Scientific Research Publishing

Journal of Biosciences and Medicines, 2024, 12, 312-329
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Multi-Organ Pathogenesis and Therapeutic Strategies for Cystic Fibrosis

Oluwaseun Babatunde[1*], Ogonna William David[2*], Richard Ifeanyichukwu Ikwugbado[2], Oluwaseyi Oyewole[3]

[1]Fetal Research and Therapy Program, Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA
[2]Department of Chemistry, University of Houston, Houston, TX, USA
[3]Biotechnology Graduate Program, School of Sciences and Engineering, The American University in Cairo, New Cairo, Egypt
Email: *obabatun@wakehealth.edu, *owdavid@cougarnet.uh.edu, riikwugb@cougarnet.uh.edu, Oyewole488@aucegypt.edu

How to cite this paper: Babatunde, O., David, O.W., Ikwugbado, R.I. and Oyewole, O. (2024) Multi-Organ Pathogenesis and Therapeutic Strategies for Cystic Fibrosis. *Journal of Biosciences and Medicines*, **12**, 312-329.
https://doi.org/10.4236/jbm.2024.125024

Received: April 29, 2024
Accepted: May 27, 2024
Published: May 30, 2024

Abstract

Cystic Fibrosis (CF) is the most common lethal autosomal inherited disorder that affects all races and ethnicities in the United States. However, it is mostly predominant in the Caucasian populace accounting for about 80% of all CF cases. CF most severe complication can be referred to as pulmonary bronchiectasis and infections of the airways, nonetheless, the devastating effects of the disease have far-reaching consequences beyond lung damage. CF is a heterogeneous disease that is caused by mutations in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The impairment or absence of this gene can affect multiple organs and systems and is characterized not only by chronic lung blockage, infections, and inflammation but also by exocrine gland dysfunction, intestinal obstruction, liver pathology, elevated sweat chloride concentration, and in males, infertility due to the congenital bilateral absence of the vas deferens. To this end, we briefly explore the pathological effects of CF and how CF mediates the destruction of several critical organs in the body and some of the gene therapeutical approaches such as gene editing and viral-based strategies available for the treatment of this multi-organ disease.

Keywords

Cystic Fibrosis, Gene Therapy, Organ Damage

1. Introduction

Long before the disease cystic fibrosis came to the frontiers of Western medicine, a popular folktale existed from Northern Europe, in ballads and children's songs in Switzerland in which the disease predominates leading to the deaths of

many children. It tells of a malediction, in which a child that tasted salty upon a brow's kiss was bewitched cursed and fated to die. This folktale delineates a crucial symptom of cystic fibrosis, which is salty sweaty glands unknown at that time.

The pathological analysis of an eleven-year-old girl purported to be bewitched by Pieter Pauw in 1595 might be the first noted record of cystic fibrosis. His findings represent an accurate description of the pancreatic lesions and abscesses that might be caused by cystic fibrosis [1]. Additional pancreatic abnormalities were also recorded by Gerardus Blasius in 1677 [2]. Perhaps, the first medical autopsy report that represents the extensive and devastating effects of what could be cystic fibrosis can be attributed to Georg Seger's examination of a three-year-old child who suffered from malnutrition, diarrhea, loss of weight, vomiting, high body temperature, and pancreatic cirrhosis [3]. Further reports by Nils Rosén von Rosenstein in 1740, describe cystic fibrosis symptoms such as swellings of the hands and feet, diarrhea, pancreatic lesions, and malabsorption [4]. Similarly, in 1878, Carl Von Rokitansky described effects such as gastrointestinal perforations [5]. However, these reports were not able to provide a direct correlation between the pathologies observed and cystic fibrosis until 1938, when Dorothy Andersen provided a detailed study of the signs and symptoms of a disease now known as cystic fibrosis [6]. Regardless of the severe histopathology of cystic fibrosis, it was thought that the disease mainly affected the pancreas and was not apparent that cystic fibrosis might be a disease that affects several organs of the body. Several more studies were required to understand the whole picture of multiorgan pathology and cystic fibrosis. The direct correlation between the cellular effects of cystic fibrosis, abnormal secretions, which block the excretory pathways of the exocrine glands might be responsible for the abscesses observed in the lungs, liver, and pancreas of patients affected by cystic fibrosis was described by Martin Bodian in 1952 [4] [7]. Concerted efforts in the 1980s finally revealed that dysfunction of the epithelial tissue was a hallmark of all tissues affected by cystic fibrosis and that the inability of the epithelial tissue to absorb chloride is responsible for salty sweats observed in patients suffering from cystic fibrosis [4] [8] [9].

2. Cystic Fibrosis Transmembrane Regulator Protein (CFTR) Mutations

Cystic Fibrosis is an autosomal recessive genetic disorder caused by over 1000 mutations in the cystic fibrosis transmembrane regulator protein (CFTR) [10], a member of the ATP-binding cassette (ABC) transporter superfamily, which couples the hydrolysis of ATP to the transport of molecules and ions across cellular membrane [11]. These mutations can be classified into subtypes based on their effects on the CFTR protein, and they affect not only the protein function, and activity but also translocation and protein turnover, regulation, and stability.

The first category of mutations results in the premature termination of the

CFTR mRNA sequence and the production of either a non-functional copy or a truncated version of the CFTR protein [12] [13]. The second category of mutations affects the post-translation modification of the CFTR protein, preventing the proper trafficking and translocation of the CFTR protein into its cellular location [12] [13]. The third category of mutations causes reduced CFTR protein channel activity in response to intracellular signaling, leading to defective protein regulation [12] [13]. The fourth category of mutations has to do with the rate of ions flow, and the duration of the CFTR protein channel opening and conduction [12] [13]. The fifth category of mutations alters the stability of the CFTR mRNA and in some cases the mature CFTR protein resulting in reduced levels of functional copies of the CFTR protein [12] [13].

3. Common Cystic Fibrosis Mutations

The most common cystic fibrosis mutation is the F508del affecting about 30% - 80% of the world populace [14] [15]. The prevalence is about 70% in the Caucasian community [15]. The F508del is a mutation that leads to the deletion of a 3 nucleotide base pair, resulting in the loss of an amino acid, phenylalanine (F) at position 508 [16]. The second most common mutation is the G542X mutation, in which the first Guanine nucleotide of the glycine codon (GGA) at position 542 in exon 11 is substituted for Thymine. This switch in nucleotide results in a stop codon with a premature or truncated CFTR protein as its consequence. This mutation type accounts for about 2.4% of the world's cystic fibrosis cases [17]. Other common mutation types such as the G551D, N1303K, and R553X are the result of a missense mutation, in which a nucleotide switch causes the incorporation of a wrong amino acid [18]. These mutations could give rise to either a truncated, or misfolded CFTR protein and, in certain cases, could give rise low abundance of the CFTR protein or alteration in the CFTR protein conformation. These ultimately result in either the absence or dysfunction of the CFTR channel.

4. Cystic Fibrosis—A Multi-Organ Disease

The mutations in the CFTR gene have far-reaching consequences, with devastating effects on several organs of the body, some of the effects of the dysfunction/absence of the CFTR gene are described below.

4.1. Cystic Fibrosis—Associated Liver Damage

The damage caused to the liver by cystic fibrosis is the third leading source of cystic fibrosis-associated mortality [19]. The mechanisms by which Cystic Fibrosis mediates liver damage are not fully understood. However, central to cystic fibrosis liver damage is the bile duct's defective CFTR protein function and cholangiocyte cytotoxicity [20]. In the liver, the cystic fibrosis transmembrane regulator protein is expressed in the epithelial cells that line the bile ducts such as the cholangiocytes and the gallbladder. In cystic fibrosis, the CFTR protein function

of regulating fluid such as water and electrolytes such as chloride and bicarbonate is altered in the bile ducts resulting in increased bile viscosity, flow, and bile salt accumulation [20]. Furthermore, abnormalities in the major macromolecular components of mucus, the mucin glycoproteins could also be a contributing factor to bile viscosity [21]. The retention of endogenous bile acids and the excretion of drug compounds and molecules that might be cytotoxic coupled with the pathogenic insults might all play key roles in the damage caused to the epithelial cells of the bile ducts [22]. Liver fibrosis and cirrhosis are the long-term implications of cystic fibrosis-mediated liver damage [22]. Complications arising from liver damage such as portal hypertension, malnutrition, and liver function failure also impair the quality of life of patients living with cystic fibrosis [23].

4.2. Cystic Fibrosis—Associated Pancreatic Damage

Pancreatic obstruction and insufficiency is a major hallmark of cystic fibrosis and it is correlated with all cystic fibrosis mutations and phenotypes [24]. The damage caused by cystic fibrosis to the pancreatic organs happens at the very onset of life, and progresses throughout life until the total destruction of the pancreatic glands [25]. Proteins synthesized in the pancreas such as the immune reactive trypsinogen (IRT), are released into the bloodstream right from birth and for a few months thereafter [26]. These excreted proteins such as the IRT served as biomarkers for the diagnosis of cystic fibrosis in neonates [26]. The destruction of the pancreas in infants takes place without any clinical symptoms [26]. How this complete devastation of the exocrine pancreas takes place is not completely understood [26]. Regardless, the damage caused to the pancreatic glands ultimately leads to the elimination of acini, mucus- and calcium-containing debris obstructing ducts, severe inflammation, and broad fibrosis [26].

CFTR is expressed mainly in the apical epithelial membranes of the small pancreatic duct. Its role is to facilitate the transportation of fluid and electrolytes such as chloride and bicarbonate. The main functions of pancreatic ductal epithelial cells are chloride uptake and bicarbonate secretion. The dysfunction of the CFTR results in decreased ductal chloride uptake, and a reduction in bicarbonate secretion, eventually leading to the decline of these anions and water in the pancreatic fluid [27]. The thickening of protein-rich acinar cell secretions leads to blockage of the proximal ducts, resulting in secondary acinar cell damage, fibrosis, and exocrine pancreatic insufficiency [27]. In addition, the bicarbonate secreted by pancreatic ductal epithelial cells serves as a neutralization buffer for the gastric acid of the stomach and provides an optimal pH for the proper functioning of the digestive enzymes [28] [29]. These processes are impaired in cystic fibrosis.

In addition, defects in the CFTR channel also lead to the destruction of the pancreatic islet cells, resulting in decreased insulin production and a predisposition to diabetes [30]. The exact mechanisms by which CFTR mediates the insulin-producing cell islets of the pancreas are not exactly understood, however,

cystic fibrosis-mediated diabetes shares some characteristics of prevalent type 1 and type 2 diabetes subtypes [31].

4.3. Cystic Fibrosis-Related Congenital Bilateral Absence of the Vas Deferens

Congenital bilateral absence of the vas deferens (CBAVD) is a form of subfertility that results from the absence, defect, or obstruction of the vas deferens, a tube whose function is to transport spermatozoa from the epididymis to the ejaculation ducts. It is responsible for about 2% of infertility cases in males [32]. CBAVD is clinically diagnosed by the presence of spermatozoa in the testicles, levels of the follicle-stimulating hormone, the evaluation of semen content such as acidity, low or undetectable levels of enzymes and molecules such as—glucosidase, fructose, and carnitine levels, and the absence of spermatozoa in the semen [33]. CBAVD can be caused by a few factors such as congenital genitourinary malformations [34] but mainly by genetic mutations [35] [36]. Genetic alteration in the CFTR gene is the main cause of CBAVD and is referred to as cystic fibrosis-related congenital bilateral absence of the vas deferens CF-CBAVD [37] [38]. This defect occurs in more than 85% of male patients suffering from cystic fibrosis. The etiology of CF-CBAVD is not fully understood, but the presence of rare and different mutations in the CFTR gene differentiates CF-BAVD from typical CF [33] suggesting the role of CFTR dysfunction in CF-CBAVD.

Similar to cystic fibrosis-mediated pancreatic damage, the deterioration of the vas deferens takes place very early in developmental life [39] [40]. The mutations in the CFTR gene, impair the function of the CFTR channel which moves chloride ions from the inside of the cells to the outside. Consequently, the inability of trapped chloride ions prevents the osmotic movement of water into mucus causing cells in the male genital tract to produce mucus that is abnormally viscous and sticky, leading to the clogging and subsequent deterioration of the vas deferens before birth [33].

4.4. Cystic Fibrosis—Mediated Intestinal Obstruction

Cystic fibrosis-mediated damage to the intestines cannot be invariably unlinked from cystic fibrosis-associated pancreatic insufficiency. The pancreas supplies the gastrointestinal tract (GI) with bicarbonate-rich pancreatic fluids, which serve as a neutralizing buffer, for digestive enzymes such as gastric acid, and the hydration of intestinal components [41] [42]. Pancreatic insufficiency and the dysfunction of the crypts and villi-rich CFTRs eventually result in reduced enzymatic activity of digestive enzymes and contribute to the maldigestion of the GI tract.

The dysfunction of the CFTR affects the GI tract, in more than one way. An adverse effect of CFTR impairment is the obstruction of the intestines, the dysregulation of chloride ions leads to the thickening and build-up of mucus, eventually resulting in the obstruction of the proximal large intestine or the ter-

minal ileum [43]. This blockage is one of the earliest symptoms of CF, and happens in infants, right from birth. It is called meconium ileus (MI), in neonates, in which the infant's first stool, meconium, (feces) blocks the ileum of the first intestine [43]. Blockage can also happen in adult patients affected by CF, in this case, it is called distal intestinal obstructive syndrome in which viscous fecal materials obstruct the terminal ileum, cecum, or ascending colon, it is often a recurrent episode and happens in 10% - 15% of adult CF patients [44]. Untreated obstruction of the GI tract ultimately could result in rupture and sepsis.

The other effect of mucus viscosity and build-up of CFTR dysfunction in the GI is the creation of niches for the abnormal colonization of the intestines by opportunistic bacteria [45]. The microbial environment of the cecum and colon, home to a wide variety of microorganisms that aid digestion is altered by the presence of these opportunistic pathogens altering the gut microflora diversity, location, and density [43] [45]. The inability of mechanisms such as gastric acid, antibacterial proteins, and intestinal fluids, to neutralize small intestinal overgrowth, and the colonization of the small intestine owing to the failure of intestinal motility to move thickened mucus-bacteria complex towards the large intestine all contribute to malnutrition, diarrhea, abdominal distension and weight loss [43] [46].

4.5. Cystic Fibrosis—Associated Lung Damage

Cystic fibrosis-mediated lung damage accounts for most of CF-related mortality. The changes in the airway interface are heavily linked to chronic and persistent infection of the lung leading to bronchitis and inflammation.

Central to the lung defense against pathogens are airway surfaces. The airway surfaces are covered by a thin film of airway surface liquid (ASL) consisting of a mucus gel and a periciliary sol (PCL). Mucociliary clearance aided by the beating actions of the cilia, together with mechanical actions such as cough and sneezing help clean the airways of contaminants, viral and bacterial pathogens.

There are two main hypotheses about CF-mediated lung pathogenesis. The first hypothesis, the high salt proposition focused on how elevated salt contents of the airway cells inhibit the innate defenses of the airway surface liquids. In this hypothesis, the dysfunction of the CFTR due to the loss of chloride conductance, results in the inability of the airways to reabsorb sodium chloride in the presence of excess water. The high salt content of ASL in CF patients impairs the innate bactericidal activities of the ASL such as lysozyme and lactoferrin [47] [48].

The second proposition is the low-volume hypothesis, which can also be referred to as the thick mucus hypothesis, focuses on the role of the failure of the CFTR protein in regulating the ENaC transporter in CF [49]. The ENaC transporter becomes hyperactivated in CF, leading to increased absorption of chloride and water from the ASL and a decline in the PCL component of the airways [8] [50]. The low liquid content of ASL in CF results in mucus dehydration and hyperconcentration. The thickened mucus impairs the beating ability of cilia of

the airways compromising mucociliary clearance, promoting infection [49] [51]. Another hypothesis worth exploring relates to the acidic environment of the ASL in CF. The CFTR is responsible for bicarbonate secretion, this function is defective in CF, bicarbonate serves as a buffer, neutralizing the acidic environment of the ASL thereby providing an optimal condition for the proper enzymatic activities of the antimicrobial peptides of the ASL [52] [53]. In fact, the inability of antimicrobial agents such as—defensin-3 and LL-37 to neutralize the effect of two of the most persistent opportunistic pathogens of the lungs, *Staphylococcus aureus* and *Pseudomonas aeruginosa* can be attributed to the low pH level of the ASL in CF in patients [54].

5. Gene Therapy Approaches for Cystic Fibrosis

The discovery of drugs that can modulate CFTRs and rectify the functioning of the defective protein have had positive impact on the life expectancy, quality of life, and given hope to many CF patients. Nonetheless, about 10% of patients are unresponsive to CFTR modulators because CFTR is not synthesized at all or is only synthesized in low quantities [55]. In addition, clinical trials (CT) show that 10% - 20% of CF patients have individual intolerance to modulator drugs [56]. In view of that, new approaches to CF management are being developed, and in this review, emphasis will be placed on the use of gene therapy methods to deliver nucleic acids to the affected cells to address the primary cause of the pathology and, through that, mitigate the course of the disease. A key strategy in CF gene therapy is to ensure that the CFTR gene is delivered to the airway epithelial cells because lungs are the main target of the gene therapy, since 90% - 95% of deaths from the disease are due to severe pulmonary lesions.

Herein, it is imperative that the delivery method should take into account the significantly reduced efficacy of aerosol administration due to the thick secretion in the bronchioles since the vector should not only ensure the effective expression of the functional CFTR protein but should also penetrate submucosal glandular cells and the superficial mucosal epithelium covered by the thick secretion [56]. It should also be noted that the airway epithelium is continuously renewed hence there is a need for repeated delivery of the target gene, and this factor restricts the use of viral vector systems, because the repeated administration often triggers an immune response resulting in vector elimination. In addition, the lack of adequate *in vivo* models for testing the efficacy of new vectors also hinders the progress in the research. Therefore, despite the initial enthusiasm, there is still no FDA approved gene therapy for CF [57]. However, with further advances in vector development and better understanding of various vector types there is a chance to come up with a more effective gene therapy for CF [58].

5.1. Adenoviral (Ad) Vectors as Gene Delivery Agent

Following the discovery of the first-generation Ad vectors, two CTs have been completed however, despite the efficacy of the approach in cell models and *in*

vivo, the CT results raised concerns about the safety of the vectors for humans. Congenital and cellular immunity hindered the long-term effect of Ad-based vectors, observations showed increased alveolar inflammation, accompanied by an increase in serotype-specific neutralizing antibodies, which rendered the repeated administration of viral particles ineffective [59]. In subsequent design, the gene was delivered using an improved Ad vector in the form of a helper-dependent adenovirus (HD-Ad) without viral genes, which made it possible to annul the T cell response to the viral protein that was a feature of the first-generation Ad vectors. Nevertheless, the adaptive immune response of CD8+ T cells with HD-Ad epitope presentation by dendritic cells remained present [60]. HD-Ad was used in the lungs in combination with lysophosphatidylcholine (LPC) with the intention to destroy the thick secretion layer and ensure better access to the basolateral cell surface for infection. This strategy resulted in lengthier gene expression *in vivo* compared to the first-generation Ad and demonstrated effective gene delivery to the airways in mice, pigs, and ferrets [58] [59].

5.2. CRISPR/Cas9 Gene Editing

Gene editing works by repairing the dysfunctional gene at the DNA level, CRISPR/Cas-9 based genome editing is a recent discovery with the potential to correct gene defect associated with cystic fibrosis [61].

Following the discovery of a natural nuclease called Cas9, accurate DNA editing is now possible. This feat is feasible because Cas9 is able to form a complex with a guide RNA (gRNA) that is specific to the target DNA, localising to the targeted DNA sequence, and then introducing a doubled-strand break (DSB) at the targeted site. The introduction of DSB then forces the DNA DSB repair mechanism known as non-homologous end-joining and homology-directed repair [62]. Donor DNA can then be provided, and this is used to repair the DSB, resulting in transgenic DNA [63]. Designing and testing gRNA has been met with high success rates and with this technology it is possible to edit the human genome for the treatment of cystic fibrosis [64]. A recent tool in CRISPR technology has been the development of Cpf1, also known as Cas12a. Relative to Cas9, Cpf1 gRNA is shorter and requires only one single gRNA molecule as opposed to Cas9 that requires two gRNAs [65]. This makes it easier to synthesize gRNA *in vitro* and package it into vectors. Cpf1 also cuts DNA to leave "sticky ends" which allows DNA insertion to be more controllable and easier to work with, while Cas9 which leaves "blunt" ends [66]. This technology has shown great success in other genetic conditions such as Duchenne muscular dystrophy, in fact, in a particular study conducted by Zhang *et al.*, they were able to successfully correct a faulty gene in both mouse models and cells derived from affected patients [66]. Maule *et al.* also provided proof of concept for the use of Cas12a in editing CFTR mutations. A minigene model was used to demonstrate that Cas12a could be utilised to effectively repair two mutations in the CFTR gene that result in splicing defects. Primary airway epithelial cells were also used to

validate the efficacy of Cas12a as delivered by a lentiviral vector [67]. Inhaled deliveries are viewed as a suitable strategy of delivering these vectors because intravenous administration only get the vectors to the alveoli, an area devoted for gas exchange, and not the surface epithelium of the bronchial tree, where the CFTR protein is more frequently expressed [67].

5.3. Adeno-Associated Viral (AAV) Vectors as Gene Delivery Agent

AAV has emerged as one of the safest and most commonly used vectors for the delivery of therapeutic genes [68]. AAV belongs to the Parvoviridae family in the *Dependovirus* genus, which depend on the coinfection of a helper virus (adenovirus or HSV) for replication in host cells [69]. In the absence of a helper virus, AAV may stably integrate but at relatively low frequency into the host gene cell and remain inactive [70]. Following the failure of the first-generation Ad vectors, Scientist began the search for alternative approaches in gene delivery to target cells. The reports from the CTs using the AAV2 vector to deliver the CFTR gene showed that introduction of the vector into the lungs of CF patients did not cause significant side-effects, but the efficacy was disappointing, since none of the CTs demonstrated significant CFTR expression or correction of pathological CF manifestations [55]. This failure can be attributed to the following reasons; insufficient efficacy of gene insertion due to the inability of viral particles to penetrate the thick secretion layer in the airways, insufficient promoter strength in the expression cassette, or immune response of the host to the introduction of the viral vector [71].

Hence, recent efforts are being directed towards improvement of the tropism of AAV vectors, identification of new serotypes, new promoters, new methods to enhance the expression of the target protein and its persistence in the lungs, as well as new approaches to immunogenicity reduction. At the same time, new *in vivo* models, including pigs [72] and mice [73], were being developed, which, along with the conventional *in vitro* tests in human epithelial cells, would make it possible to carry out more effective preclinical trials for the CF gene therapy.

Pharmaceutical companies are currently involved in the development of AAV-based CF gene therapy. According to Abeona Therapeutics [74], preclinical trials of ABO401, a new-generation capsid AAV204 developed by the company and carrying a functional copy of the human mini-CFTR gene, show that the product effectively restores the main phenotypic attribute of CF, i.e., chlorine channel functioning, in *in vitro* and *in vivo* models. In addition, AAV204 more specifically targets pulmonary cells and also transduces bronchial and nasal epithelial cells in CF patients (CFTR expression rate 3–5 times higher compared to the AAV6 vector). In addition, Spiro-2101 by Spirovant Sciences, designed for CF therapy was certified by the FDA as an orphan drug in 2020, which allowed the company to accelerate its clinical trials and take the drug to the market. Spiro-2101 also includes a new AAV capsid with improved tropism for airway epithelial cells for the delivery of a functional copy of the CFTR gene [55].

5.4. Lentiviral Vectors as Gene Delivery Agent

Lentiviral vectors are among the most intensely studied vectors utilized for virus-mediated gene transfer. Several studies have established the foundation of exploiting lentiviral vectors as vehicles for efficient gene delivery into broad range of tissues and organs. The ability to infect nondividing cells and shuttle large genetic payloads, and maintenance of stable, long-term transgene expression are attributes that have brought lentiviral vectors to the forefront of gene therapy [70]. Thus lentiviral vectors can serve as a platform for the introduction of a functional copy of the CFTR gene.

Other beneficial aspects include low immunogenicity, ability to infect various cell types and integrate consistently into the genome to ensure long-term expression and preservation of the gene in cell division. Current approaches to CF therapy using lentiviral vectors are currently undergoing preclinical trials, but recent advances in the application of improved lentiviral vectors in various CTs have shown that they are safe to use in CF therapy [75].

Studies into the primary epithelial cultures of CF patients and animal models have shown the long-term phenotype correction and low immunogenicity carried by lentiviral vectors. In a particular study, complete restoration of CFTR channel functioning in the airways of pigs after transduction with the feline immunodeficiency virus (FIV) pseudotyped with the GP64 protein was demonstrated. A significant increase in chloride ion transepithelial transport and normalization of the pH of the tracheal surface fluid and its bactericide properties were observed two weeks after FIV-CFTR aerosol administration into the nose and lungs [76]. Also, in 2017, Alton and his group analyzed the results of several preclinical trials to select the most promising vector type for initiation and planning of first CT using lentiviral transfer of the CFTR gene. The lead candidate was the lentivirus vector rSIV.F/HN since it ensured the expression of functional CFTR with efficacy of about 90% - 100% in this clinical study. Data obtained from that study supported the idea of using this vector in the first CT in CF patients [77]. Nonetheless, the CT is yet to be kick started probably due to additional preclinical trials and proof of concept on the efficiency as a therapeutic modality for CF.

5.5. mRNA Therapy

mRNA therapy involves the delivery of a new correct copy of CFTR mRNA to cells which subsequently give rise to healthy CFTR protein. mRNA therapy could potentially work for any person with cystic fibrosis regardless of the nature of CFTR mutations. The goal of mRNA therapy is to deliver CFTR mRNA to CFTR involved in the expression of epithelial cells however, due to the unstable nature of mRNA, mRNA derived therapeutics are usually formulated within a protective lipid nanoparticle [78]. One mRNA therapy currently being used for the treatment of cystic fibrosis is MRT5005, MRT5005 is a biosynthetic, codon-optimized mRNA (CO-hCFTR) encoding for the CFTR, which is delivered

by nebulization as an LNP-formulated aerosol [79]. MRT5005 is designed to have a 5' cap and a polyA tail to increase translation efficacy and prevent mRNA degradation. To mimic endogenous mRNA, the bases are left unmodified, and it is unclear whether additional modifications could further improve the potency of this drug [80]. *In vitro* and *in vivo* experiments in healthy rodents and non-human primates indicated expression of CFTR, as measured by western blot and immunohistochemical detection of CFTR in the proximal and distal airways after treatment with inhaled MRT5005 [79]. Pre-clinical studies of MRT5005 conducted *in vitro* using Fischer rat thyroid gland cells grown under polarizing conditions using chamber experiments reviewed functional restoration of CFTR activity following MRT5005 treatment and to levels that indicated the potential for bioactivity in humans. However, similar results could not be reproduced in primary human bronchial epithelial cells derived from cystic fibrosis patients. A discovery later thought to be related to the challenge of achieving substantial CFTR activity in terminally differentiated airway cells with non-viral transfection as at when this study was done [79] [80]. MRT5005 is the first clinical stage mRNA product candidate developed to treat all patients regardless of their underlying genetic mutation, including those with limited or no CFTR protein. One Phase I/II interim data of the first single ascending dose phase published showed eleven out of twelve participants who had at least one copy of F508del mutation, split into four groups receiving different dosages (8, 16, and 24 mg) with one group receiving a placebo. Following the eight-day period after dosing, the placebo group and 8 mg dose group showed no marked improvement in ppFEV1. In the 16 mg dose group, the mean maximum increase from baseline was 15.7%, with two participants in this group on a CFTR modulator during this period. In the 24 mg dose group, one patient experienced a maximum ppFEV1 increase of 21.4%, whilst two patients did not show any significant increase from their baseline [81] [82]. An advantage of mRNA therapy is that it does not alter the patient's DNA or CFTR mutations. Unlike gene editing alternatives whereby a normal copy of a defective gene is introduced into an individual's cells, there is no risk of disrupting patient's genome with this strategy however, mRNAs are naturally broken down quickly inside cells, so the effects of the mRNA therapy might last only for a short time, such as one or two weeks. This would mean that the treatment would likely need to be re-dosed regularly for it to continue to work.

To overcome this challenge, researchers have learned that chemical modifications made to RNA drugs can impact their potency and safe usage. Chemical modifications can improve the potency of RNA drugs mostly by relaxing immune response; enhancing drug stability and durability, as well as altering the affinity with which the RNA drugs bind other nucleic acids and proteins [83]. Chemical modifications made to mRNA drugs are often made at positions in the mRNA that are naturally modified in endogenous eukaryotic mRNAs [84]. For example, endogenous mRNAs are modified in human cells to include a 5' cap;

mRNA drugs are also modified at the 5' end. These 5' cap modifications have been used to decrease activation of innate immunity [85]. Endogenous mRNAs are also modified with a poly-A tail, which decreases exonuclease activity, and Scientists have developed a method, known as TAIL-Seq, to study how poly-A tails impacts mRNA stability. Researchers have used RNA sequencing to understand the distribution of poly-A tail lengths in common cell lines (HeLa and NIH 3T3). They also correlated the poly-A tail lengths to mRNA half-life and observed that longer tail lengths were correlated with an increased half-life, however not necessarily improved translation and other several studies have shown that mRNA can be modified to increase its stability and reduce immune recognition, but mRNA half-lives are still considered to be on the order of hours, not days. Hence, repeat administration of CFTR mRNA will be necessary to achieve considerable protein production in diseased patients [80].

6. Conclusion

Cystic Fibrosis is an autosomal recessive disorder characterized by classes of mutations in the CFTR channel, the dysfunction or absence of which leads to several pathological events and damage to multi-organs in the body, leading to malfunction of these organ systems and predisposition to other diseases such as diabetes and bacteria infections. Several gene therapies for the cure of cystic fibrosis hinge on either the delivery of a functional copy of the CFTR gene or the repair of the CFTR gene using gene editing tools such as CRISPR-Cas9. These approaches serve to improve the quality of lives and health of patients affected by cystic fibrosis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Busch, R. (1990) On the History of Cystic Fibrosis. *Acta Universitatis Carolinae*, **36**, 13-15.

[2] Blasius, G.L., Heiland, M., Hoffmann, M. and Wolfgang, A. (1677) Gerardii Blasii Amstelodamensis Observationes Medicae Rariores: Accedit Monstri Triplicis Historia. Apud Abrahamum Wolfgang.
https://books.google.com/books?id=QyfHzgEACAAJ

[3] Łysiak, M. (2008) From Guild Surgery to Ludwik Rydygier Surgical Clinic Collegium Medicum, Nicholas Copernicus University in Toruń. *Polish Journal of Surgery*, **80**, 227-230. https://doi.org/10.2478/v10035-008-0028-z

[4] Frías, J., Ruiz, E., López Castillo, M. and García, P. (2019) The History of Cystic Fibrosis. *Open Journal of Pediatrics and Child Health*, **4**, 1-6.
https://doi.org/10.17352/ojpch.000015

[5] Rokitansky, K. (1846) Handbuch der Pathologischen Anatomie: Handbuch der Allgemeinen Pathologischen Anatomie. Legare Street Press, London.

[6] Andersen, D. (1938) Cystic Fibrosis of the Pancreas and Its Relation to Celiac Disease: A Clinical and Pathologic Study. *The American Journal of Diseases of Children*, **56**, 344-399. https://doi.org/10.1001/archpedi.1938.01980140114013

[7] Bodian, M. (1953) Fibrocystic Disease of the Pancreas: A Congenital Disorder of Mucus Production-Mucosis. Grune & Stratton, New York.

[8] Knowles, M., Gatzy, J. and Boucher, R. (1981) Increased Bioelectric Potential Difference across Respiratory Epithelia in Cystic Fibrosis. *The New England Journal of Medicine*, **305**, 1489-1495. https://doi.org/10.1056/NEJM198112173052502

[9] Quinton, P.M. (1983) Chloride Impermeability in Cystic Fibrosis. *Nature*, **301**, 421-422. https://doi.org/10.1038/301421a0

[10] Gadsby, D.C., Vergani, P. and Csanády, L. (2006) The ABC Protein Turned Chloride Channel Whose Failure Causes Cystic Fibrosis. *Nature*, **440**, 477-483. https://doi.org/10.1038/nature04712

[11] Corradi, V., Vergani, P. and Tieleman, D.P. (2015) Cystic Fibrosis Transmembrane Conductance Regulator (CFTR): Closed and Open State Channel Models. *Journal of Biological Chemistr*, **290**, 22891-22906. https://doi.org/10.1074/jbc.M115.665125

[12] Moskowitz, S.M., Chmiel, J.F., Sternen, D.L., Cheng, E., Gibson, R.L., Marshall, S.G., *et al.* (2008) Clinical Practice and Genetic Counseling for Cystic Fibrosis and CFTR-Related Disorders. *Genetics in Medicine*, **10**, 851-868. https://doi.org/10.1097/GIM.0b013e31818e55a2

[13] Chen, Q., Shen, Y. and Zheng, J. (2021) A Review of Cystic Fibrosis: Basic and Clinical Aspects. *Animal Models and Experimental Medicine*, **4**, 220-232. https://doi.org/10.1002/ame2.12180

[14] Abou Tayoun, A.N., Tunkey, C.D., Pugh, T.J., Ross, T., Shah, M., Lee, C.C., *et al.* (2013) A Comprehensive Assay for CFTR Mutational Analysis Using Next-Generation Sequencing. *Clinical Chemistry*, **59**, 1481-1488. https://doi.org/10.1373/clinchem.2013.206466

[15] Aghamohammadi, A., Keivanfar, M., Navaei, S., Shirzadi, R., Masiha, F., Allameh, Z., *et al.* (2019) First Cystic Fibrosis Patient Registry Annual Data Report-Cystic Fibrosis Foundation of Iran. *Acta Medica Iranica*, **57**, 33-41. https://doi.org/10.18502/acta.v57i1.1751

[16] Riordan, J.R., Rommens, J.M., Kerem, B, Sheva Alon, N., Rozmahel, R., Grzelczak, Z., *et al.* (1989) Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA. *Science*, **245**, 1066-1073. https://doi.org/10.1126/science.2475911

[17] De Araujo, F., Novaes, F.C., Dos Santos, N., Martins, V., De, C., De Souza, S., Dos Santos, S., *et al.* (2005) Prevalence of DeltaF508, G551D, G542X, and R553X Mutations among Cystic Fibrosis Patients in the North of Brazil. *Brazilian Journal of Medical and Biological Research*, **38**, 11-15. https://doi.org/10.1590/S0100-879X2005000100003

[18] Tsui, L.C. (1992) The Spectrum of Cystic Fibrosis Mutations. *Trend in Genetics*, **8**, 392-398. https://doi.org/10.1016/0168-9525(92)90168-4

[19] MacKenzie, T., Gifford, A.H., Sabadosa, K.A., Quinton, H.B., Knapp, E.A., Goss, C.H., *et al.* (2014) Longevity of Patients with Cystic Fibrosis in 2000 to 2010 and Beyond: Survival Analysis of the Cystic Fibrosis Foundation Patient Registry. *Annals of Internal Medicine*, **161**, 233-241. https://doi.org/10.7326/M13-0636

[20] Debray, D., Narkewicz, M.R., Bodewes, F.A.J.A., Colombo, C., Housset, C., De Jonge, H.R., *et al.* (2017) Cystic Fibrosis-Related Liver Disease: Research Challenges and Future Perspectives. *Journal of Pediatric Gastroenterology and Nutrition*, **65**,

443-448.
https://journals.lww.com/jpgn/fulltext/2017/10000/cystic_fibrosis_related_liver_disease__research.23.aspx
https://doi.org/10.1097/MPG.0000000000001676

[21] Bhaskar, R.K., Turner, B.S., Grubman, S.A., Jefferson, D.M. and LaMont, J.T. (1998) Dysregulation of Proteoglycan Production by Intrahepatic Biliary Epithelial Cells Bearing Defective (Delta-F508) Cystic Fibrosis Transmembrane Conductance Regulator. *Hepatology*, **27**, 7-14. https://doi.org/10.1002/hep.510270103

[22] Colombo, C. (2007) Liver Disease in Cystic Fibrosis. *Current Opinion in Pulmonary Medicine*, **13**, 529-536.
https://journals.lww.com/co-pulmonarymedicine/fulltext/2007/11000/liver_disease_in_cystic_fibrosis.11.aspx
https://doi.org/10.1097/MCP.0b013e3282f10a16

[23] Lewindon, P.J., Shepherd, R.W., Walsh, M.J., Greer, R.M., Williamson, R., Pereira, T.N., *et al.* (2011) Importance of Hepatic Fibrosis in Cystic Fibrosis and the Predictive Value of Liver Biopsy. *Hepatology*, **53**, 193-201.
https://doi.org/10.1002/hep.24014

[24] Kristidis, P., Bozon, D., Corey, M., Markiewicz, D., Rommens, J., Tsui, L., *et al.* (1992) Genetic Determination of Exocrine Pancreatic Function in Cystic Fibrosis. *The American Journal of Human Genetics*, **50**, 1178-1184.

[25] Abu-El-Haija, M., Ramachandran, S., Meyerholz, D.K., Abu-El-Haija, M., Griffin, M., Giriyappa, R.L., *et al.* (2012) Pancreatic Damage in Fetal and Newborn Cystic Fibrosis Pigs Involves the Activation of Inflammatory and Remodeling Pathways. *The American Journal of Pathology*, **181**, 499-507.
https://doi.org/10.1016/j.ajpath.2012.04.024

[26] Wilschanski, M. and Novak, I. (2013) The Cystic Fibrosis of Exocrine Pancreas. *Cold Spring Harbor Perspectives in Medicine*, **3**, a009746.
https://doi.org/10.1101/cshperspect.a009746

[27] Nousia-Arvanitakis, S. (1999) Cystic Fibrosis and the Pancreas: Recent Scientific Advances. *Journal of Clinical Gastroenterology*, **29**, 138-142.
https://journals.lww.com/jcge/fulltext/1999/09000/cystic_fibrosis_and_the_pancreas__recent.7.aspx
https://doi.org/10.1097/00004836-199909000-00007

[28] Lee, M.G., Ohana, E., Park, H.W., Yang, D. and Muallem, S. (2012) Molecular Mechanism of Pancreatic and Salivary Gland Fluid and HCO_3^- Secretion. *Physiological Reviews*, **92**, 39-74. https://doi.org/10.1152/physrev.00011.2011

[29] Park, H.W. and Lee, M.G. (2012) Transepithelial Bicarbonate Secretion: Lessons from the Pancreas. *Cold Spring Harbor Perspectives in Medicine*, **2**, a009571.
https://doi.org/10.1101/cshperspect.a009571

[30] Granados, A., Chan, C.L., Ode, K.L., Moheet, A., Moran, A. and Holl, R. (2019) Cystic Fibrosis Related Diabetes: Pathophysiology, Screening and Diagnosis. *Journal of Cystic Fibrosis*, **18**, S3-S9. https://doi.org/10.1016/j.jcf.2019.08.016

[31] Coderre, L., Debieche, L., Plourde, J., Rabasa-Lhoret, R. and Lesage, S. (2021) The Potential Causes of Cystic Fibrosis-Related Diabetes. *Frontiers in Endocrinology*, **12**, Article 702823.
https://www.frontiersin.org/journals/endocrinology/articles/10.3389/fendo.2021.702823
https://doi.org/10.3389/fendo.2021.702823

[32] Hussein, T., Zakaria, N. and Zahran, A. (2011) Clinical, Laboratory and Genetic Assessment of Patients with Congenital Bilateral Absent Vas Deferens. *Andrologia*,

43, 16-22. https://doi.org/10.1111/j.1439-0272.2009.01001.x

[33] De Souza, D.A.S., Faucz, F.R., Pereira-Ferrari, L., Sotomaior, V.S. and Raskin, S. (2018) Congenital Bilateral Absence of the Vas Deferens as an Atypical Form of Cystic Fibrosis: Reproductive Implications and Genetic Counseling. *Andrology*, **6**, 127-135. https://doi.org/10.1111/andr.12450

[34] Salwan, A. and Abdelrahman, A. (2017) Congenital Absence of Vas Deferens and Ectopic Kidney. *International Journal of Surgery Case Reports*, **34**, 90-92. https://doi.org/10.1016/j.ijscr.2017.03.019

[35] Chillón, M., Casals, T., Mercier, B., Bassas, L., Lissens, W., Silber, S., *et al.* (1995) Mutations in the Cystic Fibrosis Gene in Patients with Congenital Absence of the Vas Deferens. *The New England Journal of Medicine*, **332**, 1475-1480. https://doi.org/10.1056/NEJM199506013322204

[36] Bieth, E., Hamdi, S.M. and Mieusset, R. (2021) Genetics of the Congenital Absence of the Vas Deferens. *Human Genetics*, **140**, 59-76. https://doi.org/10.1007/s00439-020-02122-w

[37] Xu, W.M., Shi, Q.X., Chen, W.Y., Zhou, C.X., Ni, Y., Rowlands, D.K., *et al.* (2007) Cystic Fibrosis Transmembrane Conductance Regulator Is Vital to Sperm Fertilizing Capacity and Male Fertility. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 9816-9821. https://doi.org/10.1073/pnas.0609253104

[38] Yang, L., Ren, Z., Yang, B., Zhou, J., Peng, Z., Fang, K., *et al.* (2020) The Association Between Variants in the CFTR Gene and Nonobstructive Male Infertility: A Meta-Analysis. *Andrologia*, **52**, e13475. https://doi.org/10.1111/and.13475

[39] Gaillard, D.A., Carre-Pigeon, F. and Lallemand, A. (1997) Normal Vas Deferens in Fetuses with Cystic Fibrosis. *gy*, **158**, 1549-1552. https://doi.org/10.1016/S0022-5347(01)64278-2

[40] Cuppens, H. and Cassiman, J. (2004) CFTR Mutations and Polymorphisms in Male Infertility. *International Journal of Andrology*, **27**, 251-256. https://doi.org/10.1111/j.1365-2605.2004.00485.x

[41] Gelfond, D., Ma, C., Semler, J. and Borowitz, D. (2013) Intestinal PH and Gastrointestinal Transit Profiles in Cystic Fibrosis Patients Measured by Wireless Motility Capsule. *Digestive Diseases and Sciences*, **58**, 2275-2281. https://doi.org/10.1007/s10620-012-2209-1

[42] Barraclough, M. and Taylor, C. (1996) Twenty-Four Hour Ambulatory Gastric and Duodenal PH Profiles in Cystic Fibrosis: Effect of Duodenal Hyperacidity on Pancreatic EnzymeFunction and Fat Absorption. *Journal of Pediatric Gastroenterology and Nutrition*, **23**, 45-50. https://doi.org/10.1002/j.1536-4801.1996.tb00301.x

[43] De Lisle, R.C. and Borowitz, D. (2013) The Cystic Fibrosis Intestine. *Cold Spring Harbor Perspectives in Medicine*, **3**, a009753. https://doi.org/10.1101/cshperspect.a009753

[44] Mavilia, M.G. and Pope, S. (2018) Distal Intestinal Obstructive Syndrome (DIOS): A Gastrointestinal Complication of Cystic Fibrosis in Adults: 1490. *American Journal of Gastroenterology*, **113**, S855-S856. https://doi.org/10.14309/00000434-201810001-01490

[45] Cotter, P.D. (2011) Small Intestine and Microbiota. *Current Opinion in Gastroenterology*, **27**, 99-105. https://doi.org/10.1097/MOG.0b013e328341dc67

[46] Singh, V.V. and Toskes, P.P. (2004) Small Bowel Bacterial Overgrowth: Presentation, Diagnosis, and Treatment. *Current Treatment Options in Gastroenterology*, **7**, 19-28. https://doi.org/10.1007/s11938-004-0022-4

[47] Smith, J.J., Travis, S.M., Greenberg, E.P. and Welsh, M.J. (1996) Cystic Fibrosis Airway Epithelia Fail to Kill Bacteria Because of Abnormal Airway Surface Fluid. *Cell*, **85**, 229-236. https://doi.org/10.1016/S0092-8674(00)81099-5

[48] Travis, S.M., Conway, B.A.D., Zabner, J., Smith, J.J., Anderson, N.N., Singh, P.K., *et al.* (1999) Activity of Abundant Antimicrobials of the Human Airway. *American Journal of Respiratory Cell and Molecular Biology*, **20**, 872-879. https://doi.org/10.1165/ajrcmb.20.5.3572

[49] Matsui, H., Grubb, B.R., Tarran, R., Randell, S.H., Gatzy, J.T., Davis, C.W., *et al.* (1998) Evidence for Periciliary Liquid Layer Depletion, Not Abnormal Ion Composition, in the Pathogenesis of Cystic Fibrosis Airways Disease. *Cell*, **95**, 1005-1015. https://doi.org/10.1016/S0092-8674(00)81724-9

[50] Mall, M., Bleich, M., Greger, R., Schreiber, R. and Kunzelmann, K. (1998) The Amiloride-Inhibitable Na^+ Conductance Is Reduced by the Cystic Fibrosis Transmembrane Conductance Regulator in Normal but Not in Cystic Fibrosis Airways. *Journal of Clinical Investigation*, **102**, 15-21. https://doi.org/10.1172/JCI2729

[51] Wine, J.J. (1999) The Genesis of Cystic Fibrosis Lung Disease. *Journal of Clinical Investigation*, **103**, 309-312. https://doi.org/10.1172/JCI6222

[52] Pezzulo, A.A., Tang, X.X., Hoegger, M.J., Abou, Alaiwa, M.H., Ramachandran, S., Moninger, T.O., *et al.* (2012) Reduced Airway Surface PH Impairs Bacterial Killing in the Porcine Cystic Fibrosis Lung. *Nature*, **487**, 109-113. https://doi.org/10.1038/nature11130

[53] Cantin, A.M., Hartl, D., Konstan, M.W. and Chmiel, J.F. (2015) Inflammation in Cystic Fibrosis Lung Disease: Pathogenesis and Therapy. *Journal of Cystic Fibrosis*, **14**, 419-430. https://doi.org/10.1016/j.jcf.2015.03.003

[54] Abou Alaiwa, M.H., Reznikov, L.R., Gansemer, N.D., Sheets, K.A., Horswill, A.R., Stoltz, D.A., *et al.* (2014) PH Modulates the Activity and Synergism of the Airway Surface Liquid Antimicrobials β-Defensin-3 and LL-37. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 18703-18708. https://doi.org/10.1073/pnas.1422091112

[55] Lomunova Gershovich, P.M. (2023) Gene Therapy for Cystic Fibrosis: Recent Advances and Future Prospects. *Acta Naturae*, **15**, 20-31. https://doi.org/10.32607/actanaturae.11708

[56] Burgener, E.B. and Moss, R.B. (2018) Cystic Fibrosis Transmembrane Conductance Regulator Modulators: Precision Medicine in Cystic Fibrosis. *Current Opinion in Pediatrics*, **30**, 372-377. https://doi.org/10.1097/MOP.0000000000000627

[57] Boyd, A.C., Guo, S., Huang, L., Kerem, B., Oren, Y.S., Walker, A.J. and Hart, S.L. (2020) New Approaches to Genetic Therapies for Cystic Fibrosis. *Journal of Cystic Fibrosis*, **19**, S54-S59. https://doi.org/10.1016/j.jcf.2019.12.012

[58] Yan, Z., McCray, P.B. and Engelhardt, J.F. (2019) Advances in Gene Therapy for Cystic Fibrosis Lung Disease. *Human Molecular Genetics*, **28**, R88-R94. https://doi.org/10.1093/hmg/ddz139

[59] Cooney, A.L., McCray, P.B. and Sinn, P.L. (2018) Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward. *Genes*, **9**, Article 538. https://doi.org/10.3390/genes9110538

[60] Cao, H., Ouyang, H., Grasemann, H., Bartlett, C., Du, K., Duan, R., *et al.* (2018) Transducing Airway Basal Cells with a Helper-Dependent Adenoviral Vector for Lung Gene Therapy. *Human Gene Therapy*, **29**, 643-652. https://doi.org/10.1089/hum.2017.201

[61] Hsu, P.D., Lander, E.S. and Zhang, F. (2014) Development and Applications of

CRISPR-Cas9 for Genome Engineering. *Cell*, **157**, 1262-1278. https://doi.org/10.1016/j.cell.2014.05.010

[62] Hille, F. and Charpentier, E. (2016) CRISPR-Cas: Biology, Mechanisms and Relevance. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **371**, Article ID: 20150496. https://doi.org/10.1098/rstb.2015.0496

[63] Hall, B., Cho, A., Limaye, A., Cho, K., Khillan, J. and Kulkarni, A.B. (2018) Genome Editing in Mice Using CRISPRCas9 Technology. *Current Protocols in Cell Biology*, **81**, e57. https://doi.org/10.1002/cpcb.57

[64] Everson, E.M., Olzsko, M.E., Leap, D.J., Hocum, J.D. and Trobridge, G.D. (2016) 1. A Direct Comparison of Foamy and Lentiviral Vector Genotoxicity in SCID-Repopulating Cells Shows Foamy Vectors Are Less Prone to Clonal Dominance. *Molecular Therapy*, **24**, S1. https://doi.org/10.1038/mt.2016.78

[65] Hodges, C.A. and Conlon, R.A. (2019) Delivering on the Promise of Gene Editing for Cystic Fibrosis. *Genes and Diseases*, **6**, 97-108. https://doi.org/10.1016/j.gendis.2018.11.005

[66] Zhang, Y., Long, C., Li, H., Mcanally, J.R., Baskin, K.K., Shelton, J.M., *et al.* (2017) CRISPR-Cpf1 Correction of Muscular Dystrophy Mutations in Human Cardiomyocytes and Mice. *Science Advances*, **3**, e1602814. https://www.science.org https://doi.org/10.1126/sciadv.1602814

[67] Katz, M.G., Fargnoli, A.S. and Bridges, C.R. (2013) Myocardial Gene Transfer: Routes and Devices for Regulation of Transgene Expression by Modulation of Cellular Permeability. *Human Gene Therapy*, **24**, 375-392. https://doi.org/10.1089/hum.2012.241

[68] Lentz, T.B., Gray, S.J. and Samulski, R.J. (2012) Viral Vectors for Gene Delivery to the Central Nervous System. *Neurobiology of Disease*, **48**, 179-188. https://doi.org/10.1016/j.nbd.2011.09.014

[69] Atchison, R.W., Casto, B.C. and Hammon, W.M. (1965) Adenovirus-Associated Defective Virus Particles. *Science*, **149**, 754-756.

[70] Kantor, B., Bailey, R.M., Wimberly, K., Kalburgi, S.N. and Gray, S.J. (2014) Methods for Gene Transfer to the Central Nervous System. *Advances in Genetics*, **87**, 125-197. https://doi.org/10.1016/B978-0-12-800149-3.00003-2

[71] Guggino, W.B. and Cebotaru, L. (2017) Adeno-Associated Virus (AAV) Gene Therapy for Cystic Fibrosis: Current Barriers and Recent Developments. *Expert Opinion on Biological Therapy*, **17**, 1265-1273. https://doi.org/10.1080/14712598.2017.1347630

[72] Steines, B., Dickey, D.D., Bergen, J., Excoffon, K.J.D.A., Weinstein, J.R., Li, X., *et al.* (2016) CFTR Gene Transfer with AAV Improves Early Cystic Fibrosis Pig Phenotypes. *JCI Insight*, **1**, e88728. https://doi.org/10.1172/jci.insight.88728

[73] Vidovic, D., Carlon, M.S., Da Cunha, M.F., Dekkers, J.F., Hollenhorst, M.I., Bijvelds, M.J.C., *et al.* (2016) RAAV-CFTRDR Rescues the Cystic Fibrosis Phenotype in Human Intestinal Organoids and Cystic Fibrosis Mice. *American Journal of Respiratory and Critical Care Medicine*, **193**, 288-298. https://doi.org/10.1164/rccm.201505-0914OC

[74] Wille, P., Rosenjack, J., Cotton, C., Kelley, T., Padegimas, L. and Miller, T. (2019) EPS1.02 Identification of AAV Developed for Cystic Fibrosis Gene Therapy That Restores CFTR Function in Human Cystic Fibrosis Patient Cells. *Journal of Cystic Fibrosis*, **18**, S39-S56. https://doi.org/10.1016/S1569-1993(19)30241-3

[75] Marquez Loza, L.I., Yuen, E.C. and McCray, P.B. (2019) Lentiviral Vectors for the Treatment and Prevention of Cystic Fibrosis Lung Disease. *Genes*, **10**, Article 218.

https://doi.org/10.3390/genes10030218

[76] Cooney, A.L., Abou Alaiwa, M.H., Shah, V.S., Bouzek, D.C., Stroik, M.R., Powers, L.S., *et al.* (2016) Lentiviral-Mediated Phenotypic Correction of Cystic Fibrosis Pigs. *JCI Insight*, **1**, e88730. https://doi.org/10.1172/jci.insight.88730

[77] Alton, E.W.F.W., Beekman, J.M., Boyd, A.C., Brand, J., Carlon, M.S., Connolly, M.M., *et al.* (2017) Preparation for a First-In-Man Lentivirus Trial in Patients with Cystic Fibrosis. *Thorax*, **72**, 137-147. https://doi.org/10.1136/thoraxjnl-2016-208406

[78] Burney, T.J. and Davies, J.C. (2012) Gene Therapy for the Treatment of Cystic Fibrosis. *Application of Clinical Genetics*, **5**, 29-36. https://doi.org/10.2147/TACG.S8873

[79] Rowe, S.M., Zuckerman, J.B., Dorgan, D., Lascano, J., McCoy, K., Jain, M., *et al.* (2023) Inhaled MRNA Therapy for Treatment of Cystic Fibrosis: Interim Results of A Randomized, Double-Blind, Placebo-Controlled Phase 1/2 Clinical Study. *Journal of Cystic Fibrosis*, **22**, 656-664. https://doi.org/10.1016/j.jcf.2023.04.008

[80] Da Silva Sanchez, A., Paunovska, K., Cristian, A. and Dahlman, J.E. (2020) Treating Cystic Fibrosis with MRNA and CRISPR. *Human Gene Therapy*, **31**, 940-955. https://doi.org/10.1089/hum.2020.137

[81] Marangi, M. and Pistritto, G. (2018) Innovative Therapeutic Strategies for Cystic Fibrosis: Moving forward to CRISPR Technique. *Frontiers in Pharmacology*, **9**, Article 396. https://doi.org/10.3389/fphar.2018.00396

[82] Jaques, R., Shakeel, A. and Hoyle, C. (2020) Novel Therapeutic Approaches for the Management of Cystic Fibrosis. *Multidisciplinary Respiratory Medicine*, **15**, Article No. 690.

[83] Gaj, T., Gersbach, C.A. and Barbas, C.F. (2013) ZFN, TALEN, and CRISPR/Cas-Based Methods for Genome Engineering. *Trends in Biotechnology*, **31**, 397-405. https://doi.org/10.1016/j.tibtech.2013.04.004

[84] Roundtree, I.A., Evans, M.E., Pan, T. and He, C. (2017) Dynamic RNA Modifications in Gene Expression Regulation. *Cell*, **169**, 1187-1200. https://doi.org/10.1016/j.cell.2017.05.045

[85] Ramanathan, A., Robb, G.B. and Chan, S.H. (2016) MRNA Capping: Biological Functions and Applications. *Nucleic Acids Research*, **44**, 7511-7526. https://doi.org/10.1093/nar/gkw551