacaftor prolongs channel opening, whereas Lumacaftor corrects misfolded proteins and transports them to the cell surface. By enhancing CFTR activity and chloride transport, they work in concert to address the underlying abnormalities of cystic fibrosis.
- **Elexacaftor** :
  Trikafta (elexacaftor, tezacaftor, and ivacaftor) was approved by the FDA in 2019 for use in patients aged 12 and up who have the F508del mutation, which is a frequent cause of cystic fibrosis. By focusing on processing errors, elexacaftor and tezacaftor increase the amount of functional protein at the cell membrane and improve illness management by correcting CFTR protein abnormalities.
  Tezacaftor, commonly known as Symdeko, enhances ion and water flow and correctly positions the CFTR protein to allow for the creation of a channel. [19]

Current Challenges in Gene Therapy For Cystic Fibrosis :

The UK Cystic Fibrosis Gene Therapy Consortium was established to advance gene therapy from proof-of-principle to clinical efficacy. Three UK research institutions—Oxford University, Edinburgh University, and Imperial College London/Royal Brompton Hospital—combine their expertise in CF gene therapy clinical trials. There are two phases to the group's research.

- **Wave -1 :-**

Through laboratory and preclinical research, we determined that Genzyme's GL67 (GL67A) was the best non-viral vector to create an efficient cystic fibrosis gene therapy. Lipids are co-formulated with GL67A to improve stability and delivery. Using the Consortium's plasmid pGM169, we are performing a single-dose safety study to evaluate lung function, gene expression, and safety. Lung function, inflammatory markers, and clinical evaluation are the main outcomes. Additionally, gene expression and function are being assessed. Following completion, a repeated-dose study will use outcome measures created in parallel observational and interventional contexts to examine therapeutic benefit. GL67A is a perfect choice for long-term CFTR expression and recurrent administration due to its safety, nebulizability, and manufacturability. [20]

- **Wave- 2 :-**

The Consortium is working to construct pseudotyped lentiviruses that express Sendai virus F and HN proteins in order to build a repeatable, long-lasting, and effective gene transfer technique for the treatment of cystic fibrosis. [20]

Gene Therapy For Cystic Fibrosis Perspectives :

Gene therapy for cystic fibrosis (CF) has issues with viral vectors like AAV due to pre-existing immunity. The UK CF Gene Therapy Consortium created lentiviral vectors, which provide long-term lung expression with a low risk of genotoxicity. They are getting ready for a first-in-human experiment in collaboration with Oxford BioMedica and Boehringer Ingelheim.[21] As an alternative, a Phase 2b trial shown promise for non-viral liposomes (pGM169/GL67A). Nebulized pGM169/GL67A or a placebo was administered to 116 CF patients, and during a 12-month period, there was a statistically significant 3.7% improvement in lung function. There were no safety issues brought up, and the active group benefited from secondary outcomes. This discovery gives patients with few treatment options hope by demonstrating the possibility of gene therapy to favorably modulate CF lung function. [21]

Future Studies Of Gene Therapy For Cystic Fibrosis :

Lung barriers are a problem for gene therapy for cystic fibrosis (CF). Researchers are creating new vectors, both non-viral and viral (AAV, lentiviral), to get around them. Treatments with oligonucleotides

and CFTR mRNA exhibit promise; mRNA has benefits such as cytoplasmic translation and a lower risk of insertional mutagenesis. [22] Although there are delivery issues, CRISPR gene editing may be able to fix CFTR mutations. More efficiency and safety are provided by non-viral methods, such as ribonucleoprotein complex delivery or mRNA. Another method is to edit epithelial progenitor cells, although this presents engraftment and expansion issues. Ionocytes with elevated CFTR levels are among the complex cell types of the airway epithelium that have been revealed by recent single-cell RNA sequencing studies. It might be essential for gene editing to target particular cell types. [23-24]

Many technologies are being explored to alleviate the pulmonary function issues associated with cystic fibrosis. Important research areas include developing efficient ways to transport nanoparticles, removing mucociliary obstacles, and improving gene editing technologies. These advancements aim to improve the outcomes of CF treatment. [25]

Conclusion :

Mutations in the CFTR gene produce the recessive genetic illness known as cystic fibrosis (CF). Treatment used to be symptomatic, but current treatments focus on the underlying problem. By restoring the function of the mutant CFTR protein, small molecule modulators (correctors, potentiators, stabilizers, and amplifiers) increase life expectancy from less than 20 to more than 50 years. However, the search for long-term remedies is fueled by their high costs and lifetime administration. Gene therapy provides a broad-spectrum, mutation-agnostic treatment by introducing functional CFTR genes into epithelial cells. Numerous gene editing methods are being developed, and the outcomes of clinical trials are encouraging. Despite difficulties in development, the benefits of gene therapy include its durability and scope of coverage. Its promise as a treatment encourages further study and enhances the quality of life that modulators provide.

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