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REVIEW

Gene therapy for the treatment of cystic fibrosis

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Abstract: Gene therapy is being developed as a novel treatment for cystic fibrosis (CF), a condition that has hitherto been widely-researched yet for which no treatment exists that halts the progression of lung disease. Gene therapy involves the transfer of correct copies of cystic fibrosis transmembrane conductance regulator (*CFTR*) DNA to the epithelial cells in the airways. The cloning of the *CFTR* gene in 1989 led to proof-of-principle studies of *CFTR* gene transfer in vitro and in animal models. The earliest clinical trials in CF patients were conducted in 1993 and used viral and non-viral gene transfer agents in both the nasal and bronchial airway epithelium. To date, studies have focused largely on molecular or bioelectric (chloride secretion) outcome measures, many demonstrating evidence of CFTR expression, but few have attempted to achieve clinical efficacy. As CF is a lifelong disease, turnover of the airway epithelium necessitates repeat administration. To date, this has been difficult to achieve with viral gene transfer agents due to host recognition leading to loss of expression. The UK Cystic Fibrosis Gene Therapy Consortium (Imperial College London, University of Edinburgh and University of Oxford) is currently working on a large and ambitious program to establish the clinical benefits of CF gene therapy. Wave 1, which has reached the clinic, uses a non-viral vector. A single-dose safety trial is nearing completion and a multi-dose clinical trial is shortly due to start; this will be powered for clinically-relevant changes. Wave 2, more futuristically, will look at the potential of lentiviruses, which have long-lasting expression. This review will summarize the current status of translational research in CF gene therapy.

Keywords: cystic fibrosis transmembrane conductance regulator (CFTR) gene, gene expression, gene transfer agents (GTAs), outcome measures

Introduction

Cystic Fibrosis (CF) is an autosomal recessive, life-limiting disease resulting from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The gene is comprised of 27 exons and is situated on chromosome 7. The protein encoded by the *CFTR* gene is a cAMP-regulated chloride channel situated in the apical membrane of exocrine epithelial cells;[1] other processes with which it is involved include regulation of the epithelial sodium channel, and bicarbonate transport. There is conflicting evidence on its role in regulating the pH of intracellular organelles and the consequences on cellular processes such as sialylation and sulfation. In patients with CF, CFTR protein function may be abnormal due to a lack of production (Class 1 mutations), failure to reach its site of action due to misfolding (Class 2; commonest Caucasian defect is Phe508Del), defects in gating (Class 3), conductance (Class 4), abnormally low channel numbers (Class 5), or decreased half-life (Class 6).

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Whilst the CFTR protein is expressed in many internal organs, the major effect of such mutations is on the respiratory, gastrointestinal, and reproductive tracts, causing, in each of these sites, obstruction by thick, viscous secretions. Pulmonary disease leads to most of the morbidity associated with CF and is the cause of death in more than 90% of patients.[2] The correlation of the molecular defect with this multi-system clinical picture is complex and not entirely understood. It has been shown that CF airway epithelia have abnormally high rates of sodium (and thus water) absorption, which dehydrates the airway surface liquid and impairs mucus transport. More recently, vibrating culture, which may recapitulate the in vivo setting better than the conventional static culture model, has demonstrated that these processes are well preserved until a "second hit" in the form of viral infection occurs.[3] Once the airway surface becomes dehydrated, mucociliary clearance (MCC) mechanisms fail to remove any inhaled bacteria, which infect the lower airways and lead to inflammation. The CF inflammatory response is abnormal in several ways, being exaggerated,[4] prolonged[5] and, at least in chronic stages of infection, ineffectual.[6] The presence of inflammatory cell contents such as DNA and elastase in the airway further increase mucus viscosity and contribute to tissue breakdown.

The existing therapeutic modalities for CF lung disease focus mainly on mechanical clearance of airway secretions and the treatment of infection. Antibiotics are the mainstay of treatment; along with improved airway clearance techniques and nutrition, these have contributed to the greatly improved life expectancy and quality of life in recent decades. However, they are palliative measures and are fraught with difficulties of bacterial resistance, cumulative toxicity and their cumbersome and time-consuming nature for patients and families. Even today, more than 90% of patients die with advanced lung disease unless they receive a transplant. There is, therefore, a pressing need for the development of newer treatment approaches. One such approach is gene therapy which addresses the basic defect rather than the downstream consequences of the disease; along with small molecules directed at CFTR protein function,[7] such an approach, if successful, has the potential to impact significantly on the natural history of the disease.

Gene therapy, the transfer of copies of the normal *CFTR* gene to the relevant cells, should theoretically be well-suited to CF as: (1) it is a single-gene disorder; (2) heterozygotes are phenotypically normal, implying that levels do not have to reach those of wild-type; (3) the main target, the airways, is easily accessible via topical routes; and (4) the lungs are normal at birth, indicating a potential therapeutic window. However, in practice, the airways are in fact very difficult targets for gene therapy. The mucociliary escalator has evolved to keep foreign particles out. The layer of mucus that is required for normal ciliary function inhibits gene transfer per se and in CF this barrier is increased as secretions are excessive and abnormally viscous. The host immune response may also be problematic: with viral gene transfer agents, recognition of viral coat proteins and the production of neutralizing antibodies leads to problems with re-administration.[8] For non-viral gene transfer agents, the plasmid DNA which has been in common use is generally rich in unmethylated CpG dinucleotides, which are likely to be recognized by humans as foreign and thus produce an inflammatory response.[9,10]

Gene transfer agents

The transfection efficiency of naked DNA is so low that gene transfer agents (GTAs) have been designed to enhance entry to the cell/nucleus. These fall broadly into viral and non-viral categories, the latter usually lipid-based, but also including nanoparticles. The assumed innate capability of viruses to infect the respiratory tract made them a natural initial choice. Engineered adenovirus (Ad) was used in early CF clinical trials[11] but at high titers, some of these reported significant inflammatory responses and unacceptable toxicity; this problem has largely been overcome with the use of new generation viruses. However, in common with many other viruses, the receptor for Ad (CAR), rather than being conveniently located on the apical cell surface, is on the basolateral surfaces[12] and therefore relatively inaccessible from the airway lumen even in the presence of the inflammation characteristic of CF. Various agents, such as polidocanol[13] and perflurochemicals,[14] have been used to break down tight junctions and allow access to such receptors, although the safety and applicability of such approaches in humans remains to be demonstrated.

Adeno-associated virus (AAV) is a small single-stranded DNA member of the *Parvovirus* family. Owing to its lack of pathogenicity it is theoretically a good vector for gene transfer. However, its receptors are serotype-specific and many are scarce on the apical surface of the airway epithelium.[15] As it is a small virus, large genes such as *CFTR* may be difficult to package. Some researchers have attempted to create a functional *CFTR* "mini gene",[16] using techniques such as cutting the *CFTR* in half and using two complementary AAVs.

Sendai virus (SeV), a single-stranded RNA virus, belongs to the family of *Paramyxoviridae*. It is highly efficient at

transfecting airway epithelial cells due to the presence of sialic acid and cholesterol receptors on their apical surface.[17] Another advantage is its cytoplasmic expression, which removes the problem of the nuclear membrane barrier. High levels of gene expression have been reported but, in common with those viruses listed above, the major problem is with repeat administration; host immune responses, both cellular and humoral, may result from the primary administration and lead to reduced or absent gene expression after subsequent exposure. As CF is a lifelong disease, repeated administration is a required feature of any gene transfer agent targeting the superficial epithelium. The only possible way of avoiding this would be permanently (with an integrating vector) to transfect the stem cells of the airways;[18] such cells are difficult to reach, being buried beneath the surface and there are justifiable concerns over the use of integrating vectors, which have been shown to induce oncogenesis in gene therapy trials for severe immunodeficiencies.[19]

Lentiviruses are highly efficient gene transfer agents.[20,21] They have certain advantages over other gene transfer methods including the ability to transfect both dividing and non-dividing cells, and long-term stable expression; they also seem, almost uniquely amongst viruses, to be repeatedly administrable.[22] However, they naturally target hematopoietic cells and do not possess the surface proteins which recognize receptors on respiratory epithelia. To address this issue, our group, the UK CF Gene Therapy Consortium has recently generated a simian immunodeficiency virus (SIV) pseudotyped with Sendai viral F and HN proteins. This novel chimeric vector leads to high levels of transgene expression, is repeatedly administrable[23] and is the focus of our future work (see below).

Non-viral approaches include cationic liposomes, compacted DNA nanoparticles and naked DNA. Cationic liposomes consist of cationic lipids that are usually mixed with cholesterol and dioleoylphosphatidylethanolamine. When combined with DNA they form particles 100–500 nm in diameter which can penetrate cell membranes and enter cells. The complex of DNA and liposomes is resistant to nuclease degradation, thus improving the success rates of gene delivery. Flu-like inflammatory responses have been generated, thought largely to be due to the presence of unmethylated CpG dinucleotides on the plasmid DNA. Our Consortium has recently reported the development of a CpG-free plasmid,[24] shown in preclinical models to abrogate this inflammatory response. A further modification, replacing the CMV promoter with a humanized one, has also conferred a significantly increased duration of expression.

Which cells to target?

Maximal CFTR expression in non-CF airways occurs in the submucosal glands and in the surface epithelium of the distal small airways.[25,26] Topical application via inhalation is likely to target the surface epithelium, but is less likely to reach the deeper submucosal gland cells. Whether or not gene transfer to these cells will be necessary for clinical effect remains to be determined. Although there is increasing understanding of the stem cell populations at various levels throughout the airway, methods to target these cells, which are usually not accessible directly on the airway surface, and to achieve long-term expression are being explored, but have not yet reached the stage of clinical trials.[27]

Current status of clinical trials

Over 20 clinical trials have been reported to date. From these, summarized in Table 1, several common themes have emerged, which are outlined below.

The proof of principle of *CFTR* gene transfer has been confirmed

Trials have largely been designed around molecular or electrophysiological outcome measures; very few trials attempt to achieve (or measure) clinical benefit. However, levels of CFTR mRNA and protein are low in healthy, non-CF patients and currently available assays may not be sensitive enough to measure a clinically-beneficial level of gene transfer. In addition, because of the complex post-translational pathway required by CFTR, mRNA levels may in fact not correlate with levels of protein or degree of functional correction. Indeed, it is unknown how well either of these measures correlates with correction of the basic ion transport defect or with markers of clinical improvement. Correction of ion transport can be measured in vivo with transepithelial (nasal or bronchial mucosal) potential difference (PD).[29] The basal PD in patients with CF is classically more negative and demonstrates a greater response to amiloride, due to the greater absorption of positively charged sodium ions into the epithelia via the epithelial sodium channel. Most sensitively, the chloride secretory capacity of the CF epithelia is blunted (in response to low chloride solution and isoproteronol, a c-AMP agonist).[30,31] However, similar to the comments above concerning molecular correction, it is not completely clear how potential difference relates to clinical disease expression or how much change is necessary in which parameter(s) (chloride or sodium) to achieve clinical benefit. Interestingly, laboratory studies have demonstrated that changes in chloride secretion can be achieved following

Table 1 Previous published trials of gene therapy in cystic fibrosis patients

Author	Route of administration	Repeated dose (yes/no)	Safety concerns	Efficacy		
				Molecular	PD	Clinical
Viral vectors: adenovirus						
1. Zabner et al[35]	Nasal	No	No	mRNA-negative	Nasal PD: decrease baseline toward normal values	
2. Crystal et al[11]	Nasal lung	No	Yes, with highest lung dose	mRNA: some positive in nose, negative in lung	Nasal PD: inconclusive	
3. Knowles et al[36]	Nasal	No	Mild mucosal inflammation at highest dose		Nasal PD: no change	
4. Hay et al[37]	Nasal	No	No	CFTR mRNA: some positive	Nasal PD: partial correction Cl^- secretion	
5. Zabner et al[9]	Nasal	Yes	No		Nasal PD: partial correction of Cl^- secretion; effects reduced with subsequent doses	
6. Bellon et al[38]	Nasal lung	No	No	mRNA: some positive both routes		
7. Harvey et al[39]	Lung	Yes	No	mRNA: some positive, decreased with subsequent dose		
8. Zuckerman et al[40]	Lung	No	Flu-like symptoms at high dose	Vector DNA: positive on Day 4, transient		
9. Joseph et al[41] and Perricone et al[42]	Lung	No	Mild, nonspecific inflammatory response	mRNA; some positive		
Author	**Route**	**Repeated dose (yes/no)**	**Safety concerns**	**Efficacy**		
				mRNA	**PD**	**Clinical**
Viral vectors: adeno-associated virus (AAV)						
1. Wagner et al[43]	Sinus	No	No		Nasal PD: partial correction of Cl^- secretion	
2. Aitken et al[44]	Lung	No	No	CFTR mRNA: negative		
3. Wagner et al[45]	Sinus	No	No		Nasal PD: no change	Sinusitis – no change, histology, IL-8: no change L-10: increase
4. Flotte et al[46]	Nasal	No	No	*CFTR* DNA: some positive	No change	
5. Moss et al[47]	Lung	Yes	No	mRNA negative DNA: some positive		FEV_1, IL-8 and IL-10: trend towards improvement.
6. Moss et al[48]	Lung	Yes	No			FEV_1, sputum markers, requirement for antibiotics: no significant improvement
Non-viral vectors						
1. Caplen et al, DC-Chol/DOPE[49]	Nasal	No	No	CFTR mRNA: negative	Nasal PD: partial correction of Cl^- secretion	
2. Gill et al DC-Chol/DOPE[50]	Nasal	No	No		Nasal PD: partial correction Cl^- secretion	

3. Porteous et al DOTAP[51]	Nasal	No	No	mRNA: some positive	Nasal PD: partial correction Cl^- secretion up to 4 weeks	
4. Hyde et al DC-Chol/DOPE[52]	Nasal	Yes	No	CFTR mRNA: some positive	Nasal PD: partial Cl^- secretion	CFTR protein: some positive
5. Zabner et al GL67 versus naked pDNA[53]	Nasal	No	No		Nasal PD: partial correction of Cl^- secretion; no difference between vectors.	
6. Alton et al GL67[54]	Nasal lung	No	Flu-like symptoms	mRNA: negative	Bronchial PD: ~25% correction Cl^- secretion	Sputum: IL-8 and neutrophils reduced
7. Ruiz et al (GL67)[55]	Lung	No	Flu-like symptoms	CFTR mRNA: 4/8 positive		
8. Konstan et al vector: DNA nanoparticles[56]	Nasal	No	No	*CFTR* DNA: positive in all actively treated subjects; cross-contamination in placebo group	Nasal PD: partial to complete Cl^- secretion correction	

Abbreviations: DC-Chol, 3ß-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol; DOPE, dioleoylphosphatidylethanolamine; DOTAP, N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate; GL67, Genzyme lipid 67; PD, potential difference.

correction of a minority of cells, whereas correction of Na^+ hyperabsorption likely reflects normal CFTR function in almost 100% of cells.[32] This may explain why improvements in chloride secretion have been observed in the absence, in general, of reduced sodium hyperabsorption (which may in fact be confused electrophysiologically with changes arising from inflammation). Residual chloride secretion is seen in some patients with classic CF and has been linked, at least in some cohorts, to milder disease,[33] although this is not a consistent finding.[34] Encouragingly in this regard, clinical trials of the CFTR potentiator, VX-770, which appears largely to improve chloride ion transport, have led to significant improvements in lung function;[35] correction of Na^+ transport in addition to Cl^- may not be required for clinical benefit. As can be seen in Table 1, molecular and electrophysiological results have been variable but with both viral and non-viral approaches, the proof of principle of *CFTR* gene transfer has been confirmed on this basis.

Gene expression is reduced after subsequent doses of viral gene transfer agents

We regard the inability to dose repeatedly to be a major limitation of current viral strategies and the main reason for the UK CF Gene Therapy Consortium to focus its first wave of research on non-viral approaches.

Even non-viral approaches may induce an inflammatory response

This may largely reflect components of the plasmid DNA, although it is possible that lipid-based complexes may trigger an inflammatory reaction, perhaps when taken up by pulmonary macrophages. Clearly, a severe inflammatory response in the already inflamed CF airway is undesirable and dosing strategies including the concomitant use of anti-inflammatory agents are under consideration.

Evidence for clinical benefit is lacking to date

The majority of clinical trials have not been designed to detect clinical benefit. The few that have included clinical outcomes have reported variable results: a reduction in inflammatory markers has been reported in both the sinuses (AAV) and sputum (cationic liposomes); one trial with AAV reported small but significant improvements in lung function, but this effect was not repeatable in a subsequent, larger trial.

Current challenges for gene therapy

We believe that the current challenge is to move gene therapy from the proof-of-principle stage into one of clinical efficacy. To this end, the UK Cystic Fibrosis Gene Therapy Consortium was established several years ago. It brought together the three clinical research centers within the UK with previous CF gene therapy clinical trial experience: Oxford University, Edinburgh University and Imperial College London (and Royal Brompton Hospital). The consortium has two waves of research:

Wave 1

We sought to identify the best, currently available gene transfer agent that could be used in a trial designed to assess clinical benefit. On the basis that such clinical benefit would require a long duration of *CFTR* expression we assumed that this would require repeated administration and therefore, based on all data available both in-house and from other groups at that time, a non-viral gene transfer system was required. In addition, the optimal agent needed to be safe, nebulizable and manufacturable to good manufacturing practice standards. On the basis of laboratory and preclinical testing, the optimal non-viral vector was found to be Genzyme's GL67 (Genzyme Corporation, Cambridge, MA) which we have co-formulated (GL67A) with the neutral lipid dioleoylphosphatidylethanolamine, to facilitate pDNA endosomal escape, along with small amounts of a polyethylene glycol-containing lipid (DMPE-PEG5000) to aid stability. We are currently testing this, in combination with the Consortium's plasmid pGM169 (CpG-depleted, long-duration, as described above) in a single application, safety, and dose-finding study. Primary outcome is safety assessed by clinical assessment, serum inflammatory markers and lung function. We are also taking the opportunity to assess gene expression (mRNA) and function (upper and lower airway potential difference). Following this, we will look for clinical benefit in a repeated dose study. Outcome measures for gauging "clinical benefit" have been assessed in parallel in both interventional and observational settings.

Wave 2

This is the more experimental and futuristic focus of the Consortium. We are designing a body of work to develop pseudotyped lentiviruses, expressing the Sendai virus F (fusion) and HN (hemagglutinin neuraminidase) as a potentially highly efficient, long-lasting and repeatable gene transfer methodology.

Conclusion

To conclude, over the years since the *CFTR* gene was discovered, the proof of principle of gene transfer to the airway has been confirmed and partial correction in ion transport achieved. We are currently poised on the brink of a new era, understanding whether and to what extent this can translate into clinical benefit for patients with this disease.

Disclosure

The authors report no conflicts of interest in this work.

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