HHS Public Access

Author manuscript

Expert Opin Biol Ther. Author manuscript; available in PMC 2018 March 19.

Published in final edited form as:

Expert Opin Biol Ther. 2017 October ; 17(10): 1265–1273. doi:10.1080/14712598.2017.1347630.

AAV gene therapy for cystic fibrosis: current barriers and recent developments

William B. Guggino and **Liudmila Cebotaru**

Departments of Medicine and Physiology, Johns Hopkins University, MD, USA

Abstract

Introduction—Since the CF gene was discovered in 1989, researchers have worked to develop a gene therapy. One of the most promising and enduring vectors is AAV, which has been shown to be safe. In particular, several clinical trials have been conducted with AAV serotype 2. All of them detected viral genomes, but identification of mRNA transduction was not consistent; clinical outcomes in Phase II studies were also inconsistent. The lack of a positive outcome has been attributed to a less-than-efficient viral infection by AAV2, a weak transgene promoter and the host immune response to the vector.

Areas Covered—Herein, we will focus on AAV gene therapy for CF, evaluating past experience with this approach and identifying ways forward, based on the progress that has already been made in identifying and overcoming the limitations of AAV gene therapy.

Expert Opinion—Such progress makes it clear that this is an opportune time to push forward toward the development of a gene therapy for CF. Drugs to treat the basic defect in CF represent a remarkable advance but cannot treat a significant cohort of patients with rare mutations. Thus, there is a critical need to develop a gene therapy for those individuals.

Keywords

gene therapy; cystic fibrosis; adeno-associated virus; preclinical testing; clinical trials

I. Introduction

The overall concept for restoring long-term function by using gene therapy for autosomal recessive diseases such as cystic fibrosis (CF) is simple: either replace or repair the defective gene; the complications with this approach arise from the methodology. Replacing the dysfunctional gene involves adding the wild-type coding sequence to the cell, along with a promoter to allow for long-term expression. To repair a mutation requires a gene editing strategy that removes and/or replaces the mutated sequence either to restore normal function or remove the malfunctioning gene product. The CF gene was discovered almost three decades ago[1–3], but despite intense efforts to develop a workable gene therapy, none has been forthcoming. The question is: why not? This review will address the hurdles that have delayed the development of an effectual gene therapy and make the case that they are

Correspondence: Liudmila Cebotaru, Department of Medicine, Division of Gastroenterology and Hepatology, Johns Hopkins School of Medicine, Hunterian Bldg., Room 417 – 725 N. Wolfe Street – Baltimore, MD Tel.: 410-614-0735, lcebotaru@jhmi.edu.

surmountable. With the new technologies that are now available and a renewed effort by CF researchers, a gene therapy for CF can still be successfully developed.

II. Cystic Fibrosis

CF is an autosomal disorder that is common among Caucasians of European descent[4]. It is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel involved in the generation and maintenance of a tiny layer of fluid on the surface of mucosal membranes of the airways, gastrointestinal tract, epididymis, liver, and pancreas[5]. In these organs, failure to produce the layer of fluid causes sticky mucus secretions, leading to chronic lung infection and inflammation, GI obstruction, male infertility, liver disease, and failure to digest food as a result of the loss of pancreatic duct function[67]. In the sweat ducts, failure to absorb chloride from the ducts leads to elevated concentrations of NaCl in the sweat, even at rest[8]. CF was considered a prime candidate for the development of a gene therapy because it is single-gene defect whose repair by gene therapy was demonstrated early on by a restoration of chloride channel function in vitro[9].

Lung disease is the primary cause of mortality in these patients, and the lungs are fortunately very accessible for the delivery of gene therapy vectors[10]. Thus, shortly after the gene was identified, intense efforts were soon undertaken in a race toward achieving a cure for CF by gene therapy[11], and several clinical trials were conducted with this purpose in mind. These clinical trials utilized full-length CFTR cDNA packaged into either adenovirus, adeno-associated virus (AAV), or liposomes and delivered to the airways.

There is still a critical need to develop a gene therapy for CF. New therapies based on chemical compounds that either activate chloride channel activity for certain gating mutants (Ivacaftor) or rescue the processing of trafficking mutants (Lumacaftor) have recently been approved by the FDA[12]. The combination, Orkambi, of the potentiator, Ivacaftor, and the corrector, Lumacaftor has been approved for the treatment of patients with ΔF508 mutations[13]. By rescuing the basic defects, these new therapies provide a substantial improvement over previous treatments, which mainly targeted the symptoms associated with this progressive disease. However, despite the progress made thus far, there is still a critical need to develop an effective gene therapy for CF. There are over 1000 different mutations in CF patients[14], and many of these mutations affect CFTR in ways that are either too severe to be treated by VX-770, 809, or their combination, or that cannot be rescued at all by the existing therapies. Moreover, it is unclear whether patients will be able to take these new drugs for their lifetime. Over long periods of time patients may accumulate drug side-effects which may limit their therapeutic usefulness. Therefore, the most fundamental way to help every patient with CF is still with a gene therapy.

Since general overviews of CF gene therapy have already been published[1516], this review will focus specifically on the efforts using adeno-associated virus.

III. Adeno-associated Virus (AAV)

AAV is a defective (DNA) parvovirus isolated from humans and primates. It is naturally defective for replication, requiring co-infection with a helper adenovirus or herpesvirus for replication. The AAV genome is a 4.68-kb, linear single strand of DNA consisting of inverted terminal repeats (ITR) and *rep* and *cap* proteins. The ITRs represent 145 nt of DNA and are absolutely required for integration, replication (ori), excision, and packaging. The *rep* gene is involved in replication, and there are four rep proteins produced by alternate splicing. The *cap* gene is required for encapsidation. There are three cap proteins, also produced by alternate splicing[17]. Single-stranded AAV must be converted into a double-stranded form for gene expression (see[18]). Studies have shown that this conversion takes place, albeit slowly, in non-dividing cells[19]. AAV is unique among the DNA viruses in that it is defective for replication and undergoes site-specific stable integration into human chromosome 19[2021].

AAV cannot be propagated by itself. Instead, its propagation requires that the host cell be co-infected with a helper virus, the most common being adenovirus. AAV usurps the function of several genes within the adenovirus genome to allow it to complete its replication cycle[22]. For human therapy, this requirement for co-infection provides a natural safety feature that helps prevent inappropriate spread of recombinant AAV (rAAV) following clinical application.

To produce rAAV, three components are needed: 1) the desired coding sequence of the gene, such as CFTR, subcloned into a plasmid flanked on both the 5’ and 3’ ends with inverted terminal repeats (ITRs); 2) a second plasmid that contains the *rep* and *cap* genes; and 3) a method for providing the helper function associated with the adenovirus[23]. In the earlier days of AAV gene therapy, AAV was produced by triple transfection, i.e., transfecting with all three of these components in separate plasmids; more recently, scalable production methods have become much more sophisticated, reducing the number of plasmids necessary to produce large quantities of AAV (see[24]). The final rAAV particle now contains the ITR-CFTR-ITR coding sequence encapsulated within the viral capsid proteins. Such particles are often called DNase-resistant particles (DRPs) because the DNA encapsulated within the viral particles, when fully formed, is DNase-resistant. Studies that have examined the fate of *rep*-deleted vector DNA have indicated that the the ITR-CFTR-ITR coding sequence is located episomally or integrated randomly at a low frequency in the host cells[25], leading to long-term expression of the recombinant protein in the infected cells.

IV. Preclinical Single-Dose Studies of AAV2 for CF

A number of preclinical studies were performed prior to the first clinical trial of the use of AAV in humans, in order to demonstrate the safety, efficacy of transduction, and long-term expression of AAV. In one of these very early studies, cells were isolated from nasal polyps of CF patients and grown in primary culture. Infection with rAAV2-CFTR showed efficient transduction of CFTR: values of 75–90% were obtained, as assayed by vector DNA transfer. The cells also expressed CFTR, as determined by immunofluorescent staining[26]. In another early study, rAAV2-CFTR was instilled into selected bronchi in rabbits via bronchoscopic

delivery. CFTR RNA and protein were detected in these bronchi for up to 6 months. The vector utilized in this study was a unique construct containing a truncated CFTR (nucleotides 486–4629) with a synthetic 60-nt oligonucleotide at the 5' end[26]. This vector produced a CFTR protein that was modified at its amino terminus to include a fusion peptide of 26 aa that is not found in native CFTR (MLLIYVHTKNQHTLIDASELFIRPGT). An antibody was raised against this peptide and used to verify the transduction of this unique sequence in both the human primary nasal epithelial cells and the rabbit cells. It is interesting that this partially synthetic CFTR construct fully complemented the CFTR defect in a CF bronchial epithelial cell line[9, 27], demonstrating that this construct was capable of restoring function to a cell line containing the common CFTR deletion known as ΔF508 CFTR.

In a subsequent study, Rhesus macaques were treated with a single dose of rAAV2-CFTR by bronchial administration using a protocol similar to that described earlier for rabbits[28]. The engineered vector used for this study were based upon AAV serotype 2 and is referred to as tgAAV2-CFTR. The vector contains the full-length coding sequence of CFTR subcloned between the AAV2 inverted terminal repeats (ITRs). The tgAAV2-CFTR vector contained a synthetic polyadenylation signal based on the murine β-globin gene. This vector utilized the intrinsic promoter of the AAV2 ITR[27] to drive CFTR expression, making it possible to package the full-length CFTR (4400 nt) into AAV AAV-CFTR was detected in monkeys that received 5×10^8, 1×10^{10}, or 1×10^{11} total particles. Remarkably, vector-specific DNA and rRNA expression were detected for up to 180 days after infection. Most importantly, there were no indications of inflammation or other toxicity.

V. Preclinical Repeat-Dosing Studies of AAV2 for CF

The development of neutralizing antibodies is a key barrier for all gene therapies involving viral vectors (reviewed by[29]). However, the lung may be one of the organs where repeat dosing is feasible. Two repeat-dosing regimens were tested in New Zealand white rabbits and Rhesus monkeys[30, 31]. The studies involved two doses of tgAAV2-CFTR, followed by a single dose of either rAAV2-CFTR or GFP. In the case of the rabbits, each animal received 5×10^9 DNase-resistant particles per dose. Three weeks after the end of the experiment, GFP expression was detected. In the rabbits, the presence of neutralizing antibodies in serum (defined as inhibition of wild-type AAV2 replication) increased after the first dose, but seroconversion (defined as a 4-fold or greater rise in titer) was not observed in all of them until after the second dose; it was sustained through the 17th week after the third dose. Interestingly, no neutralizing antibodies were detected in the bronchial lavage fluid (BAL). A similar protocol was used for the Rhesus macaques, who received ~10^{13} DNase-resistant particles per dose. At the end of the experiment, GFP expression was again detected. Neutralizing antibodies were detected in the sera of all the treated monkeys by the second time point, and the immune response persisted until autopsy at week 3. By the third dose, all the monkeys had escalating titers, with increases of more than 4-fold, consistent with seroconversion. In the monkey experiments, the presence of neutralizing antibody was defined as the ability to block vector transfer in vitro. Interestingly, a 10-fold lower titer was observed in the BAL fluid than in the serum. Although GFP expression was detected in both the rabbit and monkey studies, at least for AAV2, repeated dosing caused a significant drop

in the ability to detect vector-derived mRNA, possibly indicating that repeated dosing reduces the magnitude of vector transduction[30, 32]. One common conclusion from both the rabbit and monkey studies was that repeated dosing with AAV is safe. Armed with this body of promising data, we and others conducted a number of single-dose clinical trials.

VI. Single-Dose AAV2-CFTR Trials in Humans

The first human AAV clinical trial for the treatment of CF was a prospective, randomized, unblinded, dose-escalation phase I trial that involved instillation of tgAAV2-CFTR into one of the maxillary sinuses[33]. Ten patients who were pancreas-insufficient and had undergone bilateral antrostomies were administered the recombinant AAV2 virus. The highest levels of vector genomes were observed 2 weeks after treatment: in the range of 0.1 to 1 AAV-CFTR vector copy per cell. The vector persisted for as long as 10 weeks after treatment. To assess the functional correction of the defective CFTR, sinus transepithelial potential differences were measured. In the untreated sinus of the CF patients, the TEP measured on the surface was highly negative (approximately −57.5 mV) with respect to a ground electrode inserted under the skin. Zero Cl^- or isoproterenol solutions superfused onto the sinus had no effect, reflecting the absence of CFTR function. After the application of tgAAV2CFTR, superfusion of isoproterenol, which stimulates CFTR via an increase in cAMP, and of low Cl^--containing solutions, which increase the gradient for Cl^- across the membrane and therefore its movement via the CFTR, did succeed in producing a hyperpolarization on days 7 and 14, but not on day 28. These data indicate a restoration of function as a result of tgAAV2-CFTR treatment. No toxicity was seen to result from the treatment. This study was significant because it did demonstrate infection with tgAAV2-CFTR and its persistence and transduction in the sinus, with no evidence of toxicity. Most important was the fact that correction of the dysfunctional CFTR was also observed.

Given these promising results, a Phase II, double-blinded, randomized, placebo-controlled clinical trial was conducted in 23 CF patients[34]. In this trial, the primary endpoint was the rate of relapse of clinically defined sinusitis within a 3-month follow-up. Unfortunately, the rate of sinusitis did not differ significantly between the placebo and treated groups. Furthermore, no other secondary outcome attained statistical significance. Detection of the vector DNA varied widely between sampling periods. No statistically significant change occurred in the transepithelial potential (TEP), but the change in voltage following exposure to isoproterenol and low Cl^- were in a direction consistent with increasing CFTR function. No adverse events were associated with vector administration, again demonstrating that gene delivery via AAV2-CFTR is safe. The results of the Phase II trial were disappointing in that they indicated inefficient gene transfer and transduction with tgAAV2-CFTR. However, the results also highlighted the difficulties inherent in sampling from and conducting electrophysiological studies in the maxillary sinus.

Reported as the first trial initiated with rAAV in human lung, a phase I study in 25 adult and adolescent CF patients with mild-to-moderate lung disease was conducted in which doses of the tgAAV2-CFTR ranged from 3×10^1 to 1×10^9 replication units (RU), equivalent to approximately 6×10^4 to 2×10^{12} DNase-resistant particles (DRP)[35]. The recombinant virus was administered to one side of the nose and to the superior segment of the lower lobe of the

right lung. The patients were divided into 10 cohorts representing escalating doses. Vector genomes, as measured by DNA polymerase chain reaction (PCR), was not observed except in cohort 10 (corresponding to a nasal dose of 1×10^7 RU and 1×10^8 RU in the bronchial epithelia). Sporadic low-level copy numbers corresponded to 0.002 – 0.5 copies per cell. Several adverse events were noted before and/or after vector delivery, but most of them appeared to be related to the endogenous CF lung disease or to result from the bronchoscopic procedure. Measurements of TEP across the nasal epithelium were similar to those described above and did not result in any statistically significant differences between the treated and non-treated noses.

Finally, a fourth study was performed that involved a Phase I, single-administration, dose-escalation trial in which the tgAAV2-CFTR was administered by nebulization to the lungs of CF subjects[36]. This procedure differed from the single-dose trials in which the vector was applied directly or via bronchoscopic delivery. Twelve patients were each administered one of a series of increasing dosages of the virus ranging from 10^{10} to 10^{13} DRP, and bronchoscopies were performed throughout the study. A maximum of 0.6 and 0.1 vector copies per cell brushed from the airway during bronchoscopy was observed at 14 days and 30 days, respectively, following nebulization of 10^{13} DRP of tgAAV2-CFTR by day 90, the number of copies per cell had declined to undetectable levels. As was true for the previous studies, administration was deemed to be safe (the primary outcome in this Phase I study).

Overall, the single-dose clinical studies were similar in that vector genomes were detected in all cases, and in one instance there was functional rescue of function. The distinctly variable detection of vector genomes after infection was different from the situation observed in the single-dose monkey studies, which quite consistently showed the presence of vector genomes. Clearly, in the monkey experiments, detection was easier because the tissues examined were derived from necropsy samples. None of the studies thus far have been able to detect vector-derived mRNA expression, highlighting the difficulty in detecting gene transduction from samples of cells taken from the maxillary sinus, nose, or lungs.

VII. Repeat-Dosing AAV2-CFTR Trials in Humans

Given the positive safety data from the single-dose studies, two repeat-dosing clinical trials were performed using the tgAAV2-CFTR vector in CF patients. The first was a randomized, double-blind, placebo-controlled phase II trial[37]. Subjects were randomized into two groups, with 20 receiving at least one dose of vector and 17 receiving the placebo. The vector was administered by inhalation using a Pari-LC plus nebulizer. Patients were given three doses of 1×10^{13} DRPs at 30-day intervals. This phase II trial used a number of assessments, including spirometry, detection of lung abnormalities by high-resolution CT (HRCT), and measurement of airway cytokines, vector shedding, serum neutralizing antibody to AAV serotype 2 (AAV2), and vector genomes and expression in a subset of subjects undergoing bronchoscopy with bronchial brushings. The results of this trial were promising. Reductions in the inflammatory mediator interleukin 8 (IL-8), which is typically elevated in CF patients[38], were noted in the induced sputum samples. At Day 14, sputum IL-8 levels decreased 0.09 $\log_{10}$ ng/mL in subjects randomized to tgAAVCF, and increased 0.12 $\log_{10}$ ng/mL in placebo controls. No differences were noted, however, at days 45 and 75.

Also encouraging was the increase in FEV_1, which characteristically declines in CF patients with increasing age. What was notable was that, at day 30, 25% of the patients in the tgAAVCF group had a >10% improvement in FEV_1 vs none for placebo. At day 60, four of the patients showed a similar increase vs. one placebo subject. At day 90, three patients in the tgAAVCF group showed an improvement from baseline vs. one for the placebo patient. Although improvements in FEV_1 were evident at 60 and 90 days in some patients, the differences between the tgAAVCF and placebo groups were no longer statistically significant because of the small number of patients. It is important to note that these improvements occurred after a single dose of the vector, but they were not sustained following repeat dosing. Vector genomes were detected by PCR in all of the six patients whose epithelial cells were obtained during the bronchoscopies (29 – 100 copies of vector DNA per diploid genome).

To extend the Phase II study, a Phase IIB study was conducted in which 102 subjects over the age of 12 were treated with two doses of 1×10^{13} DNase-resistant particles of tgAAV2-CFTR or corresponding placebo, administered 30 days apart[39]. Although safety was once again documented, the study did not meet its primary endpoint of statistically significant improvement over placebo in FEV_1 at 30 days after the initial administration of tgAAV2-CFTR. There were also no significant differences seen in lung function over time, induced sputum biologic markers, or days of antibiotic use between the two groups. This study was designed on the basis of the encouraging results that were observed early in the previous study, after the administration of the first of the three treatments. The lack of effect in the large Phase IIB study was disappointing. However, there were a number of differences between the two studies that make them difficult to compare: For example, the number of treatments was reduced from three to two, and no measurements of DNA transfer or neutralizing antibodies were made in the second study. Thus, it is difficult to verify that similar amounts of tgAAV2-CFTR genomes were indeed transferred to the airways of the patents, or whether pre-existing neutralizing antibody titers were present prior to the second study.

Within the lens of hindsight one can ask whether Targeted Genetics should have moved from single to repeat-dose clinical trials without evidence of vector-specific mRNA expression in the human lung. On one hand, the quite variable experience with the phase I clinical trials perhaps should have raised enough caution to delay the repeat-dose trials until more experiments were performed to improve gene transfer and transduction in non-human primate studies. These studies could have been designed to mimic more closely the human experience such spraying the virus into the airways and sampling for gene transfer and transduction via bronchial brushing. Caution may have been particularly warranted because the non-human primate studies had already demonstrated a decrement in gene transfer and transduction in repeat-dose experiments using tgAAV2 vectors[32]. Since the phase I clinical trials already had limited success, the animal studies may have predicted a worse outcome for repeat dosing in humans. However, to evaluate in hindsight the overall decision to proceed to a repeat dosing trial, one has to look at the overall design of the Targeted Genetics repeat-dose Phase II clinical trial which included one arm with a small number of patients followed by a thorough analysis of the outcome with ample time to decide whether the safety profile and clinical outcome warranted conducting the larger study. As discussed

above the smaller study was promising which propelled the decision to move forward. Given that the whole field of gene therapy was at its early stages, the decision making at Targeted Genetics was indeed deliberate and cautionary.

One overlooked positive outcome of these early pioneering studies was that repeat dosing of AAV was shown to be safe which is ultimately why AAV is still in use in many clinical trials where the barriers to successful delivery and transduction are being overcome to treat many diseases[40].

VIII. Limitations of AAV2

A number of well-known limitations of the AAV-serotype 2 are likely to have compromised the outcomes of the clinical studies have been described. It has been shown that in AAV2, the heparin sulfate proteoglycan receptor is more abundant in the basolateral cell membrane than in the apical cell membrane, consistent with the observation that rAAV2 transduction is 200-fold more effective when the construct is applied to the basolateral surface of well-polarized epithelia rather than to the apical cell membrane[41]. The AAV2 virus that does manage to enter the cell from the apical membrane does so via a different mechanism of endocytosis, in which the viral particles are ubiquitinated and ultimately degraded in the proteasome, and therefore they do not reach the nucleus, where transduction normally occurs[42]. The current thinking is that these limitations, which were identified in vitro, are responsible for the lack of clinical effect of tgAAV2-CFTR in the human studies. This is clearly a reasonable hypothesis, since all of limitations, i.e., the lack of apical receptors and the degradation of AAV2 that enters via the apical membrane, would be expected to limit gene transfer. However, in all of the preclinical non-human primate studies and the clinical studies, significant numbers of vector genomes of rAAV2-CFTR were detected in the airway cells in vivo, and the vector genomes were present for long periods after infection. In the primate studies expression of the recombinant protein was detected suggesting that AAV2 internalization had indeed occurred, and the constructs had persisted within the cell.

One common denominator among all the clinical studies is that none of them were able to detect vector-derived mRNA expression, although it was detected in the animal studies. Thus, one likely scenario is that the promotor within the ITR of the tgAAV2-CFTR vector was too weak to generate enough mRNA to be detected in the human samples by the RT-PCR methods employed[43]. With newer generations of AAV vectors which include more powerful promotors it may be possible to detect gene transfer in future clinical studies. Thus, although the many clinical studies of AAV were widely interpreted as disappointing, the data can also be seen to provide a path forward, indicating that with improved vector transfer and improved gene expression (discussed below), gene therapy for airway disease is nevertheless feasible.

IX. Next-generation viral vectors

With the discovery of many more serotypes of AAV, including some with more tropism for the airways than is exhibited by AAV2, it is now possible to boost gene transfer using these new serotypes[44]. One of the first next-generation viruses that has been tried is AAV5[45]

which included a pseudotyped virus containing the ITRs from AAV2 and the capsid proteins from AAV5. AAV5 transduces Rhesus macaque lungs to levels much higher than those achieved with AAV2[45]. Also in macaques, AAV9 has also been demonstrated to have high tropism and to transduce lung cells with high efficiency[46]. It must be borne in mind, however, that although these results in primates are important, they may or may not be predictive of human studies.

In order to determine which vector is best for use in humans, a non-lethal study has been conducted in chimpanzees, the closest genetic relative to *Homo sapiens*. A dual-reporter assay based upon firefly and *Renilla* luciferase[47] was used, and the genes encoding these enzymes were cloned into the AAV1 and AAV5 vectors, respectively. The two luciferase enzymes differ in their substrates and cofactor requirements and can be easily distinguished in the assays. The results showed an increase in luciferase activity with time generated by transduction via gene transfer with either AAV1 or AAV5. Similar results were obtained with primary human airway cells grown in tissue culture; in these cells, AAV1 was approximately 100-fold more effective in transduction than was AAV5. As mentioned above, others have shown that AAV9 also transduces lung cells very efficiently, but primarily in the alveoli[46]. Thus for CF, AAV1 may be a better choice than AAV5.

In the studies described above, the results in the chimpanzees matched those in the primary human airways cells grown in tissue culture most likely owing to humans and chimps being close genetic relatives. However, experiments in chimpanzees are no longer possible leaving Rhesus macaques as a most commonly used species to test viral vectors. However, Rhesus macaques are genetically more distant from humans making it imperative that tropism be tested in human cultures prior to use in clinical trials.

X. New Promoters

As mentioned above, the tgAA2-CFTR virus construct utilizes an endogenous promoter located within the ITRs[27]. One reason that it was very difficult to detect vector-driven mRNA expression in the human clinical trials was that the promoter was too weak. mRNA was detected in the primate studies, however, suggesting that with better viral infection with the newer generation of vectors such as AAV1, it is still possible that tgAAVCF can be useful. To get beyond the issue of mRNA expression, new promoters have been developed. Sirninger et al. have created a rAAV-CMV enhancer/chicken β-actin promoter with intron-exon sequences (324 bp) prior to the ATG translation start site[43]. They have shown that the rAAV5-CB-promoter-driven CFTR vector can rescue CFTR-generated Cl^- transport in CF human bronchial epithelial cells (IB3-1 cells) and ameliorate the hyper-inflammatory environment in the lung of a CF mouse model infected with *Pseudomonas* containing agarose beads.

With the notion of enhancing expression from the original tgAAV2CFTR vector, Lynch and coworkers[48] at the former Targeted Genetics developed a synthetic promoter by examining a series of enhancer and promoter sequences. The constructs were transfected into IB3-1 cells, and promoter activity was measured at 48 h. With their new constructs, they were able to increase the level of transcription from 2-fold to greater than 50-fold when compared to the

ITR alone. Transduction of a rAAV2-CFTR construct containing a selected enhancer element produced an increase in CFTR RNA levels of ~5 fold in IB3-1 cells. Zhang et al.[49] tested this 83-bp construct (naming it AV2CF83) in CF human epithelial cell cultures. They found an approximately 3-fold increase in CFTR-generated currents when compared to AV2.tgCF, which used the original tg-ITR promoter element. To boost the expression even further, they identified a 100-bp enhancer (F5) from a screen of over 52,000 unique oligonucleotide sequences and created a new vector, AV2/2.F5tg83. The vector with the enhancer (AV2/2.F5tg83) showed a 17-fold increase in the expression of CFTR and an improvement in CFTR-generated Cl^- currents of ~19-fold in CF human epithelial cell cultures grown in air-liquid cultures when compared to the AV2/2.tg83 vector without the enhancer[50]. From these studies it is clear that new promoters can drastically improve the expression and function of CFTR to well above that which could be achieved with the older-generation tgAAV2-CFTR vector.

XI. Truncating CFTR

The original tgAAV2-CFTR vector was designed so that its CFTR coding sequence would fit into the packaging capacity of AAV2, which is 4.7 kb (the coding sequence of CFTR is about 4.4 kb)[28]. However, the use of the new promoters discussed above requires truncating CFTR to allow it to be packaged into AAV. Sirninger and coworkers[43] utilized a truncated CFTR (Δ264 CFTR) that was missing the first four transmembrane segments; this version was shown to be produced by additional translation initiation sequences further along in the CFTR coding sequence and to function in *Xenopus* oocytes[51]. The rAAV2-CB-Δ264 CFTR plasmid was created for packaging into AAV with a size of 5060 bp and shown to generate CFTR-generated Cl^- currents. Subsequently, a rAAV2-CB-Δ264 CFTR construct was packaged into an AAV5 capsid and sprayed into the lung of a Rhesus monkey via bronchoscope[45]. As discussed above, this treatment produced robust gene transfer and transduction.

One curious finding was an increased expression of endogenous wild-type (wt) CFTR in the monkey airways that was noted following infection with rAAV5-CB-Δ264 CFTR[45]. Further experimentation showed that the Δ264 CFTR was actually transcomplementing ΔF508 CFTR to produce wt CFTR: Transcomplementation occurs when certain truncation mutants such as Δ264 CFTR bind to ΔF508 CFTR and repair its function[43]. It was subsequently shown that Δ264 CFTR and a new construct Δ27–264 CFTR, which includes an additional 26 amino acids of the N-terminus of CFTR, do not conduct Cl^- on their own, but they can restore the function of the ΔF508 product by binding to it and repairing its trafficking and function (reviewed in[52]). Transcomplementation represents a novel combination of corrector and gene therapy.

Ostedgaard and collaborators[53] have explored a region of the regulatory (R) domain of CFTR and found a particularly interesting deletion of residues 708–759 that generates currents similar to those of wt CFTR and also retains the ability to be activated by cAMP. In their research, they created a regulated CFTR Cl^- channel, CFTRΔR, that could fit within the packaging capacity of AAV. Expressed using the fatty acid-binding protein promoter as a transgene in CFTR−/− mice, CFTRΔR was able to rescue the intestinal CF phenotype[53].

These researchers noted partial restoration of chloride transport that was similar to the restoration that occurred when they used an adenovirus containing wt CFTR for transduction. As mentioned in the previous section, Yan and colleagues[50] have created AV2/2.F5tg83-CFTRΔR and shown that transfection with this construct restores function to human CF bronchial epithelial cells. These two different strategies, the use of transcomplementation and a Cl^- channel missing part of the R domain of CFTR, seem to have solved the problem of how to truncate CFTR to fit the packaging limit of AAV and still restore function to cells containing mutations in CFTR.

XII. Trans-splicing

Spliceosome-mediated trans-splicing (SMart) is a novel idea for overcoming the inability to package CFTR into AAV with a strong promoter[54]. Normally the spliceosome converts mRNA to mRNA to enable gene expression by splicing out intronic sequences within one pre-RNA to create an mRNA which is ultimately translated into protein[55]. SMart takes advantage of cell's splicing mechanism to accomplish trans-splicing between different RNAs. Depending on how the construct is manufactured the trans-splicing can be between a donor RNA transcribed from a transgene and an acceptor endogenously produced pre-RNA. Alternately, it can occur between two different RNAs transcribed from two different transgenes packaged into individual AAV particles. Liu et al[56] took the first approach. They set out to replace exons 10–24 of CFTR containing ΔF508-CFTR mutation with the same exons containing the F508 CFTR. For this purpose they created a cassette containing regions of base pairing to intron 9 along with a 3' splice acceptor site and inverted terminal repeats from AAV2. This vector was then packaged into AAV2 or 5 and infected into polarized CF airway epithelial cells. 2 weeks post infection CFTR currents of approximately 14% of that observed in the epithelia containing wt-CFTR. The existence of wt transcripts were detected indicating that trans-splicing had occurred. Song et al.[57] took the second approach termed segmental trans-splicing. Basically, they split CFTR between exons 14a and 14b and created 5' and 3' donor and acceptor pairs. The vectors were then engineered into AAV6.2 and infected into human CF epithelial, IB3-1, cells. Using a variety of assay they estimated that the efficiency of segmental trans-splicing was approximately 4.7–12.1%. Although, in both studies trans-splicing occurred in epithelial cells grown in tissue culture the efficiency may not be high enough to be therapeutic in CF patients. Strategies have been developed to improve transduction efficiency by rational selection of the splitting site and optimization of trans-splicing vectors. Using this approach, Lai and collaborators[58] achieved wide-spread expression of a 6-kb ΔH2-R19 mini-dystrophin gene in skeletal muscle using AAV to rescue the dystrophic phenotype in mdx mouse model of Duchenne muscular dystrophy. Similar approaches may make trans-splicing more efficient for AAV-CFTR constructs where CFTR is split into two pieces.

XIII. Expert Opinion

Perhaps the most important outcome from all of the studies with AAV is that it is safe to apply to the human airways both in single and repeat dosing. Also the barriers to achieving clinical benefit are well known and, for the most part, have been surmounted by the creation of new serotypes and more power promoters. A major challenge still remains regarding how

to fit CFTR into the limited packaging capacity of AAV to accommodate the more powerful promoters. Three strategies have been proposed including transcomplementation, deletions out of the wild type sequence that still retains chloride channel function and trans-splicing. Clearly more experiments will be needed to determine which strategy will be more effective. Research into transcomplementation and trans-splicing will be particularly interesting represent new ways to repair endogenous CFTR transcripts or protein without altering the CFTR coding sequence as occurs with rapidly emerging field of gene editing[59].

To move forward a gene therapy will face fundamental challenges. The airway surface cells which possess functional CFTR have a finite lifespan[60]. Thus, any gene therapy would have to either be repeated with some frequency or target the stem cell that regenerates the surface epithelium. It would clearly be idea to target the lung stem cells which repopulate the airways. Recent progress in the identification of the stem cells in the airway has been rapid (see[61] for a review). Given that AAV viral vectors penetrate beyond the surface epithelium[28] transducing stem cells is feasible. Indeed, the ability of AAV1 and 5 to transduce conducting airway epithelial cells that persisted for extended periods of as long as 6 months has been demonstrated in mouse lung[62]. Because recombinant AAV is mostly an episomal vector[63], even if AAV were to target the stem cell, the transducing effects of AVV would be lost over time and repeat delivery would be required.

To devise a successful repeated dosing strategy, requires that the number of viral particles needed to achieve transduction will have to be optimized to reduce the neutralizing antibody response[29]. This will require continued optimization of the viral vectors to increase transduction efficiency. However, even with efficient transduction anti-AAV neutralizing antibodies (NAb) will increase with each dose. Also, pre-existing AAV antibodies are endogenous in many people who have already been exposed to AAV[29]. Thus a combination of pre-existing AAV antibodies and immunization from repeat dosing of recombinant vectors may pose a significant challenge for repeat-dosing.

One the other hand, given that treatment of CF patients with AAV would be episodic, short-term immunosuppression could be a feasible approach toward minimizing the blocking effects of NAbs. One approach that has been tried is B cell depletion with clinically available drugs such as rituximab and sirolimus which showed that B-cell ablation with rituximab prior to AAV reduced the responsiveness to both capsid and transgene in a subject with Pompe's disease[64]. However CF patients are prone to infection particularly in the lung[65]. Thus, aggressive immunosuppression to ablate the antibody response is most likely not feasible for CF. On the other hand, glucocorticoids such as methylprednisolone[66] are given to CF patients for several indications, including bronchiolitis, bronchial hyperreactivity, aspergillosis, and mild-to-moderate obstructive pulmonary disease[67]. The recommendation followed in patients is to provide the lowest effective dose and a short duration to minimize the risk of side effects[67]. Thus, although methylprednisolone does have side effects use of it as a short-term immunosuppressant for gene therapy in CF patients may be feasible. However, this approach has never been tested in CF patients thus whether it is indeed feasible in not known. Prior to testing this approach in patients more studies in animals will be necessary, particularly in non-human primates whose response to the repeat dosing may be closest to what will occur in humans. On the other hand, the CF airway is

unique environment containing mucous, inflammatory mediators, neutrophils along with compromised mucociliary clearance that is not duplicated in non-human primate models[68]. Thus additional experiments involving repeat dosing in CF ferret[69] or pig[70] models where the lung environment is more similar to CF patients may also be critical.

Where do we go from here? Interest in research into developing a gene therapy for CF once an overarching goal when the gene was first cloned received much less attention during the period of time when correctors and potentiators that target mutant CFTR trafficking and gating were identified. However, a new excitement is emerging. Given the new tools discussed above and recent advances in gene editing, the field of CF gene therapy is again at the forefront.

Acknowledgments

The authors wish to thank Dr. Deborah McClellan for editing the manuscript. Funded by NIH grant R01 HL122267

References

1. Rommens JM, Iannuzzi MC, Kerem BS, et al. Identification of the cystic fibrosis gene: Chromosome walking and jumping. sc. 1989; 245:1059–65.
2. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA [published erratum appears in Science 1989 Sep 29;245(4925)1437]. sc. 1989; 245(4922):1066–73.
3. Kerem B, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. sc. 1989; 245:1073–80.
4. Sosnay PR, Castellani C, Corey M, et al. Evaluation of the disease liability of CFTR variants. Methods Mol Biol. 2011; 742:355–72. [PubMed: 21547743]
5. Fuller CM, Benos DJ. CFTR. American Journal of Physiology: Cell Physiology. 1992; 263:C267–C86.
6. Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. Advanced Drug Delivery Reviews. 2002; 54(11):1359–71. [PubMed: 12458149]
7. Brennan M-L, Schrijver I. Cystic fibrosis: a review of associated phenotypes, use of molecular diagnostic approaches, genetic characteristics, progress, and dilemmas. The Journal of Molecular Diagnostics. 2016; 18(1):3–14. [PubMed: 26631874]
8. Collie JT, Massie RJ, Jones OA, et al. Sixty-five years since the New York heat wave: Advances in sweat testing for cystic fibrosis. ped pulm. 2014; 49(2):106–17.
9**. Egan M, Flotte T, Afione S, et al. Defective regulation of outwardly rectifying Cl− channels by protein kinase A corrected by insertion of CFTR. Nature. 1992; 358(6387):581–84. Represents the first report of AAV correction of CFTR. [PubMed: 1380129]
10. Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. New England Journal of Medicine. 2015; 372(4):351–62. [PubMed: 25607428]
11. Flotte TR. Gene therapy for cystic fibrosis. Curr Opin Mol Ther. 1999; 1(4):510–16. [PubMed: 11713767]
12. Boyle MP, Bell SC, Konstan MW, et al. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. Lancet Respir Med. 2014 Jul; 2(7):527–38. [PubMed: 24973281]
13. Wainwright CE, Elborn JS, Ramsey BW, et al. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. N Engl J Med. 2015 May 17.
14. Sosnay PR, Siklosi KR, Van GF, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet. 2013; 45(10):1160–67. [PubMed: 23974870]

15. Paul-Smith MC, Bell RV, Alton WE, et al. Gene therapy for cystic fibrosis: recent progress and current aims. Expert Opinion on Orphan Drugs. 2016; 4(6):649–58.

16**. Griesenbach U, Pytel KM, Alton EW. Cystic fibrosis gene therapy in the UK and elsewhere. Human gene therapy. 2015; 26(5):266–75. Recent efforts in gene therapy for CF. [PubMed: 25838137]

17. Samulski RJ, Muzyczka N. AAV-mediated gene therapy for research and therapeutic purposes. Annual review of virology. 2014; 1:427–51.

18*. Sanlioglu S, Monick MM, Luleci G, et al. Rate limiting steps of AAV transduction and implications for human gene therapy. Curr Gene Ther. 2001; 1(2):137–47. In depth discussion of the barriers to AAV transduction. [PubMed: 12108951]

19. Boyle MP, Enke RA, Adams RJ, et al. In utero AAV-mediated gene transfer to rabbit pulmonary epithelium. Mol Ther. 2001; 4(2):115–21. [PubMed: 11482982]

20*. Samulski R. Adeno-associated virus: integration at a specific chromosomal locus. COIGD. 1993 Definition of AAV integration.

21. Kotin RM, Linden RM, Berns KI. Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. EMBO Journal. 1992; 11:5071–78. [PubMed: 1334463]

22. Weitzman MD, Fisher KJ, Wilson JM. Recruitment of wild-type and recombinant adeno-associated virus into adenovirus replication centers. Journal of Virology. 1996; 70(3):1845–54. [PubMed: 8627709]

23*. Zolotukhin S, Potter M, Zolotukhin I, et al. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods. 2002; 28(2):158–67. Methods on how AAV is produced. Large scale production of AAV for clinical trials is still a challenge. [PubMed: 12413414]

24. Clement N, Knop DR, Byrne BJ. Large-scale adeno-associated viral vector production using a herpesvirus-based system enables manufacturing for clinical studies. Human Gene Therapy. 2009; 20(8):796–806. [PubMed: 19569968]

25. Duan D, Sharma P, Yang J, et al. Circular intermediates of recombinant adeno-associated virus have defined structural characteristics responsible for long-term episomal persistence in muscle tissue. Journal of Virology. 1998; 72(11):8568–77. Episomal localization of rAAV in cells. [PubMed: 9765395]

26*. Flotte TR, Afione SA, Conrad C, et al. Stable in vivo expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. Proc Natl Acad Sci USA. 1993; 90(22):10613–17. Early studies which led laid the foundation for the first clinical trials. [PubMed: 7504271]

27. Flotte TR, Afione SA, Solow R, et al. Expression of the cystic fibrosis transmembrane conductance regulator from a novel adeno-associated virus promoter. The Journal of Biological Chemistry. 1993; 268(5):3781–90. [PubMed: 7679117]

28. Conrad CK, Allen SS, Afione SA, et al. Safety of single-dose administration of an adeno-associated virus (AAV)-CFTR vector in the primate lung. Gene Ther. 1996; 3(8):658–68. [PubMed: 8854091]

29*. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood. 2013; 122(1):23–36. Barriers to gene therapy are discussed. [PubMed: 23596044]

30. Beck SE, Jones LA, Chesnut K, et al. Repeated delivery of adeno-associated virus vectors to the rabbit airway. Journal of Virology. 1999; 73(11):9446–55. [PubMed: 10516053]

31. Fischer AC, Beck SE, Smith CI, et al. Successful transgene expression with serial doses of aerosolized rAAV2 vectors in rhesus macaques. Mol Ther. 2003; 8(6):918–26. [PubMed: 14664794]

32. Beck SE, Laube BL, Barberena CI, et al. Deposition and expression of aerosolized rAAV vectors in the lungs of Rhesus macaques. Mol Ther. 2002; 6(4):546–54. [PubMed: 12387250]

33*. Wagner JA, Reynolds T, Moran ML, et al. Efficient and persistent gene transfer of AAV-CFTR in maxillary sinus. Lancet. 1998; 351(9117):1702–03. Among the first clincial trial for CF AAV gene therapy. [PubMed: 9734891]

34. Wagner JA, Nepomuceno IB, Messner AH, et al. A phase II, double-blind, randomized, placebo-controlled clinical trial of tgAAVCF using maxillary sinus delivery in patients with cystic fibrosis with antrostomies. Human Gene Therapy. 2002; 13(11):1349–59. [PubMed: 12162817]

35. Flotte TR, Zeitlin PL, Reynolds TC, et al. Phase I trial of intranasal and endobronchial administration of a recombinant adeno-associated virus serotype 2 (rAAV2)-CFTR vector in adult cystic fibrosis patients: a two-part clinical study. Human Gene Therapy. 2003; 14(11):1079–88. [PubMed: 12885347]

36**. Aitken ML, Moss RB, Waltz DA, et al. A phase I study of aerosolized administration of tgAAVCF to cystic fibrosis subjects with mild lung disease. Human Gene Therapy. 2001; 12(15): 1907–16. Single dose clinical trial. [PubMed: 11589832]

37. Moss RB, Rodman D, Spencer LT, et al. Repeated adeno-associated virus serotype 2 aerosol-mediated cystic fibrosis transmembrane regulator gene transfer to the lungs of patients with cystic fibrosis: a multicenter, double-blind, placebo-controlled trial. Chest. 2004; 125(2):509–21. [PubMed: 14769732]

38. Chmiel JF, Berger M, Konstan MW. The role of inflammation in the pathophysiology of CF lung disease. Clinical Reviews in Allergy and Immunology. 2002; 23(1):5–27. [PubMed: 12162106]

39**. Moss RB, Milla C, Colombo J, et al. Repeated aerosolized AAV-CFTR for treatment of cystic fibrosis: a randomized placebo-controlled phase 2B trial. Hum Gene Ther. 2007 Aug; 18(8):726–32. Repeat-dosing clinical trial often cited as inefficient gene transfer. [PubMed: 17685853]

40*. Berns KI, Muzyczka N. AAV: an overview of unanswered questions. Human Gene Therapy. 2017; 28(4):308–13. Prospects for AAV gene therapy. [PubMed: 28335618]

41*. Duan D, Yue Y, Yan Z, et al. Polarity influences the efficiency of recombinant adenoassociated virus infection in differentiated airway epithelia. Human Gene Therapy. 1998; 9(18):2761–76. Discussion of the barriers to gene therapy. [PubMed: 9874274]

42. Duan D, Yue Y, Yan Z, et al. Endosomal processing limits gene transfer to polarized airway epithelia by adeno-associated virus. J Clin Invest. 2000; 105(11):1573–87. [PubMed: 10841516]

43*. Sirninger J, Muller C, Braag S, et al. Functional characterization of a recombinant adeno-associated virus 5-pseudotyped cystic fibrosis transmembrane conductance regulator vector. Human Gene Therapy. 2004; 15(9):832–41. Improvements in the promoter for CFTR based AAV gene therapy. [PubMed: 15353038]

44. Gao G, Vandenberghe LH, Alvira MR, et al. Clades of Adeno-associated viruses are widely disseminated in human tissues. Journal of Virology. 2004; 78(12):6381–88. [PubMed: 15163731]

45. Fischer AC, Smith CI, Cebotaru L, et al. Expression of a truncated cystic fibrosis transmembrane conductance regulator with an AAV5-pseudotyped vector in primates. Mol Ther. 2007; 15(4):756–63. [PubMed: 17299412]

46. Limberis MP, Wilson JM. Adeno-associated virus serotype 9 vectors transduce murine alveolar and nasal epithelia and can be readministered. Proc Natl Acad Sci USA. 2006; 103(35):12993–98. [PubMed: 16938846]

47. Flotte TR, Fischer AC, Goetzmann J, et al. Dual reporter comparative indexing of rAAV pseudotyped vectors in chimpanzee airway. Mol Ther. 2010; 18(3):594–600. [PubMed: 19826405]

48. Lynch CM, Munson KL, Stephan TM, et al. Small synthetic promoter elements increase expression of the full-length CFTR cDNA in AAV vectors. Pediatrics Pulmonary Supplement. 1999; 19:230.

49. Zhang LN, Karp P, Gerard CJ, et al. Dual therapeutic utility of proteasome modulating agents for pharmaco-gene therapy of the cystic fibrosis airway. Mol Ther. 2004; 10(6):990–1002. [PubMed: 15564131]

50. Yan Z, Sun X, Feng Z, et al. Optimization of Recombinant Adeno-Associated Virus-Mediated Expression for Large Transgenes, Using a Synthetic Promoter and Tandem Array Enhancers. Hum Gene Ther. 2015 Jun; 26(6):334–46. [PubMed: 25763813]

51. Carroll TP, Morales MM, Fulmer SB, et al. Alternate translation initiation codons can create functional forms of cystic fibrosis transmembrane conductance regulator. The Journal of Biological Chemistry. 1995; 270(20):11941–46. [PubMed: 7538127]

52*. Cebotaru L, Guggino WB. Complement yourself: Transcomplementation rescues partially folded mutant proteins. Biophys Rev. 2014; 6(1):169–80. Review of transcomplementation. [PubMed: 24949105]

53. Ostedgaard LS, Meyerholz DK, Vermeer DW, et al. Cystic fibrosis transmembrane conductance regulator with a shortened R domain rescues the intestinal phenotype of CFTR−/− mice. Proc Natl Acad Sci USA. 2011; 108(7):2921–26. [PubMed: 21285372]
54. Mansfield S, Kole J, Puttaraju M, et al. Repair of CFTR mRNA by spliceosome-mediated RNA trans-splicing. Gene therapy. 2000; 7(22):1885. Discovery of trans-splicing. [PubMed: 11127576]
55. Mount SM. A catalogue of splice junction sequences. Nucleic Acids Research. 1982; 10:459–72. [PubMed: 7063411]
56. Liu X, Luo M, Zhang LN, et al. Spliceosome-mediated RNA trans-splicing with recombinant adeno-associated virus partially restores cystic fibrosis transmembrane conductance regulator function to polarized human cystic fibrosis airway epithelial cells. Human Gene Therapy. 2005; 16(9):1116–23. [PubMed: 16149910]
57. Song Y, Lou HH, Boyer JL, Limberis MP, Vandenberghe LH, Hackett NR, et al. Functional cystic fibrosis transmembrane conductance regulator expression in cystic fibrosis airway epithelial cells by AAV6. 2-mediated segmental trans-splicing. Human gene therapy. 2009; 20(3):267–81. [PubMed: 19257851]
58. Lai Y, Yue Y, Liu M, Duan D. Synthetic intron improves transduction efficiency of trans-splicing adeno-associated viral vectors. Human Gene Therapy. 2006; 17(10):1036–42. [PubMed: 17007565]
59. Ma Y, Zhang L, Huang X. Genome modification by CRISPR/Cas9. FEBS J. 2014; 281(23):5186–93. [PubMed: 25315507]
60. Hogan BL, Barkauskas CE, Chapman HA, et al. Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. Cell stem cell. 2014; 15(2):123–38. Recent advances in lung stem cell identification. [PubMed: 25105578]
61. Neuringer I, Randell SH. Lung stem cell update: promise and controversy. Monaldi archives for chest disease. 2016; 65(1)
62. Liu X, Luo M, Guo C, et al. Analysis of adeno-associated virus progenitor cell transduction in mouse lung. Molecular Therapy. 2009; 17(2):285–93. [PubMed: 19034263]
63. Afione SA, Wang J, Walsh S, et al. TR. Delayed expression of adeno-associated virus vector DNA. Intervirology. 1999; 42(4):213–20. [PubMed: 10567839]
64. Corti M, Elder M, Falk D, et al. B-Cell Depletion is Protective Against Anti-AAV Capsid Immune Response: A Human Subject Case Study. Mol Ther Methods Clin Dev. 2014; 1
65. Goldberg JB, Pier GB. The role of the CFTR in susceptibility to Pseudomonas aeruginosa infections in cystic fibrosis. Trends in Microbiology. 2000; 8(11):514–20. [PubMed: 11121762]
66. Thomson JM, Wesley A, Byrnes CA, et al. Pulse intravenous methylprednisolone for resistant allergic bronchopulmonary aspergillosis in cystic fibrosis. Pediatr Pulmonol. 2006 Feb; 41(2):164–70. [PubMed: 16317722]
67. Lai HC, FitzSimmons SC, Allen DB, et al. Risk of persistent growth impairment after alternate-day prednisone treatment in children with cystic fibrosis. N Engl J Med. 2000 Mar 23; 342(12):851–9. [PubMed: 10727589]
68. Boucher RC. New concepts of the pathogenesis of cystic fibrosis lung disease. Eur Respir J. 2004; 23(1):146–58. [PubMed: 14738247]
69. Sun X, Sui H, Fisher JT, et al. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. The Journal of clinical investigation. 2010; 120(9):3149–60. [PubMed: 20739752]
70. Rogers CS, Stoltz DA, Meyerholz DK, et al. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. sc. 2008; 321(5897):1837–41.

Highlights Box

- Since the cystic fibrosis gene was discovered in 1989, researchers have worked to develop a gene therapy as an ultimate cure.
- One of the most promising and enduring vectors is the adeno-associated virus (AAV), which has been shown to be safe in several clinical trials.
- A number of preclinical studies were performed to demonstrate the safety, efficacy of transduction, and long-term expression of AAV; all provide a solid foundation upon which to move toward a clinical therapy.
- An initial phase of clinical studies of AAV in CF patients were similar in that vector genomes were detected and in one instance there was functional rescue of CFTR function but no sustained clinical outcome was noted with first generation vectors.
- The limitations of initial AAV gene therapy studies are well known and strategies to surmount them are well underway.
- With many new AAV serotypes available and new promoters to drive CFTR expression the field of CF gene therapy is again at the forefront.