Therapeutic Approaches to Cure Genetic Disorders Cystic Fibrosis - A Review

Dakshayini P.N.[1], Bhushanam M.[2]* and **Abhinandini I.D[3]**

[1,2*]Department of Zoology, Maharani's Science College for Women, Bengaluru-560 001, India.
[3]Department of Zoology, Govt First Grade College, Channapatna – 571501, Bangalore University, Bengaluru, Karnataka, India.

Corresponding Email: [2*]bhushanam.honey@gmail.com

Abstract: In this review, we describe the mechanism and methodology of gene editing therapy used to cure the cystic fibrosis. It is an autosomal recessive disorder that is, caused due to the mutation in the copies of CFTR gene. The gene editing therapy, here, used is, CRISPR/Cas9. This has been already clinically tested in animals and prokaryotes. Thus, far had an attempt to edit genes in the stem cells that originated from blood lineages. The advantage of the stem cells that are originated from blood is that, they can be selected, expanded, and returned to the body. Next trials are to be likely in liver. Studies suggested that the yeast has laid foundation for the genome editing. It could suggest that, gene editing would be possible if one could direct DNA double strand to a single site in the genome.in this review, we later discuss upon zinc-finger nucleases, which is Zinc-finger nucleases are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genome. In this review, other than the information regarding the curing of cystic fibrosis by using genome editing, we will further discuss the challenges faced during the gene editing and also the information related to the gene editing therapy used in yeast.

Keywords: Genetic Disorders, Cystic Fibrosis, Stem Cells, Genes, Mutations.

1. INTRODUCTION

Genes are the basic functional and basic unit for hereditary [(1)]. They are made up of DNA. Some genes code for proteins while others doesn't. According to Human Genome Project, which is conducted in the early 90's Its estimated that there are almost 20,000-25, 000 genes in the human body. Every person has two copies of each gene, one inherited from each parent. Mostly genes are common or same in all the people, but only one percent is slightly different in people. Another term is allele, which is, different forms of the same genes, with small differences in their base-pairs. These DNAs are tightly packed into structures called as chromosomes, which exist in the nucleus.

A Brief History of Gene Editing

In just over 40 years, we've gone from simple modifications to the development of a gene drive that could eradicate an entire species. In 1973, Herbert Boyer and Stanley Cohen have created the first genetically engineered organism—a bacteria with an added gene to confer antibiotic resistance—in Cohen's Stanford University lab. Then, in 1973, The National Academy of Sciences established a moratorium on genetic engineering experiments until safety issues can be examined. In 1975, more than 100 biologists met at California's Asilomar conference center and had established the principles for risk assessment and mitigation in biotechnology that are used to this day. Among the established principles is an emphasis on public engagement and transparency. In 1982, The first genetically engineered human drug—synthetic insulin, produced by bacteria that contained the human insulin gene—is approved by the Food and Drug Administration. In 1994, Calgene introduced the first genetically

engineered food, the Flavr Savr tomato, which is engineered to stay firm when ripe. It bombs. In 1996, Monsanto released its first genetically modified crops. Within a few years, Roundup Ready corn, soybeans, cotton, sugar beets, and canola dominate the market. Thereafter, Roundup-resistant weeds begin to appear in fields where those crops are grown. In 2003, Geneticist Austin Burt is the first for proposing that a "selfish gene"—one that guarantees inheritance by most offspring—could be used as a biocontrol against another species. Burt is aware of the implications of his idea: "Wide-ranging discussions are needed on the criteria for deciding whether to eradicate or genetically engineer an entire species." But the idea remains theoretical at this time—no technology yet exists capable of designing such a gene. In 2012, Researchers at the University of California–Berkeley and the Broad Institute independently discovered that CRISPR—a bacterial immune system—can be adapted to serve as a gene-editing tool that can make specific changes to DNA anywhere in an organism's genome. In 2014, Kevin Esvelt et al. published a paper demonstrating how CRISPR could be used to drive a genetic modification through all members of a population or species, permanently altering or eradicating them. He had proposed using it to control invasive species and to eliminate diseases such as Lyme disease and malaria. In 2015, Scientists at the University of California– San Diego had build the first gene drive in a lab-based population of fruit flies, and Imperial College London builds the first gene drive in lab mosquitoes. In 2016, A National Academies of Science, Engineering, and Medicine report finds that gene drives have huge potential in agriculture, disease reduction, and conservation, but recommends more research, public engagement, and highly controlled field trials before any such organisms are released into the environment. Over the 150 groups call for a moratorium on gene drive research, but the moratorium is shot down at the United Nations Convention on Biological Diversity. In 2017, Research teams in Texas and Australia announce gene drive house mice—the first use of gene drive in a mammal. The first gene-altering treatments for cancer are approved by the FDA [(2)]. Cystic fibrosis is an autosomal recessive disorder and is chronic, progressive in nature. It is caused by mutation in CFTR gene, Cystic Fibrosis Transmembrane Conductance Regulator, which is responsible for encoding an epithelial chloride anion channel. It is a 1480 amino acid long anion transporter. The main cause of this disorder is progressive lung disease due to thick and viscous mucous, increased airway inflammations, chronic and recurrent infections thus, leading to pulmonary complications [(5)]. It is a multisystem disease due to presence of CFTR gene across the organs especially, in sinuses, lungs, Gastrointestinal tract, liver, pancreas, and testes. The understanding of this complex disease has led to a dramatic advance in treatment by using gene therapy. Recent advances have led to clinical trials in the yeast (Baker's yeast) that will be discussed in detail further in this review article.

2. METHODOLOGY

Crispr/Cas9

General Mechanism of CRISPR/Cas9

As mentioned above, CRISPR/Cas9 is RNA- guided DNA endonuclease. In bacteria, it is a diverse set of adaptive immune systems which battles infections by degrading nucleic acids. These infectious nuclei acid is degraded if a short segment of its sequence is represented in the cell's Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The short RNAs bind to an endonuclease (Cas9 in some systems) and hybridize to the incoming nucleic acids, activating a nuclease to trigger degradation. New, sequences can be added to the array in a nonlethal infection, for example those in which the host's Restriction-Modification

system, a second non-adaptive nuclease, based immune system, inactivated the phage.25 Sequence in the host CRISPR are not cut because the genomic versions lack a short, shared motif adjacent to the unique sequence. This short motif, or proto-spacer adjacent motif (PAM), is present in the original infectious DNA, but is not copied into the genome. A PAM sequence can be very simple, for example NGG. The absence or presence of the PAM sequence is the determinant by which the cell distinguishes self from non-self. The CRISPR/Cas consists of three components, namely, a guided RNA, a single stranded DNA template and a Cas 9 endonuclease. The Cas9 is in inactivated state. The Cas9 endonuclease is a large protein consists of 1380 amino acids with several functional domains. When gRNA binds to the Cas9 nuclease, it becomes activated and binds to the DNA of the cell. The DNA consists of mutation. The basic idea of gene editing is that the nuclease cleaves the mutated piece of DNA and the cell's it's own repairing machinery identifies the break or cleavage of DNA and it starts repairing by making a complementary strand to the Template strand. So, the Cas9 along with the gRNA complex, goes through or pass through the DNA to find a match of gRNA. When the gRNA sequence matches the specific sequence(hybridization), it cleaves that specific sequence and the cell's it's own repair machinery identifies this cleavage or breakage and it repairs it by making a complimentary strand of the Template strand, thus, curing the mutation, thus, curing the disorder-Cystic fibrosis. For example: Clinical trials in *Saccharomyces cereviceae* or Baker's yeast.

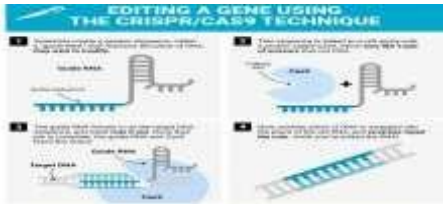

Figure 1: General mechanism of CRISPR/Cas9 (7)

Experiments performed in Yeast i.e., *Saccharomyces cerevaciae* suggested that, although it was focused on the mechanisms of DNA repair, it also suggested that gene editing would be possible if one could direct DNA double-strand breaks to a single site in the genome. Study suggested that haploid Baker's yeast can switch sex at each division. The mRNA for HO endo-nuclease will segregates into the daughter cell, where the HO protein cuts a single site in the daughter-cell genome. This DNA double-strand break will lead to replacement of sequences around the break with DNA from donor sites, one donor for each sex. The sequences of both DNA donors had homology to the recipient site. Once incorporated into the mating type locus, the recombined DNA is expressed as a protein which determines the mating type. James Haber, in his laboratory used HO-induced break to study the mechanisms of DNA repair. He showed that DNA repair by a second mechanism–non-homologous end-joining (NHEJ)–can generate small insertions and deletions (indels) at the DNA double-strand break. The mechanism of CRISPR/Cas9, endonuclease activity determinates it's both specificity and mutagenicity. Cas9 endonuclease binds to the guided RNA, into the C-terminal domain, it gains the ability to bind to double-stranded DNA, with a PAM,which is complimentary to NGG. As mentioned above, in the General mechanism of CRISPR/Cas9, when it binds to the PAM, the Cas9 endonuclease induces a kink or a cleavage in the double stranded DNA, which is complementary to the NGG. This kink or cleavage will melt the double-helix or unwound the double-helix, in order to free the base pairs and allow it to pair with guided RNA. If hybridization occurs between the nucleotides and the guided-RNA, in the strand, which is adjacent to the PAM. If hybridization is complete through the entire 20 base pairs of the gRNA, then Cas9's two nuclease domains (the HNHe and Ruv C-like nuclease domains) are activated. Each if the DNA strand-strand is

cleaved close to the PAM sequence within the region, which is bounded by guided RNA. The DNA strand paired with the gRNA is cut 3 nucleotides away from the PAM, while the unhybridized strand is cut 3 to 6 nucleotides away. Most frequently, the mutation which arises from a Cas9-generated double-strand break is a small deletion of less than 10 base pairs. The nature of some deletions is consistent with cellular enzymes removing the overhangs and ligating the two ends. In other cases, double-strand breaks are repaired by microhomology-mediated end joining, where short sequences at the break are recombined with local similar sequence to generate deletions. This, the cell's it's own repairing machinery identifies and it will repair the kink by coping the template strand by making a complimentary base pairs in the DNA.

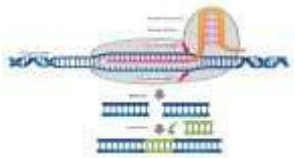

Figure 2: Mechanism of CRISPR/Cas9 9 (8)

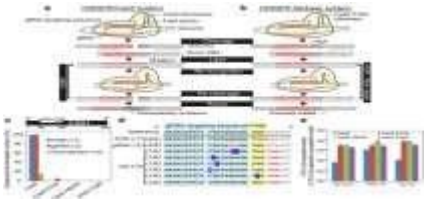

Figure 3: Mechanism of CRISPR/Cas9 (9)

Figure 4: Mechanism of CRISPR/Cas9 (10)

Gene Editing By Zinc-Finger Nucleases

Zinc-finger nucleases are artificial restriction enzyme by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains has been engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes (3). By understanding the endogenous DNA repair machinery, these can be used to precisely alter the genomes of higher organisms. The zinc-finger nucleases can be engineered and these engineered zinc-finger nucleases can induce double-stranded breaks at specific recognition sequences and promotes the insertions, deletions or substitutions at or near the cut site via homology-directed repair (HDR) with a double- and/or single-stranded donor DNA template.

ZFNs, as well as engineered homing endonucleases and transcription activator-like effector nucleases (TALENs), can be used to improve the efficiency of homology-directed repair (HDR) in a variety of different organisms and cell types.

Engineered zinc finger nucleases (ZFNs) induce DNA double-strand breaks at specific recognition sequences and can promote efficient introduction of desired insertions, deletions or substitutions at or near the cut site via homology-directed repair (HDR) with a double- and/or single-stranded donor DNA template. However, mutagenic events caused by error-prone non-homologous end-joining (NHEJ)-mediated repair are introduced with equal or higher frequency at the nuclease cleavage site. Furthermore, unintended mutations can also result from NHEJ-mediated repair of off-target nuclease cleavage sites (4).

There are 2 kinds of domains:

1. DNA-Binding Domain

The DNA-binding domains of individual ZFNs contains between three and six individual zinc finger repeats and can each recognize between 9 and 18 base pairs. If the zinc finger domains are perfectly specific for their intended target site then even a pair of 3-finger ZFNs that recognize a total of 18 base pairs can, in theory, target a single locus in a mammalian genome. The most straightforward method to generate new zinc-finger arrays is to combine smaller zinc-finger "modules" of known

specificity. The most common modular assembly process involves combining three separate zinc fingers that can each recognize a 3 base pair DNA sequence to generate a 3-finger array that can recognize a 9 base pair target site [13].

DNA-Cleavage Domain

The non-specific cleavage domain from the type IIs restriction endonuclease FokI is typically used as the cleavage domain in ZFNs. This cleavage domain must dimerize in order to cleave DNA and thus a pair of ZFNs are required to target non-palindromic DNA sites. Standard ZFNs fuse the cleavage domain to the C-terminus of each zinc finger domain. In order to allow the two cleavage domains to dimerize and cleave DNA, the two individual ZFNs must bind opposite strands of DNA with their C-termini a certain distance apart. Recent studies have begun to create zebrafish (*Daniorerio*) models for neurodegenerative diseases [14]. Zinc-Finger nuclease (ZFN) mutagenesis is currently one of the few methods to create a genetic knockout or permanent mutation in zebrafish. The lack of an effective method is a significant disadvantage in comparison to rodent models especially in relation to disease modeling where long-term mutation is relevant; however, ZFN is a potential technique in development. Embryos are injected mRNA that encodes DNA-binding proteins. The proteins then create double strand breaks in the gene of interest to which they have been targeted. Cellular repair of the induced break is prone to error leading to the desired stable mutation in the appropriate gene [15].

Gene transfer vectors for CF have not been followed the traditional pattern of drug development observed for other pharmaceuticals. One key difference between drug and gene transfer vector development is that drugs are often a single chemical entity extensively screened preclinically, while gene transfer vectors are numerous and rapidly evolving in effectiveness and other features. Several viral and non-viral vectors have been used in clinical trials and it increased the development of new and modified vectors. This review concentrates the vectors which have been used in the clinical trials [16].

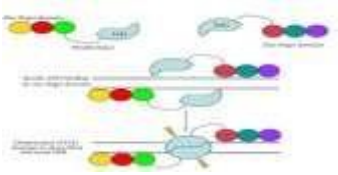

Figure. 5: Mechanism of CRISPR/Cas9 [11]

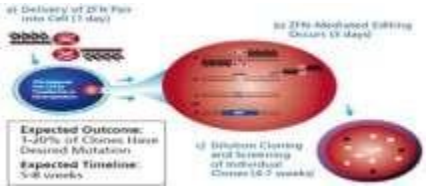

Figure 6: Mechanism of Zinc-finger nucleases [12]

Vectors used in Genome Editing

In the in vivo gene therapy vectors are used. These vectors are called as gene-delivery system. The ideal cystic fibrosis vectors are completely safe, highly efficient for entering the cells and expressing CFTR. The ideal vector for cystic fibrosis is not been recognized but as for now, there are two types of vectors-viral and non-viral vectors. Typically, and mostly, viral - viral vectors are vehicles for expression based on existing viruses, but they are genetically modified to be replication deficient and they contain an expression cassette with CFTR cDNA and an appropriate promoter. Viral vectors are vehicles for expression based on existing viruses, but they are genetically modified to be replication deficient and they contain an expression cassette with CFTR cDNA and an appropriate promoter. Typically, viral vectors have higher efficiency but are more immunogenic than nonviral vectors [17].

Viral vectors are developed specifically in order to use in CF include adenovirus and adeno-associated virus, although other viruses have been considered. Nonviral vectors are normally a combination of CFTR cDNA with a suitable promoter and lipid, protein, or other molecules, which

aids in the uptake of nonviral vectors by target cells. In contrast to viral vectors, nonviral vectors are typically less efficient and less immunogenic. Nonviral vectors developed specifically for CF include the combination of lipid and DNA called liposomes [18]. A comparison of specific vectors is shown in Table 1 and is discussed below.

Table 1: Showing virus and non-viral vector for the genome editing

	Viral		Non-viral		
Determinant	Adenovirus	AAV	Retrovirus	Liposome	Molecular conjugate
Composition	ds-DNA	ss-DNA	ss-RNA	DNA plus lipid	DNA plus polypeptide
Genome (kb)	36	5	10-20	NA	NA
Carrying capacity (kb)	7-8	<5	6–7	>10	>10
Transduction	Dividing and non-dividing	Dividing and non-dividing	Dividing only	Dividing and non-dividing	Dividing and non-dividing
Efficiency	High	Intermediate	Intermediate	Low	Low
Expression	Transient	Intermediate	Long-term	Transient	Transient
Tropism	Yes	Yes	No	Yes	No
Immune Response	High	Low	Low	Low	Low

Because of the current vector technology is, a key question is how much CFTR must be transduced in order to have a beneficial effect on the disease. CF is a recessive disorder, and heterozygotes with one mutant CF allele do not have clinical disease. The other clinical data had suggested that some phenotypically normal individuals have up to 92% abnormally spliced CFTR mRNA, which produces a defective protein product [19]. Excessive CFTR expression is always toxic in the selected in vitro experiments, but these all observations have not been confirmed with in vivo animal models.

Delivery and evaluation of the effects of gene transfer agents is another evident dilemma. The transfer vectors for CF have not followed the traditional pattern of drug development observed for other pharmaceuticals. One key difference between the drug and gene transfer vector development is that drugs are often a single chemical entity extensively screened preclinically, while gene transfer vectors are numerous and rapidly evolving in effectiveness and other features. Therefore, gene therapy development and clinical trials are occurring on multiple parallel tracts. Several nonviral and viral vectors have been reached the clinical trials, but the information has been gleaned at the bed side only and it has increased the pace of further development of new and modified vectors. We will further discuss about the vectors which have been reached the clinical trials [20, 21].

Vectors

The in vivo gene therapy which is required for Cystic Fibrosis makes special demands of a gene delivery system. An ideal CF vectors will be completely safe, highly efficient for entering cells and expressing CFTR, tropic for airway epithelial cells, capable of transducing nondividing cells as are found in the surface airway, and of low immunogenecity. The ideal vector has not yet been recognized [22]. There are two types of vectors—viral and nonviral—have been researched and each provides a unique set of advantages and disadvantages. Viral vectors act as vehicles for expression based on existing viruses, but they are genetically modified to be replication deficient and they contain an expression cassette with CFTR cDNA and an appropriate promoter. Typically, viral vectors have higher

efficiency but are more immunogenic than nonviral vectors. Viral vectors developed specifically in order for using in CF include adenovirus and adeno-associated virus, although the other viruses have been considered. Nonviral vectors are generally a combination of CFTR cDNA with a suitable promoter and lipid, protein, or other molecules, which aids in the uptake of nonviral vectors by target cells. In contrast to viral vectors, nonviral vectors are typically less efficient and less immunogenic. Nonviral vectors developed specifically for CF include the combination of lipid and DNA called liposomes [23, 24]. As of the current vector technology is dramatically inefficient, a key question is how much CFTR must be transduced in order to have a beneficial effect on the disease. As CF is a recessive disorder, and heterozygotes with one mutant CF allele do not have a clinical disease. Another clinical data suggests that some phenotypically normal individuals have up to 92% abnormally spliced CFTR mRNA, which produces a defective protein product. Similarly, preclinical experiments in monolayers of CF epithelial cells suggest that a minimum of only 6% of cells require wild-type CFTR expression in order to correct the chloride transport defect .On the other hand, close to 100% of cells require wild-type CFTR expression in order to normalize increased sodium conductance [25, 26]. If correction of chloride secretion is all that is necessary for a beneficial effect, then as little as 6–8% of airway cells will require wild-type CFTR expression; however, if other CFTR functions, like regulation of epithelial sodium channels, are necessary for clinical benefit, then a much higher percentage of cells will require transduction. Transgenic CF animal models may provide a more detailed estimate for the minimal amount of CFTR expression resulting in clinical benefit. Unfortunately, most transgenic CF models do not exactly mimic the respiratory disease process, making interpretation of these studies challenging. Gene therapy vectors for this disease have not been followed the specific pattern i. e., the traditional pattern of drug development which is observed in other pharmaceuticals. One key difference between drug and gene transfer development is that, drugs are often a single chemical entity extensively screened preclinically, while gene transfer vectors are numerous and rapidly evolving in effectiveness and other features. Therefore, the gene therapy development and clinical trials are occurring on multiple parallel tracts. Several viral and nonviral vectors have been reached the clinical trials, and information gleaned at the bedside has only increased the pace of further development of new and modified vectors. This review concentrates on those vectors that have already reached clinical trials [26, 27].

Viral vectors

Earlier, adenovirus was a favorite candidate for Cystic fibrosis. It is a double stranded DNA virus, which naturally infects the respiratory and gastrointestinal tracts of humans. There are a number of advantages of adenoviruses as it is a gene transferring agent, especially tropism for airway epithelium, ability to produce high viral titers, and ability to transduce non dividing cells. The disadvantages include transient expression of the transgene, recombination of the modified virus with wild-type viruses, shedding of the modified virus, and host immune response to the transgene or modified virus [28-30]. Adenovirus serotypes 2 and 5 are tropic for respiratory epithelium, and therefore, vectors are based on these serotypes. An early puzzle was engineering replication-deficient recombinant virus, necessary for safe administration to humans. This was accomplished by deleting early genes that code for regulatory proteins, leaving intact late genes that code for virus structural proteins. First generation, replication-deficient adenovirus vectors contain an immediate early gene (E1) deletion plus the cDNA of CFTR or a marker transgene. First-generation adenovirus vectors are effective for expression of CFTR and other

marker genes in both in vitro and in vivo animal studies. Safety studies varied from no toxicity in rodents and rhesus monkeys to lung inflammation in baboons. Inkrder to evaluate the biological efficiency and safety, human clinical trials are needed. All published human trials using nasal delivery of first-generation adenovirus vectors showed variable, transient expression of the CFTR transgene. Two of the three published single nasal administration human trials with first-generation adenovirus vectors showed variable, transient functional restoration of chloride transport as measured by nasal potential differences. The third study was indeed, a rigorous, double-blind, placebo-controlled investigation of a first-generation adenovirus vector that failed to show any functional restoration of chloride transport despite use of very high virus concentrations. The concerns regarding recombination of the modified virus with wild-type strains and shedding of the modified virus were not realized. However, transient expression and the host immune response plagued most clinical trials using first-generation adenovirus vectors. Host immune response was especially prominent in one lung subsegment trial at the highest dose of adenovirus vector used. The transient expression provided by adenovirus vectors would require repeated administrations; however, repeat administration of a first-generation adenovirus vector produced increased immune responses and decreased correction of chloride transport. The combination of immune response and transient expression have dimmed hopes that first-generation adenovirus vectors will be used for treatment of CF. Modifications of first-generation adenovirus vectors, second- and third-generation adenovirus vectors, contain further deletions of early genes in an attempt to further reduce or eliminate expression of late viral genes and, thus, reduce the host immune response. Other strategy is to turn to a different viral vector system. Adeno-associated virus (AAV) is a naturally replication-deficient single-stranded DNA parvovirus that depends on coinfection with a helper virus, such as adenovirus, for replication and is not associated with any known human disease. AAV vectors may offer theoretical advantages over adenovirus vectors, including lack of the pathogenicity of wild-type virus as well as modified AAV and longer-lasting expression. In vitro and animal studies have shown that CFTR transcripts and the CFTR protein can be detected up to six months after transduction with an AAV vector. produce sufficient viral titers (though less than adenovirus produces), and able to transduce non dividing cells. These features suggest that AAV vectors may perform well as a CFTR gene transfer agent. AAV is naturally replication deficient, but an additional technical challenge specific to AAV vectors is the small size of the viral genome and consequent limitation of transgene insert size. To make a CFTR AAV vector, the viral genes necessary for replication and encapsidation, rep and cap, respectively, must be deleted in order to fit the CFTR transgene. Because CFTR cDNA is at the upper limit of insert size, promoter selection was problematic. Fortunately, the left-hand terminal repeat, already necessary for packaging and excision of the recombinant vector, has sufficient promoter activity for transgene expression. For production, rep and cap, along with a helper virus such as adenovirus, must be provided to make recombinant virions. Besides the limitation, in the insert size, other adeno-associated virus has many disadvantages,that includes, possible vector integration, rescue or recovery of virus after infection, and the recently observed requirement for helper virus infection for leading strand synthesis, which in turn limits transgene expression. Even though, in the absence of helper virus, wild-type adeno-associated virus integrates into a site on human chromosome 19, AAV vectors predominantly persist in episomal form in vitro and in vivo. Despite, the relative lack

of integration in host chromosomes, an AAV vector can still be rescued from rhesus monkey airway epithelium by coinfection with wild-type AAV and adenovirus. The rescue of an AAV vector may portend a risk of disadvantageous shedding after gene therapy treatment. Despite the evidence for shedding, animal experiments have not demonstrated pathologic or immune alterations after AAV vector transduction. Viral shedding, safety, biological efficacy, and other issues are currently being further examined in two ongoing human phase I gene therapy trials. Because neither the adenovirus nor adeno-associated vectors fulfill all the criteria for an ideal vector, the other viruses are also have been considered as potential vectors. Retrovirus vectors, already used in other types of gene therapy studies, were initially spurned as a CF gene therapy vector because they require dividing cells for efficient transcription [(31-36)].

Non-Viral Vectors

Viral systems are not only the ones who are capable of CFTR gene transfer. There are two main kinds of non-viral vectors, which are in development and they are liposome-DNA complexes and molecular conjugates. The massively studied liposomal gene transfer method is based on cationic lipid vesicles that bind negatively charged DNA and fuse to cell membranes for DNA transfer. The DNA consists of CFTR cDNA plus a promoter together on a plasmid. The main advantages of liposomal gene transfer over viral gene transfer are the greater safety of liposomes and ease of preparation. Liposomal treatment has been shown to be safe in a variety of animals and humans. The disadvantages are decreased transduction efficiency compared with viral vectors, lack of tropism for airways epithelium, and the Liposomal-mediated gene transfer has been affected in a variety of in vitro systems and has also been shown to correct the ion transport defect in CF mutant mice. A phase I, randomized, double-blind clinical trial investigating liposomal transfer of CFTR to the nasal mucosa in CF patients assured the safety of this gene transfer technique. Variable and transient expression as well as functional restoration were observed in this trial. Three human clinical trials using liposomal gene transfer are ongoing. Observed expression and functional restoration by the liposomal vector are likely not adequate for CF gene therapy Disappointing efficiency is driving the search for more effective combinations of lipids and plasmid. One disadvantage of liposomal-mediated gene transfer is the lack of tropism for airway epithelium. Molecular conjugates are designed to take advantage of receptor-mediated endocytosis and, thus, to provide some specific delivery capabilities to this nonviral gene transfer technique. One conjugate approach cleverly uses adenovirus virions complexed with polylysine-condensed DNA to augment the uptake and expression of DNA. This complex of adenovirus particles, polylysine, and DNA plasmid is taken up by transferrin receptors. Encouraging results were obtained in vitro, but they have not been confirmed in animal models. Despite inefficient expression in vivo, this delivery system has the advantage of targeting specific cell types by exploiting cell-specific receptors. No clinical trials using molecular conjugates are ongoing [(37-40)]. In this review, it's described a path for translation of gene editing into therapy for cystic fibrosis (CF). Cystic fibrosis is caused from mutations in the CFTR gene, with one allele predominant in-patient populations. This simple, genetic cause of the disease makes gene editing appealing for treatment of this disease. There already have success in applying this technology to cystic fibrosis in cell and animal models, even though, these advances have been modest in comparison to advances for other disease. Less than six years after its first demonstration in animals, CRISPR/Cas gene editing is nowadays, in early clinical trials for several disorders. In most clinical trials, thus far, attempt to edit genes in cells of the blood lineages. The advantage of the

blood cells is that the stem cells are known, can be isolated, edited, selected, expanded, and returned to the body. The likely next trials mostly will be in the liver, which is very accessible to many delivery methods. For cystic fibrosis, the biggest puzzle is to deliver editors to other, very less accessible organs. We outline a path by which delivery can be improved. The translation of new therapies doesn't occur in isolation, and the development of gene editors is occurring as advances in gene therapy and small molecule therapeutics are being made. The advances made in gene therapy may help develop delivery vehicles for gene editing, although major improvements are needed. The approval of effective small molecule therapies for many patients with cystic fibrosis will raise the bar for translation of gene editing [(41-43)]. Cystic fibrosis (CF) is usually common genetic disorder characterized by defective epithelial chloride transport and progressive lung disease. Even though, great strides have been made in the treatment of CF, it remains lethal, often in the early adulthood. CF is one of the most extensively researched genetic diseases as a target for gene therapy development. It may also serve as an important model for gene therapy for other diseases. Preclinical and clinical research has leads to the rapid development of a variety of vectors that are designed to correct the basic defect in CF, including adenovirus, adeno-associated virus, and liposomes. Clinical studies have identified that the host immune response and low vector efficiency as key impediments to effective CF gene therapy. Further research promises to refine vector technology and bring CF gene therapy to the bedside [(44)]. Genome editing which is driven by zinc-finger nucleases (ZFNs) yields high gene-modification efficiencies (>10%) by introducing a recombinogenic double-strand break into the targeted gene. The cleavage is induced using two custom-designed ZFNs that heterodimerize upon binding DNA to form a catalytically active nuclease complex. Using the current ZFN architecture, however, cleavage-competent homodimers may also form that can limit safety or efficacy via off-target cleavage. Here we will develop an improved ZFN architecture that eliminates this problem. Using structure-based design, we engineer two variant ZFNs that efficiently cleave DNA only when paired as a heterodimer. These ZFNs modify a native endogenous locus as efficiently as the parental architecture, but with a >40-fold reduction in homodimer function and much lower levels of genome-wide cleavage. This architecture provides a general means for improving the specificity of ZFNs as gene modification reagent [(45)].

Cystic fibrosis (CF) is the most common life-limiting genetic disease in Caucasian patient population. The continued advances have led to improved survival, and adults with CF now outnumber children. As our understanding of the disease improves, new therapies have emerged that improve the basic defect, enabling patient-specific treatment and improved outcomes. However, the recurring problems continue to lead to morbidity and mortality, and new pathogens have been identified that may lead to worse outcomes. In addition to that, new complications, such as CF-related diabetes and increased risk of gastrointestinal cancers, are creating new challenges in management. For patients with end-stage disease, lung transplantation has remained one of the few treatment options, but challenges in identifying the most appropriate patients remain [(46)]. Medicine is at a turning point now. As of it is evolving and many technologies have been discovered and invented. According to the above-mentioned information, CRISPR/Cas 9 has been in clinical trials using animal and eukaryotic model such as Baker's Yeast. It's much more complicated when it comes to human beings. When it comes to human beings, there is germline editing and somatic gene editing. Somatic gene therapies involve modifying a patient's DNA in order to treat or cure certain diseases caused by a genetic

mutation. In the case of germline editing, alters the genome of a human embryo at its early stages. Those have many disadvantages including targeting the wrong gene, mosaicism etc. For these reasons, the scientific community approaches the germline with caution and have several restrictions. So now also due to ethical and safety issues the genome editing in humans are restricted rather than its success story in the clinical trial in animal models and baker's yeast [(47)].

3. CONCLUSION

Recent years have seen an explosion of research toward CF gene therapy. A fertile combination of preclinical and clinical research is producing rapid progress in defining the molecular pathophysiology of CF and improving vector technology for producing CFTR expression. Gene therapy clinical trials have refined our understanding of the barriers to CFTR gene transfer, and they will continue to impact the development of future generations of vectors in an iterative process involving both preclinical and clinical research. High expectations of gene therapy by investigators, clinicians, patients, and the lay public should not divert attention from the rapid progress that has already been made nor should it dampen enthusiasm for the necessarily iterative process of gene therapy development.

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