

Cystic Fibrosis Gene Therapy: Key Questions and Prospects

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Abstract: Cystic fibrosis is a monogenic disorder with significant morbidity and mortality, despite advances in conventional treatment. It is a good candidate for gene therapy and this field has progressed rapidly since the cystic fibrosis transmembrane conductance regulator gene was cloned. We will review the specific questions to address for successful cystic fibrosis gene therapy, such as the extra- and intracellular barriers to airway gene transfer, the target cells and the endpoints to assess efficacy. We will discuss recent advances in viral and nonviral gene transfer agents, delivery techniques and novel strategies to enhance airway gene transfer and expression.

Keywords: Cystic fibrosis, Gene therapy, Vectors

INTRODUCTION

Cystic fibrosis (CF) is the most common severe autosomal recessive disorder in the Caucasian population, with a frequency of 1 in 2,500 live births. It is caused by mutations in a gene on chromosome 7, cloned in 1989 and named cystic fibrosis transmembrane conductance regulator (*CFTR*) [1]. Since then, more than 1000 mutations have been identified that result in a wide spectrum of defects in the *CFTR* protein function. This protein is a cAMP-regulated chloride channel expressed at the apical membrane of epithelial cells in many organs. The clinical picture is dominated by dysfunctions of the respiratory and gastrointestinal tracts, related to impaired clearance and obstruction by viscous secretions. The pulmonary disease is the major cause of CF-associated morbidity and mortality and is marked by episodic exacerbations of pulmonary infection and inflammation leading to bronchiectasis and respiratory failure [2]. The pulmonary treatment so far is symptomatic, with courses of antibiotics and chest physical therapy. It is associated with pancreatic enzyme supplements and optimization of nutritional management. This comprehensive treatment program has clearly improved CF prognosis with a current median survival age of around 35 years, compared to less than 5 years in 1960. However, a treatment which addresses the underlying cause of the disease is eagerly awaited. Restoration of the wild type *CFTR* by gene therapy appears to be a realistic aim.

Cystic Fibrosis Gene Therapy: Hopes and Challenges

Gene therapy involves the introduction of a nucleic acid sequence into a cell to modify the expression of a gene in that cell. CF is theoretically an ideal candidate for gene therapy: it is due to a single gene defect; it is a recessive disease with heterozygotes being phenotypically normal; the

main pathology is in the respiratory tract, which seems to be accessible for treatment; and it is a progressive disease with a virtually normal phenotype at birth, offering a therapeutic window.

As plasmid DNA is a large and unstable molecule under *in vivo* conditions, gene delivery systems or "vectors" are necessary. The DNA must be compacted, protected from degradation and, in addition, the delivery systems should promote specific cellular uptake and hopefully efficient intracellular trafficking. For all gene therapy applications, the ideal vector system would have the following characteristics: an adequate carrying capacity; a low detection by the immune system and a low inflammatory effect; an efficiency high enough to correct the abnormal phenotype; and a long duration of expression and/or ability to be safely re-administered. Many different gene delivery systems have been developed. One strategy relies upon viral vectors because of their inherent ability to transport genetic material into cells and, in many instances, to deliver it to the nucleus for transcription. Another strategy relies on the development of chemical vectors able to compact the plasmid DNA through electrostatic interactions and to mimic key properties of viruses. Both strategies have been applied in CF gene therapy attempts.

Early expectations for the immediate success of CF gene therapy were supported by rapid demonstration of its feasibility: the *CFTR* gene was cloned in 1989 and within one year, *in vitro* studies demonstrated that introduction of *CFTR* complementary DNA into affected immortalized pancreatic cells using retroviral vectors could correct the chloride channel defect [3]. Similar results were obtained *in vivo* in CF knockout mice with cationic lipids [4]. However, efficient delivery of the *CFTR* gene to the relevant cells in humans has proven to be a difficult task. Since 1993, 30 clinical trials based on viral vectors (adenovirus and adeno-associated virus) and cationic lipids have been published (see <http://www.wiley.co.uk/wileychi/genmed/clinical>, Gene Therapy Clinical Trials Worldwide). Results have generally been disappointing, as gene transfer to human airway cells is much less effective than in rodents. Early human studies

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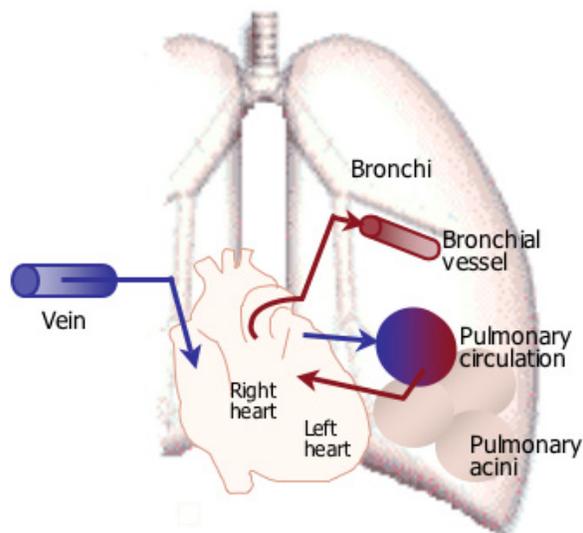


Fig. (1). Schematic drawing of mandatory routes followed by gene transfer systems after intravenous delivery to target the airways. After intravenous delivery, the gene transfer system follows the blood flow to the right side of the heart and to the pulmonary circulation. Most of gene transfer systems, particularly those positively charged are likely to become trapped in the alveolar capillaries. The neutral or negatively charged particles might be maintained longer in the circulation. If so, they continue to the left side of the heart and some will follow the blood flow to the bronchial circulation.

demonstrated some gene expression in upper and lower airway epithelia by immunocytochemistry and functional assays, and limited pulmonary toxicity. However, barriers to effective gene transfer therapy included poor efficiency, limited duration of gene expression and apparent significant immune and inflammatory responses to adenoviral vectors. During this decade of gene therapy studies, although the delivery systems that have been developed do not seem to be amenable to treat CF patients, there has been a tremendous amount of progress in the understanding of specific problems associated with CF gene therapy. We will review the key questions to address successful CF gene therapy in the future and discuss the various vector strategies that have been engineered in an attempt to answer them.

Topical or Systemic Delivery?

To target the airways, a topical delivery of the *CFTR* gene seems the most suitable approach. Indeed, CF gene therapy trials have all been based on this method. However, inhaled particles such as gene delivery vectors are promptly trapped by the thin layer of mucus covering the airway epithelium. They are then transported to the pharynx *via* airway mucociliary clearance mechanisms, driven by ciliary beating [5]. In CF, particularly at later stages of the disease, the situation is exacerbated by the presence of an abundant, thick and adhesive mucus containing inflammatory cells, cell debris, DNA and nucleases. Worries over deleterious actions of those nucleases have been put to rest by *in vitro* work showing that the plasmid DNA is in fact efficiently protected by the vector [6]. Beneath the mucus layer moving with ciliary actions, the apical surfaces of airway epithelial cells

are lined by cell surface-tethered mucins, glycolipids and proteoglycans called glycocalyx. Access to the epithelial cells is significantly harder for the gene transfer system in the presence of this mucus and glycocalyx [7, 8]. Pre-treatment with mucolytic agents, which are used in clinical practice to improve expectoration, or with neuraminidase, have been shown to increase transfection efficiency [9, 10] and could be a simple way to partly circumvent these highly efficient host defense mechanisms.

A systemic delivery based on intravenous injection may seem to be an attractive alternative option. The respiratory system has two circulatory systems, the pulmonary vasculature, which is devoted to gas exchange, and the bronchial vasculature, which supplies nutrients for the airways. However, using intravenous delivery, it is possible that the gene transfer system could be inactivated by circulating antibodies and/or complement factors [11]. If this does not occur, the vector will follow the blood flow to the right side of the heart and to the pulmonary circulation (Fig. 1). Most of gene transfer systems, particularly those positively charged are likely to become trapped in the alveolar capillaries. Gene transfer is thus achieved in alveolar endothelial cells and in some alveolar epithelial cells, but few airway cells are transfected [12-14]. The neutral or negatively charged particles might be maintained longer in the circulation. If so, they continue to the left side of the heart and some will follow the blood flow to the bronchial circulation which receives only 2% of the cardiac output from the left heart. It is obvious that only a limited amount of the injected gene transfer systems will reach the airways through this route. Moreover, once in the airways, the gene transfer systems would have to escape from the vessels and migrate through the extracellular matrix to reach the airway epithelial cells. However problematic this route appears, the feasibility has been established in rats with a vector consisting of polylysine linked to a peptide targeting the polymeric immunoglobulin receptor [15].

Which are the Target Cells?

The respiratory system can be divided into the respiratory tract including the conducting large and small airways, and the lung parenchyma where the gas exchange takes place. The human tracheobronchial tree that extends from the trachea through numerous divisions of airways is lined with a pseudostratified epithelium. It consists of several cell types, including ciliated and non-ciliated columnar cells, goblet (mucous) cells, serous cells and basal cells. The major source of mucus is the submucosal glands that are numerous beneath the epithelial surface in large airways. Secretions from serous and mucous cells in acini and tubules pass through collecting ducts and ciliated ducts before lining the surface of the airways. The bronchial epithelium eventually transitions to a bronchiolar one with a single layer of cuboidal ciliated cells and Clara cells [16].

In human large airways, *CFTR* is expressed in ciliated cells of the surface epithelium and at higher levels, in serous cells of the submucosal glands and in cells of the collecting ducts (Fig. 2). *CFTR* is also expressed in distal airways by a small population of nonciliated cells. Its expression in cells of the lung parenchyma is negligible [17, 18]. The

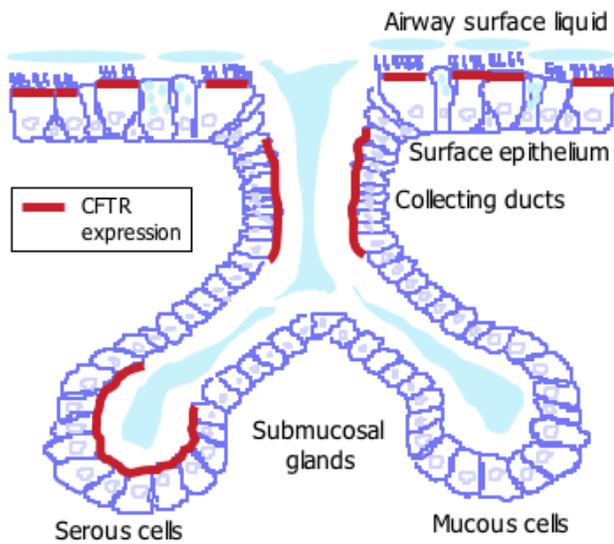


Fig. (2). Expression of CFTR in human large airways. The CFTR protein is expressed in ciliated cells of the surface epithelium, in serous cells of the submucosal glands and in cells of the collecting ducts.

expression pattern of normal CFTR suggests that CF gene therapy should target the airways. Although it is currently unclear which cell type should be the main target, it appears most appropriate to consider targeting CFTR expression to both surface epithelial cells and serous cells in submucosal glands. All those cells are highly differentiated and difficult to transfect, because differentiated cells have reduced uptake capacity [19-21]. One way to overcome this problem is to target receptors expressed at the apical surface of the airway epithelial cells. This approach has been successfully applied to adenovirus in well-differentiated airway epithelial cells *in*

vitro [22]: as coxsackie-adenovirus receptors, which are the natural adenovirus receptors, are lacking on the apical surface of airway epithelial cells, new adenoviruses are engineered to direct them to P2Y2-receptors, which are abundantly expressed on airway epithelial cells. The results suggested enhanced uptake of the modified adenovirus. Similar results were obtained with nonviral vectors coupled with a peptidic ligand [23] or carbohydrate moieties in airway ciliated cells and serous cells [21, 24].

What is the Path of the Therapeutic Gene ?

In most gene therapy studies for CF and other diseases, therapeutic gene expression does not reflect the amount of DNA/vector that have been taken up by the cells. Therefore, it has become a major aim to understand vector-cell interactions. As viruses have an inherent ability to deliver their genetic material to the nucleus for transcription, cellular entry of recombinant viruses has been extensively studied. Expression of receptors and coreceptors by the target cell and attachment of recombinant virus to these receptors has been the subject of many gene therapy studies. However, it is now understood that major rate-limiting steps occur during transduction of recombinant viruses, from receptor binding to nuclear uptake. These steps have been reviewed recently for recombinant adeno-associated virus [25].

Many studies have been devoted to unravelling the intracellular trafficking of plasmid DNA complexed with nonviral vectors. The two commonest nonviral vectors, cationic lipids [26] and polycations such as polylysine [27] and PEI [28, 29], share several features in their intracellular processing (Fig. 3). After uptake, they are enclosed in endosomal compartments that ultimately deliver their contents to lysosomes for degradation. Most of these vectors are able to cause endosomal disruption leading to plasmid release into the cytosol. There, the plasmid hardly diffuses

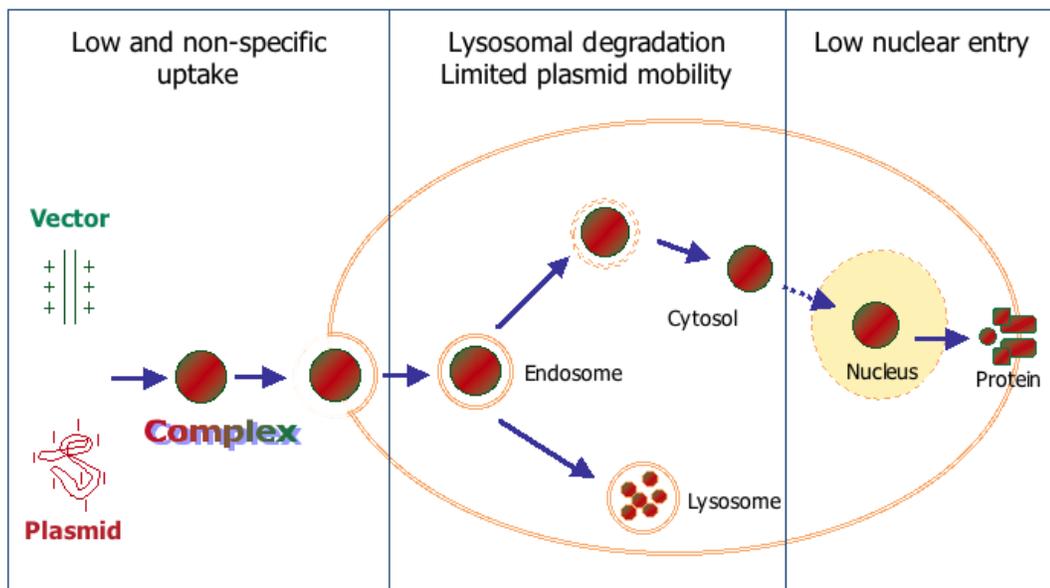


Fig. (3). Intracellular path of a therapeutic gene complexed with a synthetic vector. Plasmid DNA is condensed with the vector to form a cationic complex. The low and non-specific complex uptake might be overcome by targeting receptors expressed at the surface of airway epithelial cells. Most of the current vectors are able to cause endosomal disruption, therefore limiting lysosomal degradation. The low plasmid mobility in the cytosol and the lack of nuclear targeting remain two major barriers to an efficient plasmid expression.

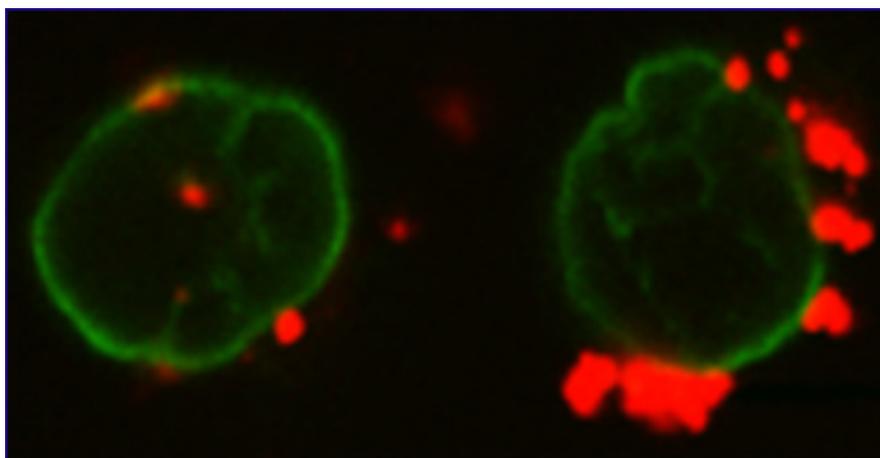


Fig. (4). Limited entry of complexes into the nucleus. Airway epithelial cells were incubated in the presence of biotinylated plasmid DNA complexed with lactosylated PEI. This vector is able to target the airway cells *via* its carbohydrate moieties and to escape from the endosomes by its "proton sponge effect". However, the nuclear membrane (which here was immunolabeled with anti-lamin A/C antibodies, followed by fluorescein conjugated antibodies and appears green) remains a major barrier to efficient gene transfer. Most of the plasmid (which was here labeled with rhodamine-labeled streptavidin and appears red) remains outside the nucleus.

and increasing its mobility by the use of microtubule-based motors is of potential interest [30]. Numerous studies have demonstrated that one of the major barriers remaining for the plasmid to cross and for gene therapists to overcome is the nuclear membrane [31] (Fig. 4). This barrier is largely eliminated in dividing cells, where the nuclear envelope disassembles during mitosis. However, the majority of airways cells are quiescent and despite numerous approaches to increase DNA nuclear import, there has been relatively little success so far. The use of peptides containing a nuclear localization signal (NLS) has proved to be controversial [32]. Other approaches that may be of interest exploit the glucocorticoid receptor [33] or the presence of carbohydrate-binding proteins, which may act as a shuttle between the cytosol and the nucleus [34]. The development of nanoparticles consisting of one molecule of DNA and 30-mer lysine polymers substituted with polyethylene glycol has given promising results. Their success is due to their small size and neutral charge density, which allow them to cross the nuclear membrane *via* the nuclear pores [35]. Although the intracellular trafficking of plasmid/nonviral vector complexes share several common features, it should be emphasized that the precise intracellular path of each complex varies with many parameters, including minor changes in the vector used and the size of complexes [36]. Therefore, the intracellular pathway of complexes made with a new nonviral vector cannot be assumed on the basis of previous studies with similar vectors, but requires thorough investigation.

How Much CFTR Expression is Required?

In vitro [37] and *in vivo* [38] studies have suggested that only a small fraction, perhaps 5-10% of CF bronchial epithelial cells need to be transfected to normalize chloride transport. However, this level of transfection had no effect on the increased sodium absorption, which is another key feature of CF ion transport abnormalities. Normalization of the increased sodium transport may require correction of nearly 100% of the affected cells [39]. In contrast with this

high percentage of transfected cells that is likely to be requested, several lines of evidence suggested that minimal levels of CFTR expression would be required to correct the ion channel defect. Respiratory epithelial cells appear to contain only 1 to 2 CFTR transcripts [40].

In order to ensure CFTR expression in appropriate cells under the control of the endogenous *CFTR* promoter, alternatives to traditional gene therapy are being developed. One example is gene repair in which only the faulty nucleotide is changed. Specific genomic sequences are targeted with small fragments of exogenous DNA that are homologous to the targeted endogenous DNA sequences except for the base pairs encoding the desired modification. Approaches using chimeroplasts (DNA/RNA hybrid oligonucleotides) [41] or small-fragment homologous recombination (SFHR) [42] have shown potential for genomic *CFTR* repair. However, these techniques are still highly inefficient, the mechanisms involved are poorly understood and it is currently uncertain if the required "repair" proteins are present in terminally differentiated airway epithelial cells.

RNA-repair strategies are also being developed (Fig. 5). Most messenger RNA precursor molecules undergo a maturation process called *cis*-splicing, during which their introns are excised and their exons joined using the spliceosome machinery. In contrast, *trans*-splicing is a process which joins two independently transcribed and excised mRNA molecules. Spliceosome-mediated RNA trans-splicing (SMaRT) is a method which can be used to correct endogenous CFTR mRNA transcripts, where the mutated region is excised and replaced with normal coding sequence [43]. However, at present, the efficiency and specificity of RNA repair vary considerably.

How to Make a Life-Long Cure?

CF is a life-long disease and it is clear that a life-long cure is needed. At present, with either integrating or non-integrating vectors, we only get to know how to target the

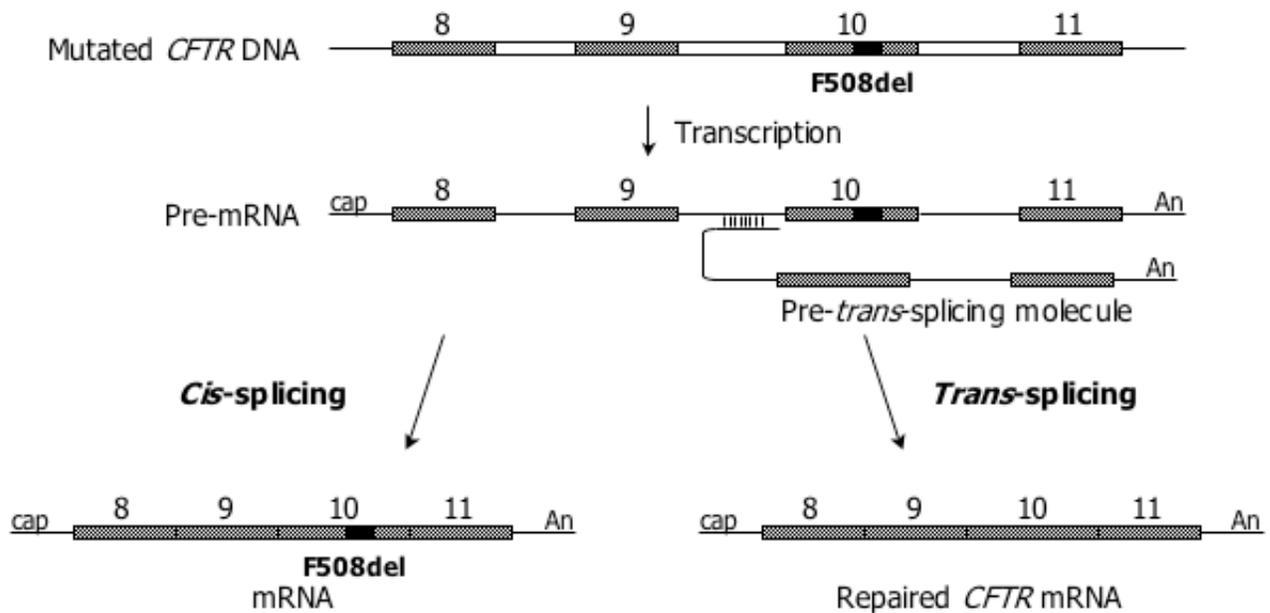


Fig. (5). Schematic representation of cis- and trans-splicing. The primary RNA transcript, pre-mRNA, containing both intron and exon sequences undergo a maturation process called *cis*-splicing during which the introns are excised and the exons joined using the spliceosome machinery. In contrast, in RNA *trans*-splicing, a pre-*trans*-splicing molecule which here in the case of *CFTR*, carries a normal tenth exon is linked through base-pairing to the ninth exon and thus generates a repaired full length *CFTR* mRNA.

differentiated airway cells. Therefore, the duration of *CFTR* expression after one administration is at the most the cell lifespan, i.e. about 120 days. This duration is rarely achieved and host immune responses initiated by viral vectors partly explain this short-term expression [44]. Cell-defense mechanisms might also take place, such as the cellular exit of complexes that has been recently described for nonviral polycationic vectors [36]. To ensure prolonged *CFTR* gene correction, the development of viral or nonviral vectors that allows repeat treatments is required.

A more attractive strategy would be to develop vectors with integrating properties that can target airway stem cells. These *CFTR* corrected stem cells would provide continuing *CFTR* gene expression in the various types of differentiated mature cells. Two vector types with integrating properties have been studied for CF application: modified adeno-associated viruses (AAV) and lentiviruses. AAV vectors are very promising gene transfer agents. However, they have a small packaging capacity. Moreover, it will probably be necessary to engineer the vector capsids for airway cell targeting, since suitable receptors are lacking on the apical surface of airway epithelial cells. In clinical trials, AAV vectors delivering the *CFTR* gene have shown satisfactory phase I safety profiles [45] and a moderate efficiency in one phase II trial [46]. While AAV has integrating properties, it was unclear in these clinical trials whether integration has occurred for recombinant AAV, and the ability of AAV to provide persistent gene expression in airway epithelium is doubtful. As with AAV vectors, lentiviral vectors are hampered by the lack of suitable receptors on the apical surface of airway epithelial cells. Recently, lentiviral vectors pseudotyped with modified Ebola envelope glycoprotein were shown both *in vitro* and *in vivo* in mice to be taken up when delivered apically and to transduce the airway

epithelium [47]. However, no clinical trials in CF have begun using lentiviruses vectors and there are clearly important safety aspects to consider, before an HIV-based virus pseudotyped with Ebola membrane proteins can be tested in human protocols. These safety issues with integrating vectors are further emphasized by the development of a leukemia syndrome in several young patients with severe combined immunodeficiency "cured" by gene therapy with a retroviral vector [48]. Ultimately, leukemia was discerned to be due to the viral vector producing insertional mutagenesis when incorporating into the patients' genome [49].

Even with integrating and safe vectors, the duration of *CFTR* expression would only last the lifetime of the transfected cell, which is quite low for differentiated airway cells. This problem has led to a great amount of work to identify the progenitor cells in the airways. These appear to be located in protected niches in murine tracheal submucosal gland ducts [50]. As there is still a long way to go before targeting airway stem cells becomes possible, other potential strategies are being developed. Murine embryonic stem cells have been reported to give rise *in vitro* to a fully differentiated airway epithelium [51]. Recently, CF adult stem cells from bone marrow stroma were transduced with a retroviral vector and were able to form a fully differentiated airway epithelium that showed apical chloride secretion in an *ex vivo* model [52]. These strategies for CF therapy require testing in *in vivo* models, but they offer new perspectives for CF cell therapy.

What are the Efficacy Endpoints? Who are the Patients?

Direct assays of *CFTR* gene transfer are one way to assess treatment efficacy. Theoretically, *CFTR* mRNA and quantification of *CFTR* protein are relevant, although their

detection does not necessarily mean functional correction. Moreover, both assays present technical difficulties well-known in the CF field [53]. Currently, the most important endpoint assay in phase I/II trials is the evaluation of CFTR chloride channel activity by transepithelial potential difference measurement. It is carried out in the nose of the patient in several laboratories. In the lower airways, it requires anesthesia and bronchoscopy. Chloride channel activity can also be measured using fluorescent chloride channel indicators in isolated airway cells harvested through brushing, but it is quite difficult to adapt this technique to primary cells.

Other efficacy endpoints are given by indirect assays of *CFTR* gene transfer. They are clearly needed because they would show an improvement in the lung disease. Development of such relevant clinical endpoints has been hampered by the lack of a good animal model for CF lung disease. Some clinical endpoints are currently being developed based on bacterial adherence, mucociliary clearance, mucus composition and inflammatory markers. The most relevant endpoint for successful CF gene therapy in later trial stages is a reduction in lung function decline over time. However, this endpoint requires long term follow-up of large patient numbers. Moreover, it implies that *CFTR* gene transfer is able to halt the lung disease evolution. It might not be the case in advanced lung disease in which CFTR activity is not likely to play a trigger role and which is characterized by an autonomous kinetics [54]. In most of the clinical trials aiming at evaluating novel vectors in CF, the patients were CF adults with advanced lung disease. They have generated very valuable information. In future CF gene therapy clinical trials, candidate patients should probably be as clinically presymptomatic as possible and endpoints, parameters indicative of prevention, such as a delay in the onset of bacterial colonization or a decrease in the frequency of bacterial proliferation episodes. This means that, rather than CF gene therapy, the goal should be preventive *CFTR* gene transfer [55].

In conclusion, CF gene therapy continues to offer great promise for the curative treatment of this life-threatening disease. Tremendous progress has been made in the understanding of the specificities of gene therapy applied to CF and novel strategies are being developed that will undoubtedly lead to meaningful results over the next decade.

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REFERENCES

- [1] Riordan JR, Rommens JM, Kerem B-S, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066-73.
- [2] Orenstein DM, Winnie GB, Altman H. Cystic fibrosis: a 2002 update. *J Pediatr* 2002; 140: 156-64.
- [3] Drumm ML, Pope HA, Cliff WH, *et al.* Correction of the cystic fibrosis defect *in vitro* by retrovirus-mediated gene transfer. *Cell* 1990; 62: 1227-33.
- [4] Alton EW, Middleton PG, Caplen NJ, *et al.* Non-invasive liposome-mediated gene delivery can correct the ion transport defect in cystic fibrosis mutant mice. *Nat Genet* 1993; 5: 135-42.
- [5] Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 2002; 109: 571-7.
- [6] Stern M, Caplen NJ, Browning JE, *et al.* The effect of mucolytic agents on gene transfer across a CF sputum barrier *in vitro*. *Gene Ther* 1998; 5: 91-8.
- [7] Kitson C, Angel B, Judd D, *et al.* The extra- and intracellular barriers to lipid and adenovirus-mediated pulmonary gene transfer in native sheep airway epithelium. *Gene Ther* 1999; 6: 534-46.
- [8] Stonebraker JR, Wagner D, Lefensty RW, *et al.* Glycocalyx restricts adenoviral vector access to apical receptors expressed on respiratory epithelium *in vitro* and *in vivo*: role for tethered mucins as barriers to luminal infection. *J Virol* 2004; 78: 13755-68.
- [9] Pickles RJ, Fahrner JA, Petrella JM, Boucher RC, Bergelson JM. Retargeting the coxsackievirus and adenovirus receptor to the apical surface of polarized epithelial cells reveals the glycocalyx as a barrier to adenovirus-mediated gene transfer. *J Virol* 2000; 74: 6050-7.
- [10] Ferrari S, Kitson C, Farley R, *et al.* Mucus altering agents as adjuncts for nonviral gene transfer to airway epithelium. *Gene Ther* 2001; 8: 1380-6.
- [11] Ogris M, Brunner S, Schuller S, Kircheis R, Wagner E. PEGylated DNA/transferrin-PEI complexes: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. *Gene Ther* 1999; 6: 595-605.
- [12] Zhu N, Liggitt D, Liu Y, Debs R. Systemic gene expression after intravenous DNA delivery into adult mice. *Science* 1993; 261: 209-11.
- [13] Song YK, Liu F, Chu S, Liu D. Characterization of cationic liposome-mediated gene transfer *in vivo* by intravenous administration. *Hum Gene Ther* 1997; 8: 1585-94.
- [14] Goula D, Benoist C, Mantero S, *et al.* Polyethylenimine-based intravenous delivery of transgenes to mouse lung. *Gene Ther* 1998; 5: 1291-5.
- [15] Ferkol T, Perales JC, Eckman E, *et al.* Gene transfer into the airway epithelium of animals by targeting the polymeric immunoglobulin receptor. *J Clin Invest* 1995; 95: 493-502.
- [16] Gail DB, Lenfant CJ. Cells of the lung: biology and clinical implications. *Am Rev Respir Dis* 1983; 127: 366-87.
- [17] Cliff WH, Frizzell RA. Separate Cl⁻-conductances activated by cAMP and Ca²⁺ in Cl⁻-secreting epithelial cells. *Proc Natl Acad Sci USA* 1990; 87: 4956-60.
- [18] Engelhardt JF, Yankaskas JR, Ernst SA, *et al.* Submucosal glands are the predominant site of CFTR expression in human bronchus. *Nat Genet* 1992; 2: 240-6.
- [19] Grubb BR, Pickles RJ, Ye H, *et al.* Inefficient gene transfer by adenovirus vector to cystic fibrosis airway epithelia of mice and humans. *Nature* 1994; 371: 802-6.
- [20] Fasbender A, Zabner J, Zeiher BG, Welsh MJ. A low rate of cell proliferation and reduced DNA uptake limit cationic lipid-mediated gene transfer to primary cultures of ciliated human airway epithelia. *Gene Ther* 1997; 4: 1173-80.
- [21] Fajac I, Thevenot G, Bedouet L, *et al.* Uptake of plasmid/glycosylated polymer complexes and gene transfer efficiency in differentiated airway epithelial cells. *J Gene Med* 2003; 5: 38-48.
- [22] Kreda SM, Pickles RJ, Lazarowski ER, Boucher RC. G-protein-coupled receptors as targets for gene transfer vectors using natural small-molecule ligands. *Nat Biotechnol* 2000; 18: 635-40.
- [23] Ziady AG, Kelley TJ, Milliken E, Ferkol T, Davis PB. Functional evidence of CFTR gene transfer in nasal epithelium of cystic fibrosis mice *in vivo* following luminal application of DNA complexes targeted to the serpin-enzyme complex receptor. *Mol Ther* 2002; 5: 413-9.
- [24] Fajac I, Briand P, Monsigny M, Midoux P. Sugar-mediated uptake of glycosylated polylysines and gene transfer into normal and cystic fibrosis airway epithelial cells. *Hum Gene Ther* 1999; 10: 395-406.
- [25] Ding W, Zhang L, Yan Z, Engelhardt JF. Intracellular trafficking of adeno-associated viral vectors. *Gene Ther* 2005; 12: 873-80.

- [26] Zabner J, Fasbender AJ, Moninger T, Poellinger KA, Welsh MJ. Cellular and molecular barriers to gene transfer by a cationic lipid. *J Biol Chem* 1995; 270: 18997-9007.
- [27] Grosse S, Tremeau-Bravard A, Aron Y, Briand P, Fajac I. Intracellular rate-limiting steps of gene transfer using glycosylated polylysines in cystic fibrosis airway epithelial cells. *Gene Ther* 2002; 9: 1000-7.
- [28] Godbey WT, Wu KK, Mikos AG. Tracking the intracellular path of poly(ethylenimine)/DNA complexes for gene delivery. *Proc Natl Acad Sci USA* 1999; 96: 5177-81.
- [29] Grosse S, Aron Y, Honore I, *et al.* Lactosylated polyethylenimine for gene transfer into airway epithelial cells: role of the sugar moiety in cell delivery and intracellular trafficking of the complexes. *J Gene Med* 2004; 6: 345-56.
- [30] Mesika A, Kiss V, Brumfeld V, Ghosh G, Reich Z. Enhanced intracellular mobility and nuclear accumulation of DNA plasmids associated with a karyophilic protein. *Hum Gene Ther* 2005; 16: 200-8.
- [31] Dean DA, Strong DD, Zimmer WE. Nuclear entry of nonviral vectors. *Gene Ther* 2005; 12: 881-90.
- [32] Escriou V, Carriere M, Scherman D, Wils P. NLS bioconjugates for targeting therapeutic genes to the nucleus. *Adv Drug Deliv Rev* 2003; 55: 295-306.
- [33] Rebuffat A, Bernasconi A, Ceppi M, *et al.* Selective enhancement of gene transfer by steroid-mediated gene delivery. *Nat Biotechnol* 2001; 19: 1155-61.
- [34] Monsigny M, Rondanino C, Duverger E, Fajac I, Roche AC. Glyco-dependent nuclear import of glycoproteins, glycoplexes and glycosylated plasmids. *Biochim Biophys Acta* 2004; 1673: 94-103.
- [35] Liu G, Li D, Pasumarthy MK, *et al.* Nanoparticles of compacted DNA transfect postmitotic cells. *J Biol Chem* 2003; 278: 32578-86.
- [36] Grosse S, Aron Y, Thevenot G, *et al.* Potocytosis and cellular exit of complexes as cellular pathways for gene delivery by polycations. *J Gene Med* 2005; 7: 1275-86.
- [37] Johnson LG, Olsen JC, Sarkadi B, *et al.* Efficiency of gene transfer for restoration of normal airway epithelial function in cystic fibrosis. *Nat Genet* 1992; 2: 21-5.
- [38] Dorin JR, Farley R, Webb S, *et al.* A demonstration using mouse models that successful gene therapy for cystic fibrosis requires only partial gene correction. *Gene Ther* 1996; 3: 797-801.
- [39] Johnson LG, Boyles SE, Wilson J, Boucher RC. Normalization of raised sodium absorption and raised calcium-mediated chloride secretion by adenovirus-mediated expression of cystic fibrosis transmembrane conductance regulator in primary human cystic fibrosis airway epithelial cells. *J Clin Invest* 1995; 95: 1377-82.
- [40] Trapnell BC, Chu CS, Paakko PK, *et al.* Expression of the cystic fibrosis transmembrane conductance regulator gene in the respiratory tract of normal individuals and individuals with cystic fibrosis. *Proc Natl Acad Sci USA* 1991; 88: 6565-9.
- [41] de Semir D, Petriz J, Avinyo A, *et al.* Non-viral vector-mediated uptake, distribution, and stability of chimeraplasts in human airway epithelial cells. *J Gene Med* 2002; 4: 308-22.
- [42] Goncz KK, Kunzelmann K, Xu Z, Gruenert DC. Targeted replacement of normal and mutant CFTR sequences in human airway epithelial cells using DNA fragments. *Hum Mol Genet* 1998; 7: 1913-9.
- [43] Liu X, Jiang Q, Mansfield SG, *et al.* Partial correction of endogenous DeltaF508 CFTR in human cystic fibrosis airway epithelia by spliceosome-mediated RNA trans-splicing. *Nat Biotechnol* 2002; 20: 47-52.
- [44] Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther* 2004; 15: 1157-66.
- [45] 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. *Hum Gene Ther* 2003; 14: 1079-88.
- [46] 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: 509-21.
- [47] Kobinger GP, Weiner DJ, Yu QC, Wilson JM. Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia *in vivo*. *Nat Biotechnol* 2001; 19: 225-30.
- [48] Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, *et al.* Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000; 288: 669-72.
- [49] Hacein-Bey-Abina S, Von Kalle C, Schmidt M, *et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; 302: 415-9.
- [50] Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, Randell SH. Evidence for stem-cell niches in the tracheal epithelium. *Am J Respir Cell Mol Biol* 2001; 24: 662-70.
- [51] Coraux C, Nawrocki-Raby B, Hinnrasky J, *et al.* Embryonic stem cells generate airway epithelial tissue. *Am J Respir Cell Mol Biol* 2005; 32: 87-92.
- [52] Wang G, Bunnell BA, Painter RG, *et al.* Adult stem cells from bone marrow stroma differentiate into airway epithelial cells: potential therapy for cystic fibrosis. *Proc Natl Acad Sci USA* 2005; 102: 186-91.
- [53] Griesenbach U, Boyd AC. Pre-clinical and clinical endpoint assays for cystic fibrosis gene therapy. *J Cyst Fibros* 2005; 4: 89-100.
- [54] Masciovecchio MV, Gabbarini J, Vega M, Drittanti L. The interactivity between the CFTR gene and cystic fibrosis would be limited to the initial phase of the disease. *Genet Med* 2000; 2: 124-30.
- [55] Drittanti L, Masciovecchio MV, Gabbarini J, Vega M. Cystic fibrosis: gene therapy or preventive gene transfer? *Gene Ther* 1997; 4: 1001-3.