Airway Epithelium Directed Gene Therapy for Cystic Fibrosis

April F. White and Selvarangan Ponnazhagan*

Department of Pathology, The University of Alabama at Birmingham, Birmingham, AL, USA

Abstract: Gene therapy is a promising therapeutic modality for the treatment of cystic fibrosis (CF). Despite a better understanding of the molecular organization of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and mutations resulting in pathophysiological and phenotypic alterations, several forms of treatments including gene therapy have failed to yield clinical success. Major limitations for the delivery of drugs and gene therapy vectors from reaching target cells in CF patients lie in physical and immunological barriers of airway epithelium. Over the last decade, non-viral and viral gene therapy approaches have been tested in preclinical studies and human clinical trials of CF. Outcomes of these studies have helped to identify hurdles that need to be overcome before such approaches can be routinely applied to patients. In addition to the physiological and immunological barriers of airway epithelium, vector transduction is also impaired by the absence or low-abundance of cellular receptors and co-receptors for viral binding and internalization. Thus, the initial enthusiasm for gene replacement therapy for CF following cloning of the *CFTR* gene dampened, as more limitations were recognized. Research directed towards improving the efficiency of gene transfer technology in CF, is focused on testing of compounds to enhance vector permeability and trafficking, identification and development of vectors which can transduce through alternate pathways, identification of airway epithelium-specific targeting ligands, and the identification of stem cells for combining cell therapy and gene therapy by *ex vivo* methods. Details provided in this article will give a comprehensive analysis of the prospects and limitations in CF gene therapy using viral and non-viral vectors.

Key Words: Airway epithelium, cystic fibrosis, gene therapy, viral vectors.

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disorder among Caucasians, affecting 1 in every 2500 live births [1]. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The *CFTR* gene encodes a chloride channel protein that is essential for regulating intracellular chloride concentrations in epithelial cells of the lungs, gastrointestinal tract and sweat glands [2]. Mutations in *CFTR* gene abolish normal protein function, causing mucus accumulation, chronic bacterial infections and inflammation. Lung function rapidly deteriorates and death from respiratory failure ensues in the second or third decade of life. Most of the currently available treatments for CF are palliative and no treatment exists for long-term correction of the disease pathology [3, 4]. Thus, newer therapies that can either phenotypically correct the disease or ameliorate severity of CF patho-physiology would greatly benefit patients suffering from this illness.

Among potential therapies for CF, gene therapy appears most promising for the following reasons: a) CF is a single gene defect and defective mutations are well characterized; b) the inheritance of the disease follows autosomal recessive pattern with heterozygotes being phenotypically normal, suggesting that gene dosage effects are not critical; c) the major target tissue with the greatest pathophysiological implication is the lung, which is easily accessible for treatment by non-surgical intervention; and d) it is a progressive disease with virtually normal phenotype at birth, thus offering a therapeutic window beginning from the neonatal period. Further, it has been clearly estimated that restoration of 5-10% of normal CFTR function can reverse the chloride channel defect in patients with CF [5].

Despite these advantages and the initial hope that CF gene therapy would progress rapidly, mainly due to the ease of non-invasive access to the lungs, successful delivery of the *CFTR* gene to the target tissue remained a difficult task due to pathological and physiological barriers and limitations inherent to gene therapy vectors.

BARRIERS TO LUNG GENE TRANSFER

Airway epithelial cells are present throughout the conducting airways of the lung, including the nasal, tracheal and bronchial regions. The cell composition in these compartments differs between species [6]. In humans, the airway epithelium is composed of ciliated, mucus-secreting goblet cells, serous cells, Clara cells, and basal cells. The luminal region of the airway is lined with pseudostratified, mucociliary epithelium, comprised of ciliated cells and goblet cells [7]. Generally, ciliated cells in both upper and lower airway regions are considered the main target cells for *CFTR* gene delivery. Recently, it has been identified that the luminal surface glycocalyx presents another barrier for vector transduction [8]. The glycocalyx on the airway epithelium luminal surface is composed of several families of carbohydrate-rich molecules including mucins, proteo-glycans and glycolipids [6]. Several mucin species present in

*Address correspondence to this author at the Department of Pathology, LHRB 513, 701, 19th Street South, University of Alabama at Birmingham, Birmingham, AL 35294-0007, USA; Tel: (205) 934-6731; Fax: (205) 975-9927; E-mail: sponnazh@path.uab.edu

1573-4064/06 $50.00+.00

the mucus in the airway lumen may prevent gene therapy vectors from reaching cell surface receptors [9].

Immunological host responses also result in a substantial reduction in the duration of gene expression *via* multiple mechanisms which ensure that initial and long-term gene expression is reduced or blocked [3, 10]. The first of these mechanisms involves airway macrophages that stimulate the host immune system as they phagocytose vector particles, and function as antigen-presenting cells. Further, induction of non-specific acute inflammatory responses during vector application results in the production of cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ which may downregulate many viral promoters and reduce gene expression in addition to vector clearance by innate immune response [11]. The cellular arm of the immune system can also be activated against the vector structural proteins *via* cytotoxic T-lymphocytes (CTL), resulting in a gradual elimination of vector-positive cells [12]. Since airway epithelial cells replenish once every few months, redosing of vector is also affected because of CTL activity on vector-tranduced cells.

GENE DELIVERY SYSTEMS

Adenovirus Vector

Adenoviral vectors (Ad) are highly efficient gene transfer vehicles and interestingly, wild-type Ad infects the respiratory epithelium during productive infection in humans. Nonetheless, application of recombinant Ad encoding a reporter or *CFTR* gene to human airway epithelium has only resulted in poor transduction. Several reasons account for the inadequacy of recombinant Ad in gene transfer to human airway epithelium and lung. Most notably, in contrast to wild-type Ad that only needs access to a small number of cells from which it can multiply and spread within the target tissue, a replication-defective recombinant (r) vector requires high efficiency transduction of a large number of viral particles to target tissues to achieve a therapeutic effect. Although *in vitro* studies using monolayer airway epithelial cell lines and differentiating cultures in semi-permeable air/liquid interface supports to induce polarization have shown remarkable transduction efficiency with Ad vectors and the correction of chloride channel defects [13], results from *in vivo* studies revealed apparent extracellular barriers to Ad infection on the human airway luminal surface that caused inefficient gene transfer. These include the mucociliary clearance system, the glycocalyceal barrier, the absence of adenoviral receptors on the airway lumen, and the slow rate of luminal endocytosis in airway epithelial cells [8, 14, 15]. In contrast, transduction of Ad through the basolateral surface successfully resulted in gene transfer to airway epithelial cells [16], and interestingly following transient disruption of epithelial tight junctions, transduction was also possible through the luminal surface [17].

From these studies, it was apparent that alternate strategies were needed to overcome the limitations of low efficiency Ad-mediated gene transfer *in vivo*. To date, two main strategies have been attempted to increase Ad gene transfer to airway epithelium. One approach involves retargeting of Ad to non-viral cellular receptors present on the apical surface of luminal epithelial cells and the other strategy attempts physical disruption of epithelial tight junctions using compounds to increase permeability and allow vector entry to the basolateral surface. Several surrogate receptors have been tested as alternate entry pathway for adenovirus [18]. However, retargeting Ad on airway epithelium requires identification of suitable receptors on the target cilitated cells. Studies in the last few years have attempted retargeting to airway epithelium using P2Y2 purinoreceptors, B2-kinin receptors, adenosine type 2b receptors, urokinase plasminogen activator receptor and the SEC-2 receptor [6, 19, 20]. Such retargeting of Ad has so far been achieved by chemical, immunological and genetic methods.

Transient disruption of epithelial tight junctions prior to vector application has been reported to facilitate vector permeation and improve exposure of Ad receptors on the apical surface. Pretreatment with the calcium chelator EGTA, short-chain fatty acid sodium caprate, and even hypotonic treatment including water, have been shown to significantly improve vector transduction in mouse models [16, 17, 21].

Adeno-Associated Virus Vectors

Adeno-associated virus vectors (AAV) are emerging as attractive therapeutic gene delivery vehicles for clinical gene therapy due to their non-pathogenicity and good safety profile. First identified in 1965 as contamination in adenovirus preparations [22], AAV has since been the subject of numerous studies undertaken to improve understanding of vector biology. However, it has only been in the last decade or so that the potential of AAV as a gene transfer vector has been recognized. In 1994, the Recombinant DNA Advisory Committee (RAC) reviewed the first AAV vector protocol and since then several clinical protocols have been approved involving rAAV [1, 23]. In view of their long-term gene expression *in vivo*, particularly in terminally differentiated cells, most of these have been directed to chronic diseases. The majority of clinical trials to date, including CF, have been conducted with vectors having serotype 2 capsid [24].

Interest in AAV for human gene therapy applications began with the realization of this vector's advantageous features of non-pathogenicity, broad tissue tropism, ability to transduce both dividing and nondividing cells, and long-term expression. Although AAV vectors possess a broad host range and were believed to transduce both dividing and non-dividing cells *in vitro* and *in vivo,* studies in the past several years have indicated significant variation in the transduction ability of AAV vectors. Most of our understanding on AAV has come from studies using AAV serotype 2. Recently, a number of new serotypes of AAV have been identified by screening human and non-human primate tissues for the presence of rescuable AAV genomes. These efforts have resulted in over 40 genomic variants [25, 26]. It appears that there are several major clades of related variants. Within these clades, many other individual variants appear to have been generated by recombination within the capsid coding sequence between different parental serotypes [27]. These serotypes use different cellular receptors for internalization based on the heterogeneity in amino-acid composition of the capsid proteins [28, 29]. A repertoire of serotype capsids with affinity to different primary attachment receptors

increases the potential application of AAV vectors in human gene therapy.

Among the viral vectors for CF gene therapy, the potential of AAV led to the initiation of several clinical trials, all with AAV2-based vectors [24]. Despite encouraging safety profiles in phase I and phase II trials, none of the primary endpoints of lung function changed significantly [30, 31]. A major limitation for this is the inability of efficient vector transduction to airway epithelial cells. Thus, it was apparent that molecular alterations to enhance AAV transduction to airway epithelium would advance their clinical utility.

Several lines of alternate approaches are being attempted with AAV vectors to achieve high-efficiency transduction of target tissues including airway epithelium. The identification and testing of newer AAV serotypes indicated that serotypes 5 and 6 were comparatively better than AAV2 in transducing airway epithelium. Although endpoint assays based on the quantitation of transgenic protein indicated significant increase using these vectors, increase in the percentage of cells transduced was not very high. Additional strategies to increase the permeability of AAV to the apical surface of airway epithelium using chemicals and a combination of transduction enhancing compounds, have resulted in significant augmentation of gene expression in airway epithelium [32-34]. Thus, it is apparent that further advancements in targeted transduction of AAV vectors to airway epithelium will greatly impact upon their clinical utility. Improvements to further increase the potential of AAV for CF gene therapy are aimed at overcoming the size constraints of AAV packaging of the *CFTR* gene, including truncation of the cDNA [35] and trans-splicing of a larger gene packaged in two different vectors following transduction [36-38].

Considering the pathophysiological barriers and lack of sufficient AAV receptors in airway epithelium, one approach will be to make molecular alterations to the AAV capsid to increase gene transfer to airway epithelium and lung. In fact, such modified vectors can be used in combination with permeability and transduction enhancing compounds to synergize increased transgene expression. Over the last few years, both genetic and conjugate approaches have been attempted with AAV vectors for targeted gene transfer. Conjugate based retargeting methods have employed high-affinity viral surface binding molecules such as monoclonal antibodies [39] and biotin-avidin bridges [40]. Although these approaches are generally not limited by capsid complexity, issues such as *in vivo* stability and toxicity, homogeneity of modified vector, and concerns of immunogenicity pose obstacles to therapeutic applications. A genetic or direct targeting approach, on the other hand, overcomes these limitations since cell-specific targeting of the vector by this method is mediated by a ligand that is directly inserted into the viral capsid. We have recently reported that a peptide ligand, identified by phage-display analysis on human airway epithelial cell line [41], significantly enhanced gene transfer to monolayer and polarized human airway epithelial cells when inserted in AAV2 capsid [42]. Further, this increment in transduction efficiency was found to occur independently of heparin sulfate proteoglycan receptor, the primary receptor for AAV2 infections.

RNA Virus Vectors

Although retroviral vectors were attractive for CF gene therapy because of their ability to integrate into the host cell genome for long-term expression, a main limitation for their use is the requirement for proliferating cells. The epithelial cell proliferation in human airways is extremely low, accounting for less than 0.2%. Advances in vector design and production, including the development of human and animal lentiviruses that transduce non-dividing cells, have raised hopes for retroviral approaches for the treatment of CF. Based on the fact that the human retrovirus, human immunodeficiency virus (HIV), was capable of infecting nondividing cells, a first replication defective lentivirus was created by Naldini and coworkers [43]. To increase the tropism of the lentiviral vector, chimeric vectors using glycoprotein envelopes from other viruses were subsequently generated [44]. Pseudotyping of the lentivirus surface with the glycoprotein envelope of vesticular stomatitis virus (VSV-G) has been shown to result in efficient and stable gene transfer to many organs *in vivo*. However, this pseudotyped vector was still inefficient in transducing differentiated airway epithelial cells *via* the apical surface, which was attributed to the lack of receptor for VSV-G in sufficient amounts on the apical surface of airway epithelium [45]. Kobinger *et al.* [46] tested surface glycoproteins from a number of unrelated viruses including rhabdovirus, oncoretrovirus, filovirus, orthomyxovirus, paramyxovirus, arenavirus, hep-DNA virus, and coronavirus. The results indicated maximum transduction efficiency when HIV vector was pseudotyped with the glycoprotein envelope from the Ebola Zaire (EboZ) filovirus. With this vector, high efficiency gene transfer was seen throughout the conducting airways and in cells lining the submucosal glands. Recently, domains have been identified within the EboZ envelope that confers high levels of gene transfer into the airway [47].

The murine parainfluenza virus type 1 (or Sendai virus [SeV]), the human respiratory syncytial virus (RSV) and the human parainfluenza virus type 3 (PIV3) have also been shown to efficiently transduce airway epithelial cells *via* the apical membrane using sialic acid and cholesterol, which are abundantly expressed on the apical surface of the airway epithelium [48, 49]. A first generation recombinant SeV carrying the *CFTR* gene was shown to produce functional CFTR chloride channels *in vitro* and in the nasal epithelium of CF knockout mice [48]. Further improvements in the SeV vectors have been made by deleting the F-protein from the viral backbone (ΔF), which rendered the second-generation viruses transmission-incompetent. Inoue *et al*. [50] further improved the ΔF/SeV vector by introducing mutations into the matrix and hemaglutinin-neuraminidase proteins.

Non-Viral Vectors

Non-viral vectors for CF gene therapy have the potential to avoid some of the critical problems observed with viral vectors, such as the immune response, limited packaging capacity, and random integration [51]. Three major non-viral vector systems are currently being used: cationic liposomes, DNA-polymer conjugates and naked DNA. To date, only cationic liposome-based systems have been tested in clinical trials of CF patients.

Cationic liposomes form large complexes in which their positively-charged side chains interact with DNA and the hydrophobic lipid portion of the liposome enhances fusion with the host cell membrane. Cationic liposomes that have been used successfully *in vivo* include DOTAP [1,2-bis (oleoyloxy)-3-(trimethylammonio)propane] [52], DC-Chol {3b[*N*-(*N', N'*-dimethylaminoethane)-carbamoyl]cholesterol} mixed with DOPE (dioleoyl phosphatidylehtanolamine) [53], and EDMPC (1,2-dimyristoyl-P-O-ethylphosphatidylcholine) mixed with cholesterol [54]. Clinical trials of liposome-mediated gene therapy in cystic fibrosis patients have demonstrated evidence of vector-specific CFTR expression with some functional changes towards normality, but this has been variable and at low levels [55]. Variation in end point measurement techniques, particularly potential difference assessment and vector-specific mRNA detection, makes comparisons between studies difficult [54, 56]. Although there was no evidence of an immune response or any reduction in effectiveness in a repeat dosing clinical trial [57], current levels of gene transfer efficiency are too low to result in clinical benefit [56]. Thus, further improvements are required to achieve the efficacy necessary to result in a cure for CF.

PRECLINICAL ANIMAL MODELS AND *EX VIVO* GENE THERAPY FOR CF

The availability of authentic animal models is a key requirement in developing gene therapy for CF. Currently, the CF mouse model is the only available animal model and although these mice do not develop the characteristic CF lung disease, they have the same ion transport defect as CF patients in their nasal epithelium. In addition, non-CF primates [58, 59], pigs [60], and sheep [61] have been used to optimize airway gene transfer and allow clinically relevant delivery methods, such as nebulization, to be assessed.

A noteworthy alternative for CF therapy is based on utilizing stem cells capable of proliferating into airway epithelium. Although research in this area is in the beginning stages, identification of stem and progenitor cells would allow the development of gene-modified cell-based therapy by *ex vivo* methods. Studies to date have indicated a close relationship between progenitor and stem cell phenotypes in the surface airway epithelia and submucosal glands, and thus it has been suggested that submucosal glands serve as a protective niche for surface airway epithelial stem cells [62]. Further, the pluripotent stem/progenitor cells that exist in the surface epithelium have the ability to differentiate into ciliated, secretory, intermediate and basal cells, and also have the developmental capacity to form submucosal glands. In addition to the identification of airway epithelial stem cells, significant effort is also being placed on utilizing embryonic stem cells and mesenchymal stem cells to generate airway epithelial tissue [63, 64]. Future developments in this area should provide alternate resources for the correction of CF, combining cell- and gene-based approaches.

CONCLUSION

Preclinical and clinical trials of gene therapy for CF have so far demonstrated proof of principle, but a major deficiency encountered has been low efficiency gene transfer, irrespective of vector system. Whereas viral vectors suffer from low abundance of receptors, accessibility through apical surface and immunological barriers of human airway epithelium, non-viral vectors are limited by inefficient nuclear delivery and duration of transgene expression. Recent studies to overcome these deficiencies are encouraging but are still far from attaining full potential for treating patients with CF. The next few years will likely provide exciting prospects and outcomes in this area.

ACKNOWLEDGEMENT

Financial support from the Cystic Fibrosis Foundation grant CFF-SORSCH98SO, and NIH grant AR050251 is greatly acknowledged.

REFERENCES

[1] Hildinger, M.; Auricchio, A. *Eur. J. Hum. Genet.,* **2004**, *12*, 263.
[2] Rowe, S.M.; Miller, S.; Sorscher, E.J. *N. Engl. J. Med.,* **2005**, *352*, 1992.
[3] Driskell, R.A.; Engelhardt, J.F. *Annu. Rev. Physiol.,* **2003**, *65*, 585.
[4] Gonçalves, M.A. *J. Virol.,* **2005**, *2*, 43.
[5] Johnson, L.G.; Boyles, S.E.; Wilson, J.; Boucher, R.C. *J. Clin. Invest*., **1995**, *95*, 1377.
[6] Pickles, R.J. *Proc. Am. Thor. Soc*., **2004**, *1*, 302.
[7] Randell, S.H. *Chest,* **1992**, *101*, 11S.
[8] Pickles, R.J.; Fahrner, J.; Petrella, J.; Boucher, R.; Bergelson, J. *J. Virol*., **2000**, *74*, 6050.
[9] Arcasoy, S.M.; Latoche, J.; Gondor, M.; Watkins, S.C.; Henderson, R.A.; Hughey, R.; Finn, O.J.; Pilewsky, J.M.; *Am. J. Respir. Cell. Mol. Biol.,* **1997**, *17*, 422.
[10] Ferrari, S.; Griesenbach, U.; Geddes, D.M.; Alton, E. *Clin. Exp. Immunol., **2003**, 132*, 1.
[11] Ritter, T.; Brandt, C.; Prosch, S.; Vergopoulos, A.; Vogt, K.; Kolls, J.; Volk, H.D. *Cytokine*, **2000**, *8*, 1163.
[12] Yang, Y., Su Q.; Wilson, J.M. *J. Virol*., **1996**, *70*, 7209.
[13] Johnson, L.G.; Pickles, R.J.; Boyels, S.E.; Morris, J.C.; Ye, H.; Zhou, Z.; Olsen, J.C.; Boucher, R.C. *Hum. Gene Ther.,* **1996**, *7*, 51.
[14] Pickles, R.J.; McCarty, D.; Matsui, H.; Hart, P.J.; Randell, S.H.; Boucher, R.C. *J. Virol.,* **1998**, *72*, 6014.
[15] Zabner, J.; Freimuth, P.; Puga, A.; Fabrega, A.; Welsh, M.J. *J. Clin. Invest.,* **1997**, *100*, 1144.
[16] Walters, R.W.; Grunst, T.; Bergelson, J.M.; Finberg, R.W.; Welsh, M.J.; Zabner J. *J. Biol. Chem.,* **1999**, *274*, 10219.
[17] Coyne, C.B.; Kelly, M.M.; Boucher, R.C.; Johnson, L.G. *Am. J. Resp. Cell Mol. Biol*., **2000**, *23*, 602.
[18] Gaden, F.; Franqueville, L.; Magnusson, M.K.; Hong, S.S.; Merten, M.D.; Lindholm, L.; Boulanger, P. *J. Virol*., **2004**, *78*, 7227.
[19] Kreda, S.M.; Pickles, R.J., Lazarowski, E.R.; Boucher, R.C. *Nat. Biotechnol.*, **2000**, *18*, 635.
[20] Drapkin, P.T.; O'Riordan, C.R., YI, S.M.; Chiorini, J.A.; Cardella, J.; Zabner, J.; Welsh, M.J. *J. Clin. Invest.*, **2000**, *105*, 589.
[21] Johnson, L.G.; Vanhook, M.K.; Coyne, C.B.; Haykal-Coates, N.; Gavett, S.H. *Hum. Gene Ther*., **2003**, *14*, 729.
[22] Atchison, R.W.; Casto, B.C.; Hammon, W.M. *Science*, **1965**, 149, 754.
[23] Athanasopoulos, T.; Graham, I.R.; Foster, H.; Dickson, G. *Gene Ther*., **2004**, *11*, S109.
[24] Carter, B.J. *Hum. Gene Ther.,* **2005**, *16*, 541.
[25] Gao, G.P.; Alvira, M.R.; Wang, L.; Calcedo, R.; Johnston, J.; Wilson, J.M. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 11854.
[26] Gao, G.; Alvira, M.R.; Somanathan, S.; Lu, Y.; Vandenberghe, L.H.; Rux, J.J.; Calcedo, R.; Sanmiguel, J.; Abbas, Z.; Wilson, J.M. *Proc. Natl. Acad. Sci. USA,* **2003**, *100***,** 6081.
[27] Gao, G.; Vandenberghe, L.H.; Alvira, M.R.; Lu, Y.; Calcedo, R.; Zhou, X.; Wilson, J.M. *J. Virol.,* **2004***, 78*, 6381.
[28] Buning, H.; Ried, M.; Perabo, L.; Gerner, F.; Huttner, N.; Enssle, J.; Hallek, M. *Gene Ther.,* **2003**, *10*, 1142.
[29] Buning, H.; Braun-Falco, M.; Hallek, M. *Cells Tissues Org.,* **2004**, *177*, 139.

[30] Aitken, M.L.; Moss, R.B.; Waltz, D.A.; Dovey, M.E.; Tonelli, M.R.; McNamara, S.C.; Gibson, R.L.; Ramsey, B.W.; Carter, B.J.; Reynolds, T.C. *Hum. Gene Ther.,* **2001**, *12*, 1907.

[31] Wagner, J,A.; Moran, M.L.; Messner, A.H.; Daifuku, R.; Conrad, C.K.; Reynolds, T.; Guggino, W.B.; Moss, R.B.; Carter, B.J.; Wine, J.J.; Flotte, T.R.; Gardner, P. *Hum. Gene Ther.,* **1998**a, *9*, 889.

[32] Duan, D.; Yue, Y.; Yan, Z.;Yang, J.; Engelhardt, J.F. *J. Clin. Invest.,* **2000**, *105*, 1573.

[33] Ding, W.; Yan, Z.; Zak, R.; Saavedra, M.; Rodman, D.M.; Engelhardt, J.F. *J. Virol.,* **2003**, *77*, 7361.

[34] Yan, Z.; Zak, R.; Zhang, Y.; Ding, W.; Godwin, S.; Munson, K.; Peluso, R.; Englehardt, J. *J. Virol.,* **2004**, *78***,** 2861.

[35] Ostedgaard, L.S.; Rokhlina, T.; Karp, P.H.; Lashmit, P.; Afione, S.; Schmidt, M.; Zabner, J.; Stinski, M.F.; Chiorini, J.A.; Welsh, M.J. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*, 2952.

[36] Nakai, H.; Storm, T.A.; Kay, M.A. *Nat. Biotechnol.,* 2000, *18***,** 527.

[37] Sun, L.; Li, J.; Xiao, X. *Nat. Med.,* **2000**, *6,* 599.

[38] Yan, Z.; Zhang, Y.; Duan, D.; Engelhardt, J.F. *Proc. Natl. Acad. Sci. USA,* **2000**, *97***,** 6716.

[39] Bartlett, J.S.; Kleinschmidt, J.; Boucher, R.C.; Samulski, R.J., *Nat. Biotechnol.,* 1999, *17*, 181.

[40] Ponnazhagan, S.; Mahendra, G.; Kumar, S.; Thompson, J.; Castillas, M. *J. Virol*., **2002**, *78*, 12900.

[41] Jost, P.J.; Harbottle, R.P.; Knight, A.; Miller, A.D.; Coutelle, C.; Schneider, H. *FEBS Lett*., **2001***, 489***,** 263.

[42] White A.F, Mazur, M.; Sorscher, E.J.; Ponnazhagan, S. *Mol. Ther.,* **2005**, *11*, S197.

[43] Naldini, L.; Blomer, U.; Gallay, P.; Ory, D.; Mulligan, R.; Gage, F.H.; Verma, I.M.; Trono, D. *Science*, **1996**, *272*, 263.

[44] Wilson, J.M. *Proc. Am. Thorac. Soc*., **2004**, *1*, 309.

[45] Goldman, M.J.; Lee, P.S.; Yang, J.S.; Wilson, J.M. *Hum. Gene Ther*., **1997**, *8*, 2261.

[46] Kobinger, G.P.; Weiner, D.J.; Yu, D.C.; Wilson, J.M. *Nat. Biotechnol*., **2001**, *19*, 225.

[47] Medina, M.F.; Kobinger, G.P.; Rux, J.; Gasmi, M.; Looney, D.J.; Bates, P.; Wilson, J.M. *Mol. Ther*., **2003**, *8*, 777.

[48] Ferrari, S.; Farley, R.; Munkonge, F.; Griesenbach, U.; Smith, S.N.; You, J.; Tokusumi, T.; Iida, A.; Wainwright, B.; Gray, M.; Wright, A.; Verdon, B.; Argent, B.; Geddes, D.M.; Hasegawa, M.; Alton, E.W.F.W. *Mol. Ther*., **2003**, *7*, S38.

[49] Zhang, L.; Pepples, M.; Collins, P.; Pickles, R. *Mol. Ther.*, **2003**, *7*, S36.

[50] Inoue, M.; Tokusumi, Y.; Ban, H.; Kanaya, T.; Shirakura, M.; Tokusumi, T.; Hirata, T.; Nagai, Y.; Iida, A.; Hasegawa, M. *J. Virol*., **2003**, *77*, 6419.

[51] Ratko, T.A.; Cummings, J.P.; Blebea, J.; Matuszewski, K.A. *Am. J. Med.*, **2003**, *115*, 560.

[52] Leventis, R.; Silvius, J.R. *Biochim. Biophys. Acta,* **1990**, *1023*, 124.

[53] Gao, X.; Huang, L. *Biochem. Biophys. Res. Commun*., **1991**, *179*, 280.

[54] Noone, P.G.; Hohneker, K.W.; Zhou, Z.; Johnson, L.G.; Foy, C.; Gipson, C.; Jones, K.; Noah, T.L.; Leigh, M.W.; Schwartzbach, C.; Efthimiou, J.; Pearlman, R.; Boucher, RC.; Knowles, M.R. *Mol. Ther*., **2000**, *1*, 105.

[55] Lee, T.W.R.; Matthews, D.A.; Blair, G.E. *Biochem. J*., **2005**, *387*, 1.

[56] Ferrari, S.; Geddes, D.M.; Alton, E.W.F.W. *Adv. Drug Deliv. Rev.*, **2002**, 54, 1373.

[57] Hyde, S.C.; Southern, K.W.; Gileadi, U.; Fitzjohn, E.M.; Mofford, K.A.; Waddell, B.E.; Gooi, H.C.; Goddard, C.A.; Hannavy, K.; Smyth, S.E.; Egan, J.J.; Sorgi, F.L.; Huang, L.; Cuthbert, A.W.; Evans, M.J.; Colledge, W.H.; Higgins, C.F.; Webb, A.K.; Gill, D.R. *Gene Ther*., **2000**, *7*, 1156.

[58] Beck, S.E.; Laube, B.L.; Barberena, C.I.; Fischer, A.C.; Adams, R.J.; Chesnut, K.; Flotte, T.R.; Gugginoet, W.B. *Mol. Ther*., **2002**, *6*, 546.

[59] Lerondel, S.; Vecellio None, L.; Faure, L.; Sizaret, P.-Y.; Sene, C.; Pavirani, A.; Diot, P.; Le Pape, A. *J. Aerosol. Med.,* **2001**, *14*, 95.

[60] Cunningham, S.; Meng, Q.-H.; Klein, N.; McAnulty, R.J.; Hart, S.L. *J. Gene Med*., **2002**, *4*, 438.

[61] Emerson, M**.;** Renwick, L.; Tate, S.; Rhind, S.; Milne, E.; Painter, H.; Boyd, A.C.; McLachlan, G.; Griesenbach, U.; Cheng, S.H.; Gill, D.R.; Hyde, S.C.; Baker, A.; Alton, E.W.; Porteous, D.J.; Collie, D.D.S. *Mol. Ther.*, **2003**, *8*, 646.

[62] Liu, X.; Driskell, R.R.; Engelhardt, J.F. *Curr. Top. Dev. Biol.*, **2004**, *64*, 33056.

[63] Coraux, C.; Nawrocki-Raby, B.; Hinnrasky, J.; Kileztky, C.; Gaillard, D.; Dani, C.; Puchelle, E. *Am. J. Respir. Cell. Mol. Biol*., **2005**, *32*, 87.

[64] Wang, G.; Bunnell, B.A.; Painter, R.G.; Blesilda, C.; Quiniones, B.C.; Tom, S.; Lanson, N.A.; Spees, J.L.; Bertucci, D.; Peister, A.; Weiss, D.J.; Valentine, V.G.; Prockop, D.J.; Kolls, J.K. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 186.

Received: 09 January, 2006 Revised: 23 March, 2006 Accepted: 24 March, 2006