CRISPR/Cas9-Mediated Gene Editing: A Novel Approach for Cystic Fibrosis

Section 1: Introduction to CRISPR Cas9 systems and Cystic Fibrosis

The idea of CRISPR – clustered regularly short palindromic repeats – was discovered in the DNA sequence of *Escherichia coli* bacteria in Osaka University of Japan in 1987 [11]. Later, in 2012, Jennifer Doudna along with her collaborator Emmanuelle Carpenter published a groundbreaking paper that described CRISPR-cas9 system's potential as a gene-editing tool. But prior to their collaboration each respective scientist was doing their own research which led them to their investigation into CRISPR. Doudna, an American biochemist at the University of California, Berkeley had already been established in the scientific community for her work in RNA and its role with biological processes. Early in her career, Doudna worked to analyse the three-dimensional structure of RNA to provide insights on RNA catalytic activity. She later started investigating the control that RNA molecules have on genetic information, leading to her fascination with the CRISPR process [12]. Charpentier, a French microbiologist, was studying a harmful bacterium, *Streptococcus pyogenes*, when she discovered a new molecule called tracrRNA. Her past work demonstrated that the tracrRNA molecule was part of a bacterial immune system known as CRISPR/Cas which could protect the bacteria from occupying viruses by splitting their DNA [15]. After the discovery of the tracrRNA molecule, Charpentier knew she needed a partner to exchange their expertise on the topic of CRISPR. Emanuelle and Charpentier met at a conference in 2011 at Puerto Rico and had both realized they had complementary scientific research on the genome. Together, they set out to understand the molecular mechanisms behind the CRISPR-cas9 system. Their primary goal was to understand how this system would be able to conduct precise gene editing without major off-target effects. The CRISPR process starts out with a ribonucleoprotein (RNP) complex that consists of a cas9 protein and guide RNA. The cas9 protein is an endonuclease enzyme that plays a crucial role in genetic engineering applications. Cas9 is a type of DNA nuclease that can precisely cleave double-stranded DNA [18]. The guide RNA is made up of trans-activating RNA (tracrRNA) and CRISPR RNA (crRNA). The tracrRNA binds to the crRNA, which creates more stability in the gene when the tracrRNA guides the cas9-protein to the correct sequences. The crRNA has a complementary sequence of around twenty-thirty base pairs that “unlocks” the gene that needs to be cut out of the genome. Next, the guide RNA is mixed with the crRNA and incubated until the cas9 enzyme can be activated. By using the crRNA and tracrRNA, the cas9-system can more efficiently and accurately target specific DNA sequences. The tracrRNA properly positions the cas9 protein at the target DNA site. Once the cas9 enzyme is bonded to the target DNA, it can introduce a double-strand break at the specific location. This double-stranded break opens segments of the target DNA and finds a complementary match, allowing the gene found to be spliced. The double-stranded break can either disrupt a gene (inactivating it) or can be used to insert or delete specific sequences in DNA, thereby altering the genetic information. Splicing the gene means the PAM (protospacer adjacent motif) sequence, recognition site for cas9, can be cut. The specific order of the PAM sequence matters because it determines whether the cas9-protein will be able to bind correctly to a particular site in the genome. The specificity of the PAM sequence (the most common one being NGG) helps reduce off-target effects and ensures that the CRISPR-cas9 system edits only the intended target site [13].

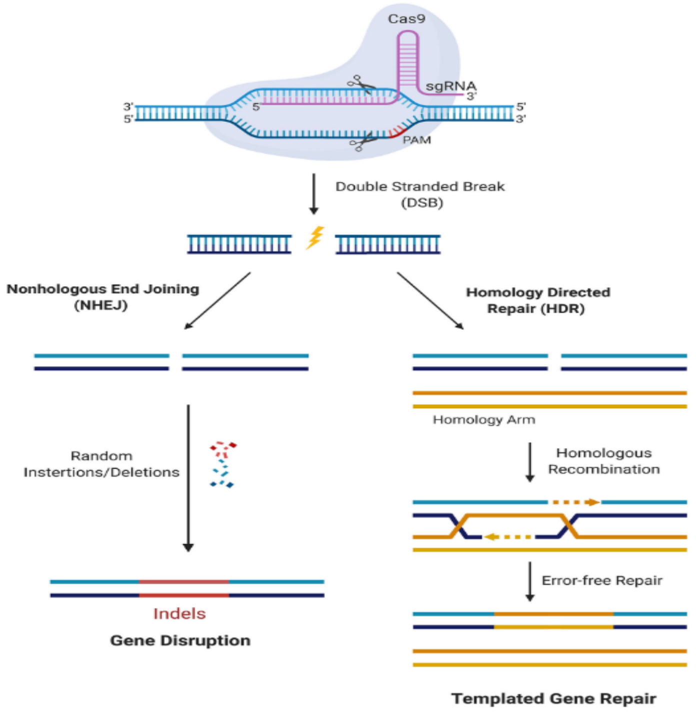

FIGURE 2 | CRISPR/Cas9 mediated gene editing. Cas9 in complex with the sgRNA targets the respective gene and creates DSBs near the PAM region. DNA damage repair proceeds either through the NHEJ pathway or HDR. In the NHEJ pathway, random insertions and deletions (indels) are introduced at the cut side and ligated resulting in error-prone repair. In the HDR pathway, the homologous chromosomal DNA serves as a template for the damaged DNA during repair, resulting in error-free repair.

Figure 1: CRISPR/cas9 gene editing mechanism. Cas9, guided by sgRNA, creates a double-strand break (DSB) in DNA. The DSB can be repaired by Nonhomologous End Joining (NHEJ), which introduces random insertions/deletions (indels) leading to gene disruption, or by Homology Directed Repair (HDR), which uses a DNA template for precise, error-free repair [7].

NHEJ (Non-Homologous End Joining) and HDR (Homology-Directed Repair) are two distinct DNA repair pathways that cells use to fix double-strand breaks in DNA. While NHEJ directly joins the broken pieces of DNA together without needing a homologous base template, HDR uses a homologous sequence as a template (sister chromatid) to repair the break more accurately. In summary, NHEJ is quicker, more error-prone while HDR is a slower but more precise way to repair DNA [21] (Figure 1).

Before the introduction of gene therapies, CRISPR's biological function was to protect prokaryotes from viruses. Prior to the discovery of using CRISPR for gene-editing, the biological function of CRISPR's system hadn't been illuminated, but scientists proposed using it to genotype various strains of bacteria. They started out by using *Mycobacterium tuberculosis* [7] and later used *Streptococcus pyogenes* [10]. The results demonstrated that the loci had a high degree of polymorphism, occurrence of different forms for a single living organism, which allows the identification of bacterial strains in clinical conditions. The CRISPR-cas9 system was mainly designed to prevent diseases caused by gene mutations in species. Genome editing, which includes the use of the CRISPR-cas9, has two major ways of introducing the cas9-complex into embryos [6]. First, is micro injection where the cas9 protein is injected directly into the cytoplasm of fertilized embryos. Second, electroporation, which is electric stimulation to the fertilized embryo in the presence of the cas9-complex. While those are the most common methods of introducing the cas9-complex into embryos, there are methods such as the use of viral vectors and lipid nanoparticles. Cas9 and gRNA are delivered using engineered viruses (lentivirus, adenovirus, etc.) and is efficient for in vivo gene editing, can target specific tissues or cells. Some challenges to using viral vectors is the risk of mutagenesis, potential immune responses, and less control over the integration site. The cas9 mRNA and gRNA can also be encapsulated in lipid nanoparticles that bind together with cell membranes to deliver their cargo. Although with the use of nanoparticles there is relatively low toxicity, there is also lower efficiency compared to microinjections [16].

Currently, the CRISPR-cas9 system is being tested through numerous trials on embryos (mostly animal) to cure genetic diseases such as sickle-cell disease and even bone regeneration illnesses. It allows defective genes to be cut out from the genome and discarded to repair genome damage (from mutations) in a variety of species. Most clinical trials of CRISPR-cas9 have been done with smaller animal embryos such as mice or rats, since they resemble human embryos more closely than other animal species. This is due to morphological similarities during early development in both mouse and human embryos. Both embryos undergo processes such as gastrulation (process that morphs an embryo from a single-layered sphere into a multi-layered structure), organogenesis (development of organs in an animal), and neurulation (formation of the neural tube in an animal). In terms of the genomes themselves, both species have about 3.1 billion base pairs with a large portion consisting of non-coding DNA, often known as "junk DNA". Approximately 85% of the protein-coding regions in the human and mouse genomes are identical and are homologous to each other. This high level of similarity is because mice and humans share a common ancestor from about 80 million years ago [17].

The reason cystic fibrosis has one of the most in-demand cures is because it is one of the most common genetic disorders in the United States, occurring in one of every 3,200 births. More than 30,000 adults and children in the United States deal with cystic fibrosis, with another 70,000 people who have cystic fibrosis live in other countries [1]. As early as 1595, was when historical documents suggested that cystic fibrosis started to be noticed in children. Babies who had cystic fibrosis were described as having "salty skin", as one of the common symptoms of cystic fibrosis is an extreme change in skin colour, shortness of breath, and frequent lung infections. In the 1930s, doctors from Switzerland officially recognized "cystic fibrosis of the pancreas" as a disease [1]. Cystic fibrosis is a genetically passed down disease from a genetic defect from the autosomal recessive pattern of inheritance. This means children would need to inherit one copy of the gene from each parent for them to develop cystic fibrosis. This disease mainly causes damage to the lungs and digestive system. Because of the destruction of the lungs, it causes major breathing problems, lung inflammation, and the buildup of large amounts of mucus. Normally, in human bodies, mucus acts as a lubrication agent because of its thin and slippery nature. However, cystic fibrosis patients have stickier and thicker layers of mucus (sputum) that plug up ducts, tubes, and several passageways in their body. Primarily, it blocks passageways in the lungs and pancreas causing organ failure and difficulties in breathing. The increased amount of sticky sputum creates an ideal environment for the growth of dangerous bacteria. This puts cystic fibrosis patients at a higher risk of contracting bacterial chest infections and pneumonia. In addition to the destruction of the lungs, cystic fibrosis also causes severe damage to the pancreas The combination of sputum with the previous damage prevents crucial nutrients from reaching to a person's digestive tract. This is due to not being able to release digestive enzymes [1].

Cystic fibrosis is a genetic disease caused by mutations in the CFTR gene. The CFTR gene encodes a protein that regulates the secretion of chloride and bicarbonate, which regulates the body's acid-base balance. This is why patients with cystic fibrosis have extremely high amounts of mucus built up in their lungs, causing difficulty in breathing. Current therapeutic approaches include CFTR modulators known as ivacaftor and lumacaftor which deliver functional CFTR DNA to correct the gene defect in the patient's body. Many researchers have also chosen to use rabbit embryos to test potential solutions as opposed to mice or rats as their amino acid sequence for the CFTR gene (gene that causes cystic fibrosis) holds 92% identity with the human CFTR gene [20]. This means it was relatively more accurate than other animal models. There are also challenges that come with using CRISPR on cystic fibrosis, which are extracellular barriers and intracellular barriers [4]. Extracellular barriers cause gene transfer into the lung to be difficult because of barriers such as mucus and other immune responses. Specifically, for cystic fibrosis, the type of extracellular barrier that is most important is the mucous barrier, which discharges mucus through epithelial cells and goblet cells. Intracellular barriers cause nuclear membranes to prevent effective gene delivery because of the vast size of the CRISPR-cas9 system, which compared to other systems can be seen as less suitable (Marangi and Pistritto 2018). Overall, Cystic Fibrosis is a complex disease that impacts individuals on multiple physiological levels. However, its high level of genetic specificity allows for potential genetic therapeutic strategies to intervene or completely mitigate disease progression.

Section 2: What have been some past experiments done involving CRISPR and cystic fibrosis?

Many past experiments involving the use of CRISPR to help cure cystic fibrosis have been conducted using animal embryos as human embryos are much more difficult to acquire. Human embryos are not used for clinical trials due to ethical concerns, legal restrictions, and lack of patient safety.

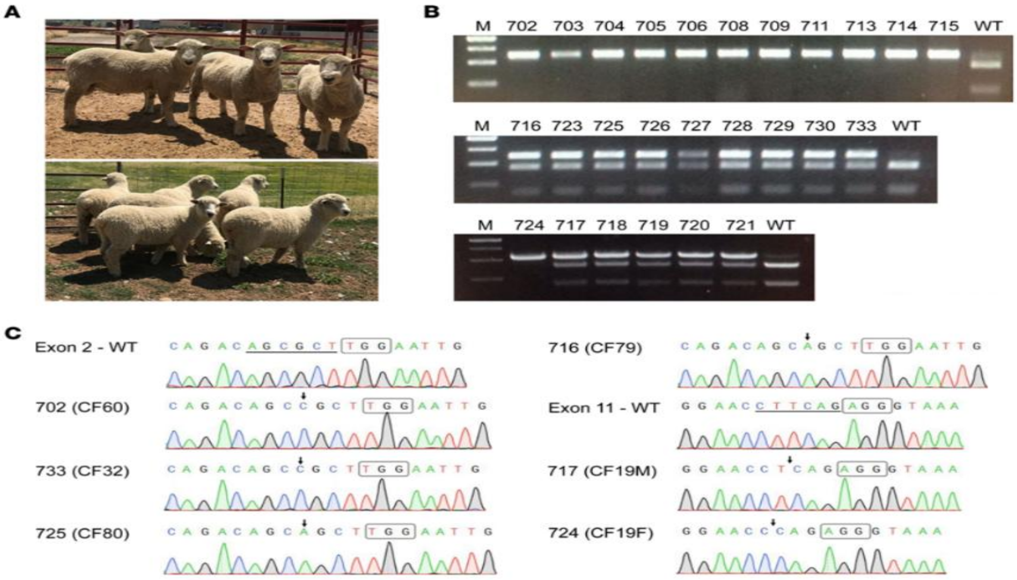

Figure: (A) Photographs of sheep, both in a group and individually, displaying a phenotype of interest in an agricultural or biomedical study. (B) Agarose gel electrophoresis images showing PCR amplification of targeted DNA regions in different sheep samples, indicated by their corresponding numbers (702-733, 716-733, 724-721) compared to the wild type (WT). (C) DNA sequencing chromatograms for different sheep samples (702, 716, 717, 724, 725, 733), illustrating genetic variations across Exon 2 and Exon 11 when compared to the wild type sequences. The sequencing results highlight specific mutations or polymorphisms present in the studied sheep, with color-coded peaks representing nucleotide bases [4].

Meanwhile, animal models help scientists understand fundamental biological processes and developmental pathways, which can provide useful insights into human development and disease. This knowledge is essential for developing treatments and cures for a variety of diseases. For example, during the study of gene expression in embryo development in animals, it can reveal important information about human genetic disorders. In a recent study, sheep and pig models were used to generate an animal model of cystic fibrosis to investigate its early disease pathology [4]. Until the development of CRISPR/cas9 technologies, it was not easily possible to target the sheep CFTR locus (the gene mutation which activates cystic fibrosis in one’s body). The loss of function of the CFTR gene in the sheep model was severe as it was dominated by intestinal obstruction (side effect of cystic fibrosis). This phenotype can be managed through dietary changes in mice animal models but is not possible for animal models such as a pigs or sheep models that include cystic fibrosis (Figure 2) induce driven by an intestine-specific promoter such as fatty acid binding protein (FABP) must be used to alleviate intestinal disease. CFTR expression in the intestinal passageway without pancreatic correction was sufficient to bring back function in the CFTR gene for pig models, and a similar approach is likely to be effective in sheep. At this point in research, it is hard to predict whether sheep with cystic fibrosis will be able to recreate human cystic fibrosis lung disease over time. It is important to note that lung disease is not present in pigs with cystic fibrosis at birth though they do experience lung inflammation, tissue remodelling, and the accumulation of mucus. Sheep models have similar cystic fibrosis disease pathology to humans because the lung disease is already well advanced from birth. Newborn cystic fibrosis pigs experience higher incidence of liver and gallbladder disease than in human CF. A major side effect for human males that are diagnosed with cystic fibrosis is infertility. Infertility is due to the loss of the vas deferens and obstruction of the epididymis. Male lambs also showed similar side effects when they contracted cystic fibrosis. When sheep are born with cystic fibrosis, the loss of genital ducts (causing infertility) suggests the presence of a utero event (obstruction of ducts with large amounts of mucus secreted). Since cystic fibrosis pathology begins in utero, intervention during this event may offer optimistic possibilities to effectively treat this disease [4].

Through research with animal models, the viability of curing cystic fibrosis increased. Similarly, cystic fibrosis experiments have been conducted with intestinal stem organoids. Intestinal stem organoids are derived from intestinal stem cells and mimic the structure and function of a human intestine. To grow the intestinal stem organoids in the patients, a protein named R-spondin1 was used to help the stem cells multiply and maintain the cultures. This system has been adapted for creating organoid cultures for various tissues such as stomach and liver in both mice and humans. R-spondin1 is crucial for stem organoids as it is needed to help maintain Lgr5+ stem cells (type of stem cell found in various tissue including the intestine). CRISPR/cas9 was used to knockout APC, a negative regulator of the Wnt pathway (network of proteins that plays a role in cell growth, differentiation, and embryonic development), which led to stem cell organoid growth in the absence of the R-spondin1. They have also described the optimized protocol for targeting the APC gene (tissue regulating gene) and the following selections of edited organoids. Steps include first culturizing of the intestinal organoids and then dissociating the organoids into single cells using trypsin (an enzyme that breaks down proteins and supports the growth of intestinal stem cells) [19]. Next, the cas9 protein and guide RNAs were specifically designed to target the APC gene (tissue regulating gene) to get the most precise target as possible. In this experiment, only the Lgr5+ stem cells, which are capable of replicating and forming their own organoids, could grow out “secondary” organoids. This selective growth means that the

CRISPR/cas9 system was able to precisely choose which gene to mutate and was successful. Compared to wild-type organoids, the edited organoids showed morphological differences which confirmed successful CRISPR/cas9 gene editing. Using this data, it may be possible in the future to create gene therapies for hereditary diseases using stem cell organoids [19].

In addition to the use of stem cell organoids to find a cure, there has also been research on the genetics for cystic fibrosis patients in lung epithelial cells, which are generated from patient iPSCs (stem cells that can be generated directly from a somatic cell). To start, they generated iPSCs (induced pluripotent stem cells) from patients with cystic fibrosis carrying a homozygous deletion of F508 (common mutation type) in the CFTR gene, which causes loss of function in the CFTR gene. All the iPSCs that were generated have the same characteristics as regular iPSCs. Next, a customized CRISPR system consisting of a plasmid encoding the cas9 protein and a separate plasmid containing gRNA was created. Essentially, the gRNA was created to target sequences in the vicinity of the F508 mutation in the CFTR gene. This mutation was able to be corrected using CRISPR to target sequences in relation to the CFTR locus. The non-mutated iPSCs were substantially differentiated into lung epithelial cells where the CFTR’s normal function was reverted to its wildtype phenotype (Figure 3). This process has been used to correct CFTR in adult intestinal stem cells, however these cells cannot be used to study the pathology of lung-related diseases. Since the intestine is a major site of its pathology, especially regarding the buildup of mucus and nutrient absorption. Lung epithelium (tissue) has a unique cellular architecture and environment, which influences how CFTR functions and how its mutations create disease. Even if CFTR is corrected in intestinal stem cells, these cells do not exhibit the same properties to the dysfunction of CFTR that lung cells do. For example, the amount of thick mucus builds up seen in the lungs of patients with cystic fibrosis cannot be studied in intestinal cells [5]. The difficulties of using this research on cystic fibrosis remain as lung disease is a major part of the disease’s pathology. This iPSCs-model based system could be adapted or changed for the development of other gene-therapy approaches. [5].

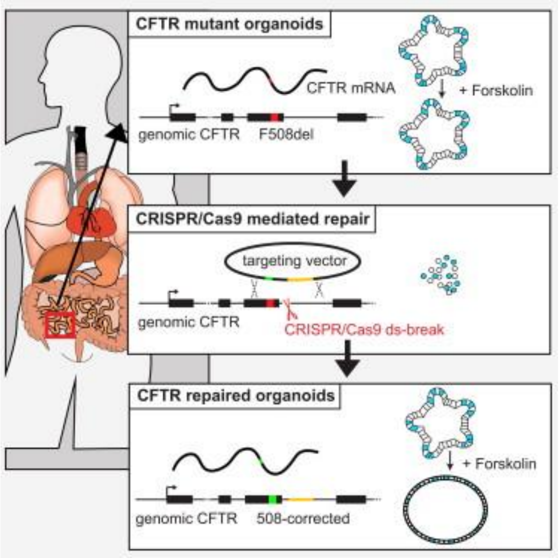

Figure 3: Diagram illustrating the use of CRISPR/cas9 for correcting the CFTR gene mutation in organoids derived from cystic fibrosis patients. The top panel shows CFTR mutant organoids with the F508del mutation, leading to defective CFTR function, as shown by the impaired response to forskolin. The middle panel depicts the CRISPR/cas9-mediated repair process, where a targeting vector corrects the mutation at the genomic level. The bottom panel shows the corrected CFTR gene in organoids, resulting in restored CFTR function, as evidenced by the positive response to forskolin [5].

Building on previous experiments, another study was used to mediate CFTR knockouts in human macrophages (type of white blood cells in humans). This was used to research how CFTR regulates the function of the macrophage. Macrophages have an important role in cystic fibrosis immune dysfunction as they often exhibit reduced phagocytic activity, meaning they are less efficient at protecting the body from intruding pathogens. This function can lead to persistent bacterial infections, which is important to note especially for patients who already have extremely weakened immune systems [14]. There have been limited strategies to allow reliable and efficient gene editing in human macrophages due to their terminally differentiated state (protein is constantly having to change its function). One of the study's conclusions was that macrophage effector functions are directly dependent on CFTR. Therefore, a CFTR knockout would reflect similar observations in primary human cystic fibrosis macrophages. CFTR knockout macrophages had higher rates of apoptosis, like earlier findings where cystic fibrosis macrophages showed higher apoptosis rates but was reduced with ivacaftor treatment. Macrophages in cystic fibrosis sputum (mucus) had lower apoptosis rates due to adaptation to the lung environment and differences in immune cell populations. These findings could be used to help improve immune responses to infections caused by cystic fibrosis (CF) in patients' bodies. Additionally, it suggested that many aspects of cystic fibrosis macrophage dysfunction are CFTR-dependent which is key to understanding how to regulate immune responses from cystic fibrosis [22].

Section 3: What have been the main challenges in using CRISPR and using it with cystic fibrosis specifically?

Despite CRISPR's advantages, there are also places where CRISPR is not the most efficient example of gene therapy. This includes low HDR efficiency, off-target effects, and body immune responses. HDR (homology-directed repair) gene editing creates the option of inserting a transgene (artificially created gene inserted into the genome) into the desired locus (position). The HDR gene editing process can also be applied to gene knockouts, point mutation corrections, and introducing more beneficial mutations [21]. Also, most HDR gene editing processes have not made it to clinical trials, which includes the use of CRISPR. Although the CRISPR/cas9 system is designed for high specificity, there are still possibilities for off-target effects (three to five base pair matches in the sgRNA-guiding sequence). Off-target effects are not wanted in the process of CRISPR because it can create unintended mutations that disrupt the function of the gene and can lead to harmful consequences (such as other diseases) [24]. Also, unintended changes like off-target effects can lead to genomic instability which might result in chromosomal rearrangements like deletions and duplications. This can lead to the development of fatal diseases such as cancer [23]. Several methods have been used to identify these off-target effects, each with varying levels of accuracy. Most of these methods start out with the process of DNA sequencing. The goal of DNA sequencing is to investigate the base pair specificity of a loci of interest, allowing researchers to compare DNA between organisms and compared/contrast genetic relationships between species. For the study of cystic fibrosis, one of the methods used is the T7 Endonuclease I Assay. This assay detects mismatches in DNA (an off-target effect), but it only identifies off-target effects occurring at a frequency above 1%. This was not cost effective for large-scale screening, as it could only detect low-frequency mutations. Next, was the technique of deep sequencing that can detect frequencies between 0.01% to 0.1%. Deep sequencing can still miss off-target sites, especially with sequences that are less similar to the intended target. The downside of using this technique is that it is biased towards known or predicted off-target sites. Digenome-seq, now considered the “gold standard” for detecting off-target effects, involves in-vitro digestion of genomic DNA with cas9 and subsequent whole-genome sequencing. Although this method is highly sensitive, it can detect off-target mutations with a frequency of 0.1% or lower. This provides a comprehensive profile of cas9 activity throughout the genome, while also being cost effective [8]. While significant progress has been made to minimize off-target effects, there are still other issues that make CRISPR/cas9 editing difficult: body immune responses. The cas9 protein is guided to the target DNA sequences with a guide RNA (gRNA) but can either be delivered through a viral or non-viral delivery method. Whether its viral or non-viral delivery can decide if there will be an immune response upon administration. Viral vectors have high efficiency in gene delivery and expression but create a higher risk of immunogenicity and carcinogenicity risks. Non-viral vectors are safer to edit with but have much lower delivery efficiencies. Since cas9 is a large foreign protein to the human body created from bacteria it can trigger effector and memory adaptive immune responses, meaning cells aren’t able to remember when they first came across a dangerous antigen to better protect against them the second time. Also, it is very common for the gRNA to induce immune responses through pattern recognition receptors. Additionally, in-vitro delivery has been shown to trigger cytotoxicity, creating an unhabitable space for healthy cells. Cytotoxicity turns many cells toxic, making it extremely dangerous for the species. gRNA immunogenicity needs to be considered while completing the CRISPR process, because it can create serious side effects in one’s body, as explained above. Adeno-associated viruses (AAV) that are used to deliver various gene therapies, less harmful than other viral vectors, can adapt to immune responses that show up within different serotypes (groups within a single species of microorganisms) [3]. The physical effects of the immune responses include the creation of antibodies in humoral immunity (protecting the body from pathogens), cell death by cytotoxicity, and high levels of inflammation [3]. Figuring out a strategy to reduce the immunogenicity of cas9 proteins is essential to ensuring the safety of all patients that would one day be utilizing CRISPR/cas9 genome editing in a clinical setting.

Section 4: Potential Solutions

The integration of AI has played an important role for opening possibilities to understanding the function of genes and improving medical treatments. The use of AI would create enhanced efficiency, precision, and affordability of gene editing tools, especially when discussing genetic diseases, such as cystic fibrosis (one of the most common genetic diseases internationally). AI models have been used in designing gRNAs for CRISPR cas9 systems, which is vital for avoiding off-target effects that can lead to serious repercussions in the immune system. AI models, such as DeepCRISPR and CRISTA, predict the most optimal gRNAs considering factors such as Cas protein type, on-target/off-target scores, and the predicted outcome of the gene-editing technology (GED). Using various machine learning and deep learning techniques, they can provide valuable guidance for researchers choosing to use GED (gene editing) technologies. These techniques can introduce precise and more “programmable” changes to DNA sequences, eliminating the need for homology-directed repair pathways or donor DNA templates. AI models can select optimal editors for target sequences, genomic contexts, mutation types, off-target effects, and the potential impacts on the function of different phenotypes [2]. Another factor in the use of AI models is how they can create personalized treatments based on certain genetic profiles. It can analyse patients’ genomic data identifying various mutations and biomarkers that are found in certain diseases (Alzheimer’s, cancer, diabetes, and cystic fibrosis). AI models can predict how the patient will react to different drugs and gene editing therapies by considering efficacy and toxicity based on the patient’s past health information. Additionally, AI models can help in designing efficient vectors, promoters, and enhancers which improve the specificity of delivery to various tissues and cell types. Promoters are DNA sequences located near the transcription start site of a gene. They act as “binding” sites for RNA polymerase and initiate the process of transcription in the genome. Enhancers are DNA sequences that are located further from the gene they intend to regulate (as compared to promoters). They play a crucial role in increasing the transcriptional activity of a gene by containing multiple binding sites for transcriptional activators (proteins that bind to the enhancer and increase the rate of transcription). These activators are often interacting with the promoter mechanism through a looping mechanism of the DNA [9]. In addition to the AI therapies listed above, there is also AlphaFold which is a multicomponent artificial intelligence system that uses machine learning to predict the 3D-structure of a protein based on the primary amino acid sequence. Since AlphaFold is not a homology-based tool, it can successfully operate without the use of any template structure and can foresee previously unknown protein folds. Additionally, it is difficult to have a digital image of membrane proteins (CFTR) as they are folded in-between phospholipids bilayers. These bilayers make it hard to scan the actual CFTR protein and acquire a digital image of it. Through AlphaFold, since it can take a picture of the membrane protein with needing to use new crystallographic data for its predictions. On the other hand, crystallography takes digital images of proteins through high-powered x-rays that can isolate the protein, apply radiation and determine the structure of the protein. The downside to this method is that it is a less accurate prediction method than the AlphaFold method mentioned above. More on AlphaFold, it analyses the amino acid sequence of a new protein by aligning it with sequences from similar proteins. This helps identify sections that evolve together, suggesting they interact and are likely close in the protein’s 3D structure. Within minutes (or longer for larger proteins or complexes), AlphaFold2 generates a prediction of the sequence’s 3D structure [9]. Although AI is an achievable solution for the cure of cystic fibrosis, it still requires much needed testing for widespread clinical usage.

Section 5: Discussion

Finding a cure for cystic fibrosis has been a long and complex process. While efforts continue, no definitive treatments or therapies currently exist to fully eliminate hereditary diseases from the body. A lasting cure for hereditary diseases, as opposed to current therapies that merely manage the condition, is needed. Substantial research has been conducted using animal models of pigs and sheep rather than the usual mice or rats. This is because of the pathology of cystic fibrosis. In mice, cystic fibrosis does not fully attack the lungs while in human pathology they do, which is one of the key factors to a diagnosis of the disease. Since this is a crucial part of CF, animals which exhibit similar pathology such as pigs and sheep were crucial to gain a better understanding of the way cystic fibrosis attacks the body so severely. In addition to the use of animal models, the use of organoids derived from stem cells (able to mimic the structure and function of a real organ) while testing gene therapies became increasingly common as their use helps model human diseases and understand the mechanisms behind those diseases. Also, these organoids can be derived from a patient's cells to create a personalized model of their disease which can then be used to test the effectiveness of various possible treatments. iPSCs also came into use as a potential gene therapy because those differentiated CFTR-corrected iPSCs into airway epithelial cells were demonstrated successful. The mutation in the CFTR gene was able to be successfully corrected without leaving any trace or footprint behind. The corrected cells also displayed normal functions, suggesting a more viable approach for gene therapy in cystic fibrosis (or hereditary diseases in general). A study on macrophages (type of white blood cell in humans) was done to create a stable CFTR knockout in human macrophages to study how the CFTR gene regulates the macrophage function. This knockout process mimics similar pathology observed in macrophages obtained from people with cystic fibrosis which suggested that the dysfunction of the macrophage is dependent on the CFTR gene. These findings could be used to help improve immune responses to infections caused by cystic fibrosis. Along with the potential solutions described for cystic fibrosis, there are also multiple challenges including off-target effects, immune responses to the CRISPSR cas9 system, and low HDR efficiency. The combination of these three effects is much of the reason as to why CRISPR cannot be distributed to the public without intensive clinical trials.

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