CURRENT AND FUTURE APPLICATIONS OF GENE THERAPY IN RESPIRATORY DISEASE*

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The first clinical trial of gene therapy was approved in 1989; it involved the transfer of human cDNA encoding adenosine deaminase to a patient with severe combined immune deficiency (SCID).[1] The demonstration of the therapeutic potential of this technology has inspired enormous (often unfounded) expectation, particularly for the treatment of other autosomal recessive disorders such as cystic fibrosis (CF).[2] It is hardly surprising that gene therapy has not kept pace with the heady optimism which heralded its arrival;[3-5] nevertheless, in the ensuing decade, a gene therapy approach has been taken in several thousands of patients with various clinical conditions.[6] Collective experience gathered over this period, coupled with technological advances in the laboratory and the potential power and specificity of this therapeutic approach, suggests that gene therapy will increasingly pervade the clinical domain. This is especially true in relation to the respiratory tract which is particularly suited to gene therapy; the nose and sinuses are readily accessible and even the constituents of the pulmonary gas exchange membranes - the alveolar epithelium and the capillary endothelium - are approachable via airway instillation or intravenous infusion, respectively. For these reasons the lung has been the target organ in many landmark studies in gene therapy.[7]

The purpose of this article is to outline the basic principles of gene therapy, to illustrate the pre-clinical and clinical trials using gene therapy administered via the nose, sinuses, lung and pleural cavity, and to provide insights into the therapeutic applications that can be expected as a result of important recent research developments.

PRINCIPLES OF GENE THERAPY

Gene therapy may be loosely defined as the transfer of genetic material to effect a desired biological response. This usually entails transfer of chosen cDNA (or genomic DNA), under the control of an appropriate promoter, into host cells. Once inside the cell, the host's transcriptional and translational apparatus effects production of the desired protein (Figure 1). An alternative strategy is to introduce engineered nucleic acids which specifically bind host transcripts preventing protein production (antisense oligonucleotides), or bind and enzymatically cleave host transcripts (ribozymes).

Successful transfer of therapeutic genetic material to a cell of interest requires that the genetic material reaches the target cell and then navigates the cell membrane, cytoplasm and nuclear membrane. In general this requires packaging of genetic material into 'vectors' designed to overcome these barriers (Figure 1); unpackaged, or 'naked' DNA plasmids can cross cell membranes but the efficiency of such transfer is generally low.

Vectors are traditionally classified as viral or non-viral (Table 1). Non-viral vectors principally comprise cationic liposomes, which are effectively cationic lipid bilayers capable of enveloping (anionic) plasmid DNA.[8] Liposomes can incorporate into cell membranes, liberating their DNA content into the cytoplasm. Liposomes have found favour in many quarters because they are relatively easy to produce and administer, and they do not carry the theoretical risks of oncogenesis or superinfection that may accompany the use of some viruses.[8] Furthermore, liposomes have traditionally been considered biologically inert, and thus amenable to repeated administration without inducing inflammatory or immune responses, although this position has been challenged recently.[9] A major limitation of liposome vectors is that the vast majority of the nucleic acid carried into cells is directed into, and degraded by, endosomes before reaching the nucleus. In addition, the small proportion of DNA reaching the nucleus is usually only transiently and weakly expressed.[8] Finally, in the context of the lung, effective dispersal of liposomes throughout the bronchial tree is a daunting practical prospect.

In contrast, viral vectors make use of the sophisticated machinery employed by viruses specifically to deliver DNA to host nuclei. An increasing array of viruses is available for gene therapy (Table 1), with certain vectors suited to particular clinical applications (for example adeno-associated viruses efficiently target hepatocytes, while Herpes simplex viruses efficiently transfect targets in the nervous system).[10-12] However, most experience to date has been obtained with retroviruses and adenoviruses.

Retroviruses are potentially important vectors for gene therapy because viral DNA is integrated into the host genome, resulting in stable transfection thus circumventing the need for re-administration.[3,13] Unfortunately, large-scale production of retrovirus for therapeutic purposes is technically difficult, although improvements are emerging.[14] In addition, retroviruses are critically dependent upon cell division for transfection - this favours the use of retrovirus in the setting of malignant disease, where indeed it has found its principal utility in gene therapy, but largely precludes its use in the surface bronchial epithelium which is characterised by terminal differentiation and a low rate of cell division.

Adenoviruses, on the other hand, efficiently transfect non-dividing cells and have a natural tropism for respiratory

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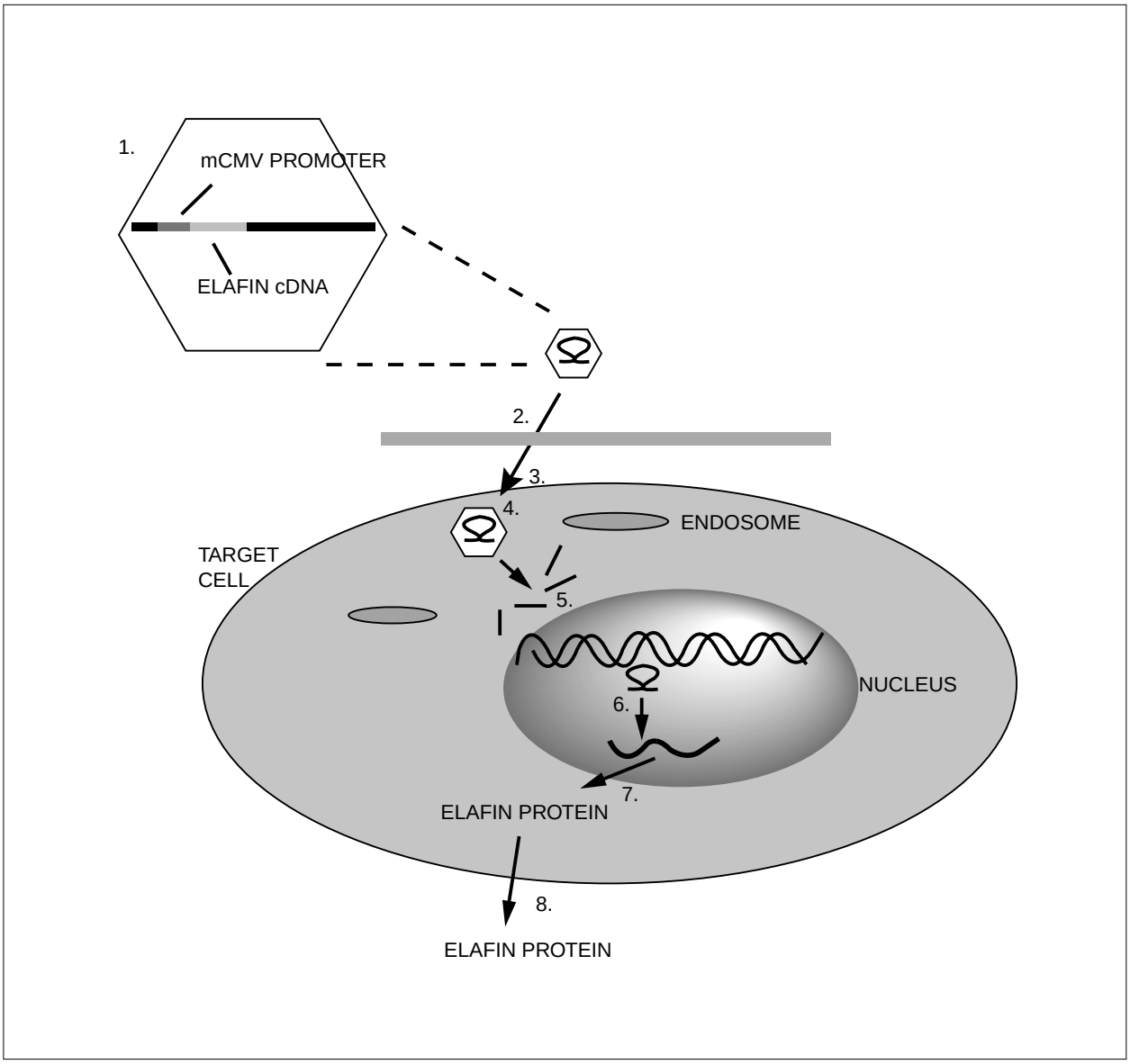

FIGURE 1
Principles of successful gene therapy.
The transgene of choice (in this case human elafin* cDNA under the control of the murine cytomegalovirus (mCMV) promoter)[16] is packaged within a vector (in this example a replication-deficient adenovirus) (1). The vector must overcome physicochemical barriers such as mucus (2) and access the target cell, where it must penetrate the cell membrane (3), traverse the cytoplasm evading endosomal degradation (4), access the nuclear membrane, and disassemble, allowing DNA to enter the nucleus (5). The DNA (in this case epichromosomal) must then be transcribed to mRNA (6), before translation to protein (7). Protein is then secreted from the cell into the appropriate milieu or incorporated into the cell membranes (8).
*elafin (elastase specific inhibitor) is an endogenous inhibitor of human neutrophil elastase, and comprises part of the lung's 'anti-elastase shield'.

epithelium making them ideal candidates for gene delivery to the lung.[15] Adenoviruses can efficiently transfect cells of both the proximal and distal airways both *in vitro* and *in vivo* and once inside host cells, they efficiently access the nuclear membrane, and liberate their genetic material.[16] Techniques have been devised to yield extremely high titres of adenovirus rendered incapable of replication, but capable of efficiently transfecting human cells. These techniques involve removing the only region of the adenoviral genome absolutely required for viral replication (the E1 region). Deletion of E1 also makes space for therapeutic transgene to be inserted.[17] Generation of sufficient virus to allow transfection of organs in large mammals such as man, along with high levels of transgene expression, have made adenoviruses extremely attractive therapeutic tools.[15]

By far the biggest problem facing adenoviral gene therapy is the host immune response it evokes.[15] Adenoviral DNA adopts an epichromosomal, non-integrated, location in the host nucleus, making transfection short-lived which means that re-administration is necessary for long-term therapy. Both cellular and humoral immunity present hurdles to the effective administration and re-administration of adenovirus, principally in the form of MHC Class I restricted cytotoxic T lymphocytes which eliminate host cells

TABLE 1
The principle vectors used in gene therapy.

Non-viral	Viral
Cationic liposomes*	Adenovirus*
Ligand-DNA conjugates	Retroviruses*
	Adeno-associated virus (AAV)*
	Herpes simplex virus
	Vaccinia virus
	Sendai virus
	Polio virus

*denotes those vectors which have been employed in clinical trials in the lung. DNA has also occasionally been administered in clinical trials in the absence of a vector, in 'naked' plasmid form.

transfected with virus, and neutralising antibodies which eliminate virus entering the airways.[18-23] In addition, a range of other immune mechanisms are activated, against both the virus and the transgene.[18,24,25] Overcoming these problems remains the biggest challenge facing adenoviral gene therapy, an issue returned to later in this article. Other theoretical concerns surround adenoviral gene therapy (for example adenoviruses can disrupt the cell cycle in human cells; they may cause fulminant pneumonia and decline in lung function in immunocompromised hosts; replication competent adenoviruses may be generated during laboratory production of virus; and recombination could occur in hosts harbouring E1 from previous wild type infection).[26-30] However these concerns have largely not been realised in practice, and adenoviral gene therapy has generally proved safe to date, both in gene therapy protocols and in large-scale vaccination programmes.[9,15]

The following section reviews the progress made in clinical gene therapy for respiratory diseases in recent years. A few caveats are worth bearing in mind when considering this information. Firstly, respiratory gene therapy remains in its infancy, and as such many of the studies described represent feasibility studies rather than full scale attempts to reverse a disease phenotype. Secondly, gene therapy protocols are devised for diseases for which no known cure exists, which are often at an advanced stage when gene therapy is administered (for example CF and lung cancer), and it could thus be argued that the odds are stacked against effecting meaningful phenotypic correction.[7] Finally, the examples shown illustrate the evolving utility of gene therapy, which was originally developed to provide definitive genetic replacement in monogenic disorders, but which is rapidly being turned towards diseases of increasingly complex genetic aetiology.

CLINICAL TRIALS OF GENE THERAPY IN THE NOSE, SINUSES, LUNG AND PLEURA

Cystic fibrosis

Cystic fibrosis is the commonest fatal autosomal recessive disorder in the Western world. The principal burden of morbidity and mortality in CF is attributable to lung disease characterised by thick, inspissated mucus, colonisation with bacterial pathogens, bronchiectasis and progression to respiratory failure.[31] The underlying defect consists of homozygous mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR),[32,33] which is thought to play a critical role in chloride ion transport across pulmonary epithelium, and which may have other important functions.[31] CF became an obvious target for gene therapy because the genetic defect is well characterised, because the organ bearing the most important burden of disease is readily accessible, and because of the high morbidity and inevitable early mortality attributable to the disease. Furthermore, the development of CFTR 'knock-out' mouse models has generated an abundance of important pre-clinical information.[34-36]

Four studies have reported the administration of adenovirus containing the CFTR gene to the nasal cavity of patients with CF,[37-40] and a further two the administration to both nose and lung.[41,42]

In each of the nasal studies, some (but not all) of the patients studied showed evidence of gene transfer as evidenced by the polymerase chain reaction. More importantly, in three of the studies, evidence of partial functional correction was obtained, as measured by electrophysiological changes in membrane potential.[37-39] These three studies therefore provide proof of principle for the functional transfer of CFTR to the human CF epithelium *in vivo*. However, the fourth study did not find evidence of electrophysiological correction.[40]

No viral shedding (and thus, by implication, no replication-competent virus) was found in any of the studies. However one study (using the highest dose of virus) described nasal inflammation and irritation,[40] and inflammation also arose after repeated administration of adenovirus in the only study administering more than one dose.[38] Interestingly, partial functional correction did occur after multiple administration, but there was strong circumstantial evidence to suggest that inflammation hampered functional improvement.[38]

Four published controlled trials have studied nasal administration of CFTR with cationic liposomes.[43-46] No evidence of vector-induced inflammation could be found in three of the trials, although nasal administration of vector or liposome alone was associated with a rise in white blood count in the fourth.[46] Successful gene transfer was demonstrated in all of these trials, though curiously one study described CFTR gene transfer in a placebo-treated patient.[43] Partial functional correction was obtained in some, but not all, patients. Importantly, early indications suggest that repeated administration is safe and partially corrective electrophysiologically.[43-45]

In summary, nasal gene transfer of CFTR with consequent functional correction is apparently feasible using both adenoviral and liposomal vectors, but neither system provides either consistent or complete phenotypic correction. It should be remembered, however, that the nasal epithelium has been used principally as an exceptionally accessible surrogate for the bronchial epithelium, and that the true challenge for CF gene therapy remains treatment of the pulmonary airways.

The first pulmonary trial of human CF gene therapy was initiated in 1992 and involved adenoviral transfer of CFTR to the lungs of four patients, using bronchoscopic instillation to one or other lower lobe.[41] The study demonstrated that gene transfer to the airways with subsequent protein expression was feasible, and that viral shedding did not occur. At the time, sophisticated techniques to measure changes in potential difference across airway epithelium *in situ* were not available.[46] Unfortunately,

in the patient receiving the highest dose of adenovirus, pneumonia occurred in the treated lobe, with associated decline in lung function.[41] Although the pneumonia resolved, and lung function returned to baseline, the study raised serious safety concerns for localised high-dose adenoviral administration to the lung. In many ways, this finding held back further studies of adenovirus-CFTR in the CF lung.

However, more recently, a meticulous study described aerosolisation of adenovirus in lower doses than that which had caused pneumonia in the previous study.[42] This trial was the first attempting to deliver gene therapy to all regions of the airways. The study described gene transfer to the nose in all six patients studied; in addition, CFTR DNA was detected in bronchoalveolar lavage (BAL) fluid from all patients, with evidence of transcription and/or protein expression in three. Importantly, no viral shedding was described, and no inflammation or other adverse effects directly attributable to the treatment could be detected.

Recently, cationic liposomes carrying the CFTR gene have also been administered by nebulisation to the lung in CF.[46] Mild, transient, flu-like symptoms were common but not limiting, and a decline in lung function commonly followed nebulisation. Importantly, however, this thorough study demonstrated that partial electrophysiological correction can be produced in the airways, and that inflammatory markers can be ameliorated. These data provide enormous encouragement for successful future CF gene therapy, especially if the findings can be reproduced in the context of repeated administration.[9]

Lastly, adeno-associated virus (AAV) has also been given to patients with CF in clinical trials. Dose-dependent gene transfer was demonstrated when AAV-CFTR was applied to the sinuses (frequently involved in CF) of patients who had undergone bilateral antrostomies.[47] Repeated administration proved successful and a trend towards electrophysiological correction was described.[47] Results of trials using AAV-CFTR in the lung are awaited; however barriers to successful transfection have already been described.[48]

In summary, gene transfer to the airways in CF is eminently feasible, but to date it has proved to be an inefficient process. Although considerable achievements have been made in gene therapy for CF to date, several problems require to be overcome if this early promise is to be translated into effective treatment.

Transfer efficiency certainly has the potential to improve considerably in future with clarification of the physical and cellular barriers to transfection, and with selective engineering of vectors (as discussed in more detail below). However considerable debate continues to revolve around the question of exactly which cells in the airway should be targeted in CF. In the healthy lung CFTR is expressed throughout the respiratory epithelium, including the submucosal glands. The latter are effectively inaccessible to currently available vectors applied topically, although submucosal gland epithelium can be transfected *in vitro*.[49] Whether restoration of CFTR function in submucosal glands is required to effect phenotypic correction is keenly contested, but not yet clear, though efforts are being made to resolve the issue. In addition, apical respiratory epithelium is thought to be relatively resistant to adenoviral transfection as compared with the basal respiratory epithelium.[50] A major challenge then, is to define precisely which cells in the airway require phenotypic correction in CF, and to devise vector modifications and/or routes of application to access these cells efficiently. Encouragingly animal studies suggest that only a small proportion of CF cells need be transfected to secure phenotypic correction, though this observation requires confirmation in man.[51]

If gene targeting can indeed be optimised, improvements in the duration of gene transfer seem highly likely. As discussed in the final section of this article, advances in technology have led to prolonged expression of transgene in animals using modified adenovirus, AAV and liposomes. These advances seem set to find their way into clinical application.

One remaining caveat to consider is that CF lung disease begins in early childhood, and gene therapy trials for CF have recruited only adults thus far. The challenge now is to improve gene therapy for CF and extend it to a younger population.

Alpha-1 antitrypsin deficiency

Alpha-1 antitrypsin (α-1 AT) deficiency is an autosomal recessive condition characterised by production of dysfunctional α-1 AT.[52-54] Alpha-1 AT is produced mainly in the liver, secreted into the circulation and sequestered into organs such as the lung, where it is thought to exert a protective effect against proteolytic enzymes, in particular human neutrophil elastase.[55] Several different alleles for α-1 AT variants lead to a propensity for premature spontaneous emphysema with a predilection for the lower lobes, but by far the commonest abnormality is the PiZ phenotype.[54]

Correction of the single gene defect in α-1 AT deficiency has long seemed an appropriate goal for gene therapy. However, certain features of the disease limit the potential for gene therapy. Firstly, the natural history remains poorly understood.[54] Secondly, treatment with intravenous recombinant α-1 AT is under continued evaluation,[54] and thirdly, the optimal target organ for gene delivery in α-1 AT deficiency remains contentious, with the liver, the vascular endothelium and the lung all being mooted as candidates.[56] Arguments can also be made for intramuscular delivery, which, under appropriate conditions, can result in sustained release of protein into the circulation (thus modelling hepatic release of α-1 AT) and has proved successful in other clinical applications.[57]

Promising studies have demonstrated the feasibility of human α-1 AT gene delivery to the airways in animal models, one using intratracheal instillation of adenovirus, the other using nebulisation of liposome complexes.[58,59] However, levels of human α-1 AT in excess of the widely accepted threshold for protection against elastase have not yet been demonstrated. Nevertheless, the first clinical trial, using cationic liposomes, is now underway, and its findings are awaited with interest.[56]

Bronchogenic carcinoma

Bronchogenic carcinoma is responsible for an enormous burden of morbidity and mortality, with five-year survival rates of approximately 5% being described.[60] The only conventional prospect for cure is confined to surgery in cases presenting early, but the large majority of lung cancers present long after the potential for curative resection.[60] In this context, innovative gene therapy strategies have been proposed for lung cancer, drawing on recent advances in the understanding of immune mechanisms, molecular

biology and tumour genetics. The diversity of these strategies is reviewed in detail elsewhere, and is summarised in Figure 2.[7,61]

Reports from clinical trials of gene therapy for bronchogenic carcinoma are now emerging. To date these have mainly focused on intratumoural gene delivery. Concerns have been raised as to whether gene therapy will advance sufficiently to allow systemic delivery of tumour specific vectors which could influence pulmonary metastases (both those secondary to lung cancer, and metastases from a distant non-respiratory primary). Nevertheless, important principles have been established.

Two French trials established the feasibility of directly transfecting human non-small cell lung cancer (NSCLC) deposits with adenovirus expressing marker transgene.[62,63] These trials suggested that adenovirus itself may be responsible for regression of transfected nodules in a high proportion of cases, and implied that this may arise because of the immune response elicited. Thus the immunity which blights adenovirus administration in, for example, CF may be used to advantage in some cancer applications.

Of the therapeutic transgenes used, the best characterised clinically is the gene encoding p53, a molecule with tumour suppressant and pro-apoptotic properties, which is mutated in over 50% of cases of NSCLC.[64] The first clinical trial of gene therapy in lung cancer studied injection of retroviruses encoding p53 into endobronchial and metastatic NSCLC, and resulted in tumour regression or stabilisation in six of seven patients amenable to assessment.[65] The same group recently applied a similar protocol using an adenoviral vector encoding p53 and described clinical improvements.[66] A further trial using adenovirus and p53 described clinical responses in four of six tumours efficiently transfected.[67] These trials suggest that high doses of adenovirus are required to deliver sufficient p53 to effect a response, but that, at least when locally delivered, such high doses appear effective and well tolerated.

Important findings have also been reported in clinical studies of gene therapy for lung cancer using different approaches. One trial involved harvesting malignant pleural effusions associated with lung cancers, and separating out tumour-specific T lymphocytes.[68] These were then transfected with retrovirus encoding interleukin (IL)-2 and re-injected into the pleural cavity. In 60% of patients treated in this way, effusions failed to re-accumulate within one month, and in one patient the primary tumour decreased in size.[68] Interestingly, regression of untreated regional lymph nodes has been observed in patients with subcutaneous metastases transfected with liposomes encoding HLA-B7/β_2-microglobulin.[69] Such anecdotal reports of biological effects distant to the site of vector administration offer encouragement for the treatment of metastases.

Interpretation of these encouraging early trials must be tempered with caution however. These trials have not been controlled, and it is not possible to be certain whether the effects observed were attributable to the transgene used, to concomitant therapies, to the vector employed, or even to the physical stresses of injection.

Non Hodgkin's lymphoma (NHL)

This disease process commonly involves the mediastinum. A British study used antisense oligonucleotides against Bcl-2 (an inhibitor of apoptosis expressed in several human tumours) in NHL, and demonstrated regression or stability of tumour deposits in certain cases.[70] Although by no means all patients improved, and treatment was attended by side-effects, the importance of this study rests in the administration being systemic (subcutaneous) rather than local.

Malignant mesothelioma

Malignant mesothelioma is associated with a dismal prognosis, no satisfactory treatment is available[71] and mortality is expected to continue to rise in the UK over the next 20 years.[72] Gene therapy trials are well suited to mesothelioma for a number of reasons.[61] Pleural mesothelioma is a peculiarly 'localised' malignancy, the pleural cavity is readily accessible, disease monitoring can be achieved by consecutive computerised tomography and mesothelial cells can be transfected *in vitro* and *in vivo*.[61,73] Furthermore, 'suicide gene therapy' is particularly applicable to mesothelioma. Suicide gene therapy involves transfecting tumour cells with genes encoding enzymes which do not occur naturally in human cells, and which convert inactive pro-drugs into cytotoxic metabolites.[74] The archetypal system involves transfection with thymidine kinase derived from Herpes simplex virus (HSV-tk), followed by treatment with gancyclovir. Crucially, this treatment is associated with a so-called 'bystander effect' whereby non-transfected tumour cells become susceptible to the toxic effects generated in neighbouring transfected cells.[75] As a result, only a relatively small proportion of mesothelioma cells need be transfected.

The first clinical experience with this technology is now being reported. One group administered adenovirus encoding HSV-tk intrapleurally, followed by gancyclovir systemically, to 21 patients with mesothelioma.[76] Gene transfer was practicable, and indeed efficient at high doses. However, high doses were associated with clinically important side-effects. Interestingly, gene expression persisted despite humoral and cellular immune responses to the vector. The authors conducted a painstaking clinical assessment of response, which indicated no regression of any tumour.[77] At the time of publication, the three patients receiving high dose vector had not been studied long enough to determine whether clinical improvement had occurred. In six other patients disease appeared to stabilise or progress slowly, but it was impossible to attribute any effect to gene therapy.

Another group has initiated a fascinating clinical trial instilling ovarian cancer cells stably transfected with HSV-tk intrapleurally, then administering gancyclovir.[78] This strategy relies entirely upon the 'bystander effect', and has been shown to prolong survival significantly in a murine model of mesothelioma.[79] Results of the clinical trial are eagerly awaited.

In summary, unique circumstances offer potential for effective gene therapy in mesothelioma. Early results suggest the requirement for randomised, controlled trials.

Pre-clinical data with potential clinical application

In the laboratory, gene transfer is being applied to increasingly complex pulmonary pathologies. The findings generated are beginning to suggest possible novel clinical applications.

For example, in an age of increasing antimicrobial resistance, pulmonary clearance of *Pseudomonas aeruginosa* from the lungs of rats was enhanced by prophylactic

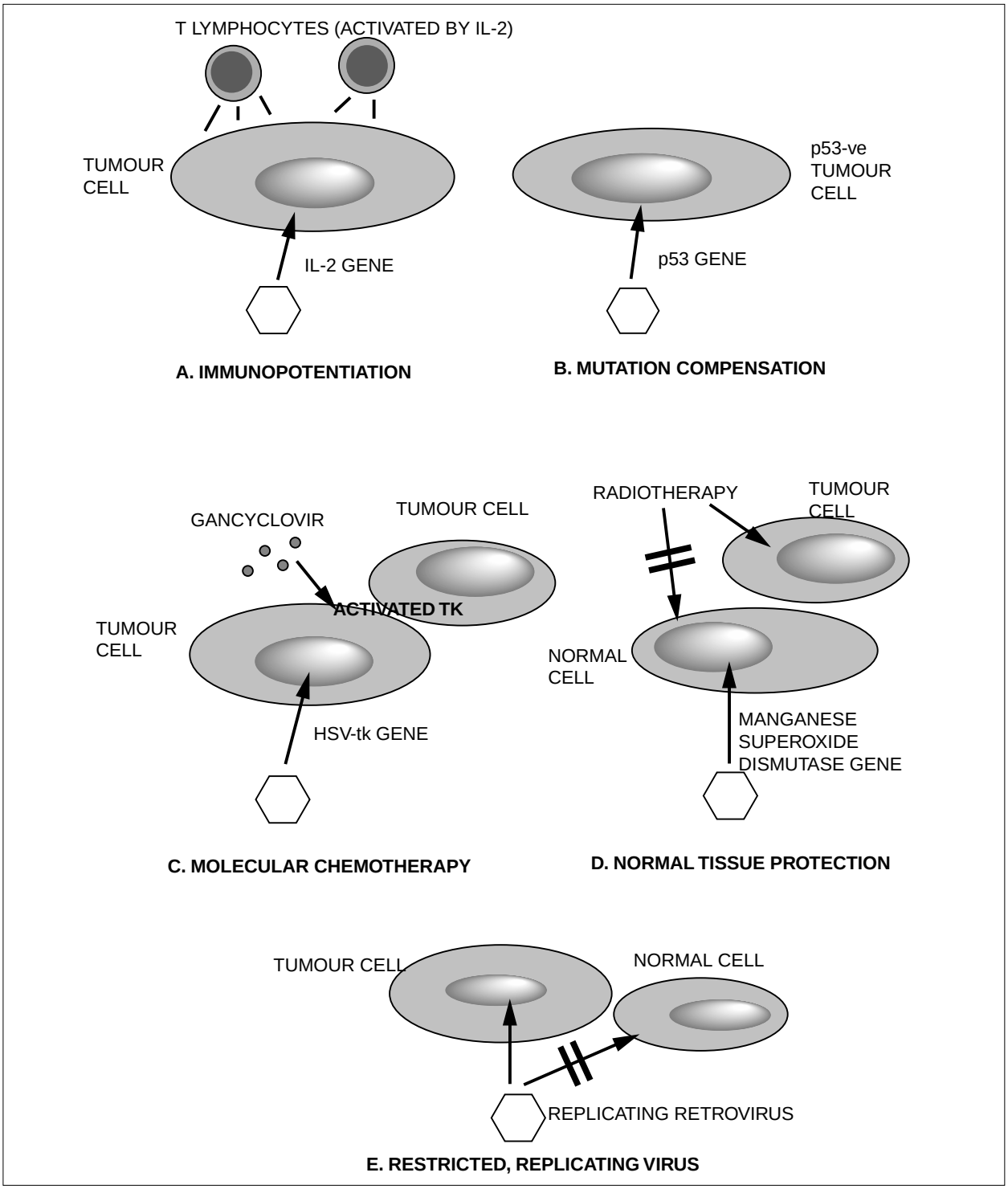

FIGURE 2

Gene therapy strategies for the treatment of cancer (adapted from references.)[7,61]

A. Tumour cells can be destroyed by introducing transgenes which, when expressed, activate immune effector cells (such as tumour specific T lymphocytes and natural killer cells), as illustrated using the example of IL-2 transfection, a strategy which has been employed clinically.[68]

B. Tumour cells with known mutations in tumour suppressor genes can be transfected with the functional gene, for example p53, a strategy used in several clinical applications. [65-67]

C. Tumour cells can be transfected with genes encoding toxic viral enzymes which are activated by specific chemical agents (for example HSV-tk is activated by gancyclovir, resulting in death of transfected cells, and in destruction of neighbouring untransfected tumour cells using the 'bystander effect');[75] this strategy has been applied clinically.[76-77]

D. Normal cells can be transfected with protective genes (for example superoxide dismutase) leaving tumour cells susceptible to radiotherapy; no clinical trials using this method in the lung have been reported.

E. Replicating retroviruses infect and lyse dividing cells only, sparing non-dividing cells; this strategy has not been used clinically in the human lung.

administration of adenovirus encoding γ-interferon.[80] An ingenious extension of this protocol demonstrated improved clearance of *Pneumocystis carinii* from CD4 depleted mice with an associated reduction in mortality.[81] Similarly, adenovirus encoding IL-12 protected mice against lethal doses of *Klebsiella pneumoniae*.[82]

Extending the theme of antimicrobial gene therapy, the generation of transgenes encoding microbial products has raised hopes of developing more effective vaccines against, for example, influenza and tuberculosis.[83] In animal studies DNA vaccines encoding single mycobacterial antigens have proved to be as successful as vaccination with the complex BCG vaccine.[84,85]

Gene therapy may also find a role in preventing lung transplant rejection.[86] Prophylactic transfection of donor lungs with adenovirus encoding immunosuppressant genes has significantly improved engraftment in mice.[87]

Respiratory failure is an inevitable consequence of many progressive myopathies. The dystrophin gene is defective in Duchenne's muscular dystrophy (DMD). In animal models of DMD, functional improvement has been achieved by transfection of the dystrophin minigene into myocytes either directly, or *ex vivo* before transplantation into muscle beds.[88] This technology is feasible for respiratory muscles in animal models.[89]

Gene therapy can also provide insights into mechanisms of pulmonary disease. For example, intratracheal instillation of adenovirus encoding transforming growth factor β (TGF-β) in rats produces florid pulmonary fibrosis, suggesting both mechanistic pathways and potential novel therapies for cryptogenic fibrosing alveolitis in man.[90]

Our own group has a longstanding interest in the role of antiproteases in inflammatory lung disease, with particular reference to the recently identified anti-elastases elafin and SLPI (secretory leukocyte protease inhibitor) which, along with α-1 AT, comprise the 'anti-elastase shield' in the human lung.[91-93] We are using gene therapy strategies in an attempt to elucidate the functions of these molecules in pulmonary inflammation, and also to suggest novel therapeutic applications.[94,95] These molecules appear to harbour a variety of biological actions in addition to their antiproteolytic function which could potentially be augmented in the appropriate clinical setting.[96-99] Furthermore, in association with local collaborators, we are exploring novel gene therapy strategies for CF and pulmonary infection, and investigating the potential of adenoviral gene therapy to encode molecules central to immune tolerance in allergic models of asthma.[100,101]

ADVANCES IN THE TECHNOLOGY OF GENE THERAPY, AND ITS FUTURE CLINICAL APPLICATION

The ideal vector would access its cell of interest selectively, and allow sufficient, sustained, and regulatable expression of transgene(s) without detriment to the host. Each component of this equation has now been addressed with some success in the laboratory, offering enormous hope for future clinical practice.

Much more is now known about the mechanisms by which viral vectors enter cells, locate the nucleus, and traverse the nuclear membrane.[102-104] The optimal viral vector for a given application can thus begin to be identified more readily. This knowledge has also allowed the generation of chimeric ligands which can, for example, expand the tropism of viral or liposomal vectors.[8,105,106] Furthermore, proteins used by viruses to evade endosomal ingestion can be fused to liposomes, thus potentially improving transgene delivery by this method.[8]

Technology now exists to engineer transgenes under the control of more powerful promoters. We have previously demonstrated the importance of selecting the appropriate promoter for a given transgene[16] and cell- and tissue-specific promoters are now becoming available, allowing more selective targeting of transgene expression.[107,108] More excitingly still, inducible promoters have been described which are responsive to chemical 'switches' such as oral tetracycline or ecdysone.[109,110] These systems allow both temporal and quantitative regulation of transgene expression.

Perhaps the most intensely investigated question in gene therapy has been how to abrogate or at least down-regulate the immune response generated by administration of adenovirus. This has been addressed in two ways: by making the vector less immunogenic, and by attempting to reduce the host immune response (Table 2). Major advances have been made in both approaches. Ingenious methods have been devised to construct adenovirus deleted of virtually the entire viral genome (nicknamed 'gutted viruses'), thereby freeing up almost 30 kb of genetic 'space', and radically reducing immunogenicity.[111-113] Such vectors can allow expression of transgene for at least a year after a single administration, as has been demonstrated for human α-1 AT in a murine model, and permit successful re-administration.[114] With regard to modulating recipient immunity, co-administration of a plethora of agents has shown augmented and prolonged expression of transgenes.[115-123] Particularly impressive has been the use of an inhibitor of T-cell co-stimulatory interactions, CTLA4Ig, which allows improved transgene expression and successful re-administration in the lung.[124] An alternative technique may be to use adenoviruses of different serotypes in sequential dosing programmes.[125]

Increasingly imaginative routes of vector delivery are also evolving. For example, apparently permanent reversal of the CF phenotype and dramatically increased survival

TABLE 2
Methods employed to facilitate successful re-administration of adenoviral vectors.

↓ Immunogenicity of vector	Transiently abrogate host immune response
• Deletion of all viral coding sequences ('gutted vectors')	• Give ligands interfering with co-stimulatory signals CTLA4Ig Anti-T cell receptor antibody • Augment endogenous immune suppressants IL-12 • Induce tolerance Oral tolerisation of adenovirus • 'Conventional' therapeutic immunosuppression Glucocorticoids FK506 Deoxyspergualin

have been achieved through single intra-amniotic instillation of adenovirus encoding CFTR in CF knock-out mice.[126] Although pathology in this knock-out model is primarily gastrointestinal rather than pulmonary, the implications would be staggering if this principle could be extended to humans.

Thus it is now possible to imagine a single intravenous (or even intra-amniotic!) dose of liposome or gutted adenovirus encoding CFTR under the control of a tissue-specific, inducible promoter, allowing carefully regulated gene expression at selected, critical points in development. This flight of fancy may be far off in its clinical application, but each individual component is presently feasible on technological grounds.

CONCLUSIONS

Pulmonary gene therapy has advanced enormously since the first CF patient was treated in 1992, although unfortunately its achievements fall short of the expectations of the late 1980s. However as has been noted elsewhere few, if any, conventional therapeutic modalities could have progressed as quickly from drawing board to bedside as has gene therapy.[2] The principle of safe gene transfer has been achieved in all of the pulmonary conditions studied to date. Gene therapy has apparently 'jumped the gun' to some extent in lung cancer, in that considerable clinical improvement, almost certainly attributable to treatment, has been observed in phase I clinical trials which did not set out with the intention of monitoring disease regression, thus setting the scene for prospective, randomised, controlled trials of great potential importance. Additionally, gene therapy is now being applied to a wide range of pulmonary conditions for which no ideal conventional therapies exist. All of the advancing clinical applications owe their existence, and their exciting future, to innovative and meticulous laboratory data generated in disciplines as diverse as cell biology, molecular biology, virology, genetics, and membrane biochemistry. All things considered, the future of human pulmonary gene therapy appears bright, and realistically it may find its first accepted clinical application by the time it has doubled in age.

Key points

- Gene therapy strategies have been applied clinically to cystic fibrosis, α-1 antitrypsin deficiency, bronchogenic carcinoma, malignant mesothelioma and non Hodgkins lymphoma.
- The principle of successful transgene transfer has been established in these conditions.
- Localised, functional improvements have been achieved in selected cases of cystic fibrosis (improved electrophysiology *in situ*) and in lung cancer (tumour regression).
- Significant technological advances in gene therapy have produced promising results pre-clinically, suggesting novel applications for gene therapy; these refined technologies are set to be applied in clinical trials, and should greatly increase the capabilities of clinical gene therapy.

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REFERENCES

1 Blaese RM, Culver KW, Miller AD *et al*. T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. *Science* 1995; 270:475-80.
2 Alton EW, Geddes DM. Prospects for gene therapy. *Br J Hosp Med* 1997; 58:47-9.
3 Crystal RG. Transfer of genes to humans: early lessons and obstacles to success. *Science* 1995; 270:404-10.
4 Verma IM, Somia N. Gene therapy - promises, problems and prospects. *Nature* 1997; 389:239-42.
5 Weatherall DJ. Scope and limitations of gene therapy. *Br Med Bull* 1995; 51:1-11.
6 Marcel T, Grausz JD. The TMC worldwide gene therapy enrollment report, end 1996. *Human Gene Therapy* 1997; 8:775-800.
7 Curiel DT, Pilewski JM, Albelda SM. Gene therapy approaches for inherited and acquired lung diseases. *Am J Respir Cell Mol Biol* 1996; 14:1-18.
8 Schofield JP, Caskey CT. Non-viral approaches to gene therapy. *Br Med Bull* 1995; 51:56-71.
9 Alton EWFW, Geddes DM, Gill DR *et al*. Towards gene therapy for cystic fibrosis: a clinical progress report. *Gene Therapy* 1998; 5:291-2.
10 Mulligan RC. The basic science of gene therapy. *Science* 1993; 260:926-32.
11 Snyder RO, Miao CH, Patijn GA *et al*. Persistent and therapeutic concentrations of human factor IX in mice after hepatic gene transfer of recombinant AAV vectors. *Nature Genetics* 1997; 16:270-6.
12 Goins WF, Krisky D, Marconi P *et al*. Herpes simplex virus vectors for gene transfer to the nervous system. *J Neurovirology* 1997; 3(Suppl 1):S80-S88.
13 Miller AD. Retroviral vectors. *Curr Topics Microbiol Immunol* 1992; 158:1-24.
14 Miyanohara A, Abi A, Friedmann T. Partial cell-free assembly of VSV-G pseudotyped retrovirus particles. *Keystone Symposia: Molecular and Cellular Biology of Gene Therapy* 1998; J7:34.
15 Kremer EJ, Perricaudet M. Adenovirus and adeno-associated virus mediated gene transfer. *Br Med Bull* 1995; 51:31-44.
16 Sallenave J-M, Xing Z, Simpson AJ *et al*. Adenovirus-mediated expression of an elastase-specific inhibitor (elafin): a comparison of different promoters. *Gene Therapy* 1998; 5:352-60.
17 Bett AJ, Haddara W, Prevec L, Graham FL. An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proc Natl Acad Sci USA* 1994; 91:8802-6.
18 Van Ginkel FW, Liu C, Simecka JW *et al*. Intratracheal gene delivery with adenoviral vector induces elevated systemic IgG and mucosal IgA antibodies to adenovirus and β-galactosidase. *Human Gene Therapy* 1995; 6:895-903.
19 Kaplan JM, St George JA, Pennington SE *et al*. Humoral and cellular immune responses of nonhuman primates to long-term repeated lung exposure to Ad2/CFTR-2. *Gene Therapy* 1996; 3:117-27.
20 Yang Y, Nunes FA, Berencsi K *et al*. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci USA* 1994; 91:4407-11.
21 Kaplan JM, Armentano D, Sparer TE *et al*. Characterization of factors involved in modulating persistence of transgene expression from recombinant adenovirus in the mouse lung. *Human Gene Therapy* 1997; 8:45-56.

[22] Song W, Kong H-L, Traktman P, Crystal RG. Cytotoxic T lymphocyte responses to proteins encoded by heterologous transgenes transferred *in vivo* by adenoviral vectors. *Human Gene Therapy* 1997; 8:1207-17.

[23] Yei S, Mittereder N, Tang K *et al*. Adenovirus-mediated gene transfer for cystic fibrosis: quantitative evaluation of repeated *in vivo* vector administration to the lung. *Gene Therapy* 1994; 1:192-200.

[24] Worgall S, Wolff G, Falck-Pedersen E, Crystal RG. Innate immune mechanisms dominate elimination of adenoviral vectors following *in vivo* administration. *Human Gene Therapy* 1997; 8:37-44.

[25] Worgall S, Leopold PL, Wolff G *et al*. Role of alveolar macrophages in rapid elimination of adenovirus vectors administered to the epithelial surface of the respiratory tract. *Human Gene Therapy* 1997; 8:1675-84.

[26] Wersto RP, Rosenthal ER, Seth PK *et al*. Recombinant, replication-deficient adenovirus gene transfer vectors induce cell cycle dysregulation and inappropriate expression of cyclin proteins. *J Virol* 1998; 72:9491-502.

[27] Hierholzer J. Adenoviruses in the immunocompromised host. *Clin Microbiol Rev* 1992; 5:262-74.

[28] Rosenecker J, Harms KH, Bertele RM *et al*. Adenovirus infection in cystic fibrosis patients: implications for the use of adenoviral vectors for gene transfer. *Infection* 1996; 24:5-8.

[29] Elliott WM, Hayashi S, Hogg JC. Immunodetection of adenoviral E1A proteins in human lung tissue. *Am J Respir Cell Mol Biol* 1995; 12:642-8.

[30] Fallaux FJ, van der Eb AJ, Hoeben RC. Who's afraid of replication-competent adenoviruses? *Gene Therapy* 1999; 6:709-12.

[31] Davis PB, Drumm M, Konstan MW. Cystic fibrosis. *Am J Respir Crit Care Med* 1996; 154:1229-56.

[32] Geddes DM. Cystic fibrosis: epidemiology and pathogenesis. In: Brewis RAL, Gibson GJ, Geddes DM, eds *Respiratory medicine* London: Baillière Tindall, 1990; 760-9.

[33] Rommens JM, Iannuzzi MC, Kerem B *et al*. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; 245:1059-65.

[34] Grubb, BR, Boucher RC. Pathophysiology of gene-targeted mouse models for cystic fibrosis. *Physiol Rev* 1999; 79:S193-S214.

[35] Kent G, Iles R, Bear CE *et al*. Lung disease in mice with cystic fibrosis. *J Clin Invest* 1997; 100:3060-9.

[36] Davidson DJ, Dorin JR, McLachlan G *et al*. Lung disease in the cystic fibrosis mouse exposed to bacterial pathogens. *Nature Genetics* 1995; 9:351-7.

[37] Zabner J, Couture LA, Gregory RJ *et al*. Adenovirus-mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis. *Cell* 1993; 75:207-16.

[38] Zabner J, Ramsey BW, Meeker DP *et al*. Repeat administration of an adenovirus vector encoding cystic fibrosis transmembrane conductance regulator to the nasal epithelium of patients with cystic fibrosis. *J Clin Invest* 1996; 97:1504-11.

[39] Hay JG, McElvaney NG, Herena J *et al*. Modification of nasal epithelial potential differences of individuals with cystic fibrosis consequent to local administration of a normal CFTR cDNA adenovirus gene transfer vector. *Human Gene Therapy* 1995; 6:1487-96.

[40] Knowles MR, Hohneker KW, Zhou Z *et al*. A controlled study of adenoviral-vector-mediated gene transfer in the nasal epithelium of patients with cystic fibrosis. *N Eng J Med* 1995; 333:823-31.

[41] Crystal RG, McElvaney NG, Rosenfeld MA *et al*. Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis. *Nature Genetics* 1994; 8:42-51.

[42] Bellon G, Michel-Calemard L, Thouvent D *et al*. Aerosol administration of a recombinant adenovirus expressing CFTR to cystic fibrosis patients: a phase I clinical trial. *Human Gene Therapy* 1997; 8:15-25.

[43] Caplen NJ, Alton E, Middleton PG *et al*. Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Nature Medicine* 1995; 1:39-46.

[44] Porteous DJ, Dorin JR, McLachlan G *et al*. Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Therapy* 1997; 4:210-18.

[45] Gill DR, Southern KW, Mofford KA *et al*. A placebo-controlled study of liposome-mediated gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Therapy* 1997; 4:199-209.

[46] Alton EWFW, Stern M, Farley R *et al*. Cationic lipid-mediated CFTR gene transfer to the lungs and nose of patients with cystic fibrosis: a double-blind placebo-controlled trial. *Lancet* 1999; 353:947-54.

[47] Wagner JA, Reynolds T, Moran ML *et al*. Efficient and persistent gene transfer of AAV-CFTR in maxillary sinus. *Lancet* 1998; 351:1702-3.

[48] Flotte T, Poirier A, Wang J *et al*. Excessive levels of neutrophil elastase and neutrophil defensin in cystic fibrosis BAL fluid inhibit rAAV transduction. *Am J Respir Crit Care Med* 1999; 159:A433.

[49] Jiang C, Finkbeiner WE, Widdicombe JH *et al*. Restoration of cyclic adenosine monophosphate stimulated chloride channel activity in human tracheobronchial submucosal gland cells by adenovirus-mediated and cationic lipid-mediated gene transfer. *Am J Respir Cell Mol Biol* 1999; 20:1107-15.

[50] Pickles RJ, Barker PM, Ye H, Boucher RC. Efficient adenovirus-mediated gene transfer to basal but not columnar cells of cartilaginous airway epithelia. *Human Gene Therapy* 1996; 7:921-31.

[51] 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. *GeneTherapy* 1996; 3:797-801.

[52] Laurell CB, Eriksson S. The electrophoretic α1-globulin pattern of serum in α1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963; 15:132-40.

[53] Hutchison DCS. Chronic obstructive pulmonary disease (COPD): Proteases and antiproteases. In: Brewis RAL, Gibson GJ, Geddes DM (eds). *Respiratory medicine* London: Baillière Tindall, 1990; 485-96.

[54] Stoller JK. Clinical features and natural history of severe α1-antitrypsin deficiency. *Chest* 1997; 111:123S-128S.

[55] Gadek JE, Fells GA, Zimmerman RL. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J Clin Invest* 1981; 68:889-98.

[56] Alton EWFW, Geddes DM. Gene therapy for respiratory diseases: potential applications and difficulties. *Thorax* 1995; 50:484-6.

[57] Baumgartner I, Pieczek A, Manor O *et al*. Constitutive expression of phVEGF$_{165}$ after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation* 1998; 97:1114-23.

[58] Rosenfeld MA, Siegfried W, Yoshimura K *et al*. Adenovirus-mediated transfer of a recombinant α1-antitrypsin gene to the lung epithelium *in vivo*. *Science* 1991; 252:431-4.

[59] Canonico AE, Conary JT, Meyrick BO, Brigham KL. Aerosol and intravenous transfection of human α1-antitrypsin gene to lungs of rabbits. *Am J Respir Cell Mol Biol* 1994; 10:24-9.

[60] Spiro SG. Tumours: lung tumours. In: Brewis RAL, Gibson GJ, Geddes DM (eds). *Respiratory medicine* London: Baillière Tindall, 1990; 832-79.

[61] Albelda SM. Gene therapy for lung cancer and mesothelioma. *Chest* 1997; 111:144S-149S.

[62] Tsurz T, Cesne AL, Baldeyrou P *et al*. Phase I study of a recombinant adenovirus-mediated gene transfer in lung cancer patients. *J Natl Cancer Inst* 1996; 88:1857-63.

[63] Gahery-Segard H, Molinger-Frenkel V, Le Boulaire C *et al*. Phase I trial of recombinant adenovirus gene transfer in lung cancer. Longitudinal study of the immune responses to transgene and viral products. *J Clin Invest* 1997; 100:2218-26.

[64] Gazzeri S, Bramilla E, Caron de Fromentel C *et al*. p53 genetic abnormalities and myc activation in human lung carcinoma. *Int J Cancer* 1994; 58:24-32.

[65] Roth JA, Nguyen D, Lawrence DD *et al*. Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. *Nature Medicine* 1996; 2:985-91.

[66] Roth JA, Swisher SG, Merritt JA *et al*. Gene therapy for non-small cell lung cancer: a preliminary report of a phase I trial of adenoviral p53 gene replacement. *Semin Oncol* 1998; 25 (Suppl 8):33-7.

[67] Schuler M, Rochlitz C, Horowitz JA *et al*. A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung cancer. *Human Gene Therapy* 1998; 9:2075-82.

[68] Tan Y, Xu M, Wang W *et al*. IL-2 gene therapy of advanced lung cancer patients. *Anticancer Res* 1996; 16(4A):1993-8.

[69] Heo DS, Yoon SJ, Kim WS *et al*. Locoregional response and increased natural killer activity after intratumoral injection of HLA-B7/beta2-microglobulin gene in patients with cancer. *Human Gene Therapy* 1998; 9:2031-38.

[70] Webb A, Cunningham D, Cotter F *et al*. BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. *Lancet* 1997; 349:1137-41.

[71] Rudd RM. Asbestos-related disease. In: Brewis RAL, Gibson GJ, Geddes DM (eds). *Respiratory medicine* London: Baillière Tindall, 1990; 1241-65.

[72] Peto J, Hodgson JT, Matthews FE, Jones JR. Continuing increase in mesothelioma mortality in Britain. *Lancet* 1995; 345:535-9.

[73] Hwang HC, Smythe WR, Elshami AA *et al*. Gene therapy using adenovirus carrying the herpes simplex-thymidine kinase gene to treat *in vivo* models of human malignant mesothelioma and lung cancer. *Am J Respir Cell Mol Biol* 1995; 13:7-16.

[74] Tiberghien P. Use of suicide genes in gene therapy. *J Leukocyte Biol* 1994; 56:203-9.

[75] Dilber MS, Smith CIE. Suicide genes and bystander killing: local and distant effects. *Gene Therapy* 1997; 4:273-4.

[76] Sterman DH, Treat J, Litzky LA *et al*. Adenovirus-mediated herpes simplex virus thymidine kinase/gancyclovir gene therapy in patients with localized malignancy: results of a phase I clinical trial in malignant mesothelioma. *Human Gene Therapy* 1998; 9:1083-92.

[77] Molnar-Kimber KL, Sterman DH, Chang M *et al*. Impact of preexisting and induced humoral and cellular immune responses in an adenovirus-based gene therapy phase I clinical trial for localized mesothelioma. *Human Gene Therapy* 1998; 9:2121-33.

[78] Schwarzenberger P, Harrison L, Weinacker A *et al*. The treatment of malignant mesothelioma with a gene modified cancer cell line: a phase I study. *Human Gene Therapy* 1998; 9:2641-9.

[79] Schwarzenberger P, Lei D, Freeman SM *et al*. Antitumor activity with the HSV-tk-gene-modified cell line PA-1-STK in malignant mesothelioma. *Am J Respir Cell Mol Biol* 1998; 19:333-7.

[80] Kolls JK, Lei D, Nelson S *et al*. Adenoviral-mediated murine interferon gene transfer compartmentally activates alveolar macrophages and enhances bacterial clearance. *Chest* 1997; 111:104S.

[81] Kolls JK, Habetz S, Vazquez C *et al*. Interferon-γ and CD8+ T-cells restore host defences against *P.carinii* in mice depleted of CD4+ T-cells. *J Immunol* 1999; 162:2890-4.

[82] Greenberger MJ, Kunkel SL, Strieter RM *et al*. IL-12 gene therapy protects mice in lethal *Klebsiella* pneumonia. *J Immunol* 1996; 157:3006-12.

[83] Dickler HB, Collier E. Vaccines: new approaches and concepts. *J Allergy Clin Immunol* 1997; 97:896-906.

[84] Tascon RE, Colston MJ, Ragno S *et al*. Vaccination against tuberculosis by DNA injection. *Nature Medicine* 1996; 2:888-92.

[85] Huygen K, Content J, Denis O *et al*. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. *Nature Medicine* 1996; 2:893-8.

[86] Trulock RP. Lung transplantation. *Am J Respir Crit Care Med* 1997; 155:789-818.

[87] Chapelier A, Danel C, Mazmanian M *et al*. Gene therapy in lung transplantation: feasibility of *ex vivo* adenovirus-mediated gene transfer to the graft. *Human Gene Therapy* 1996; 7:1837-45.

[88] Yang L, Lochmuller H, Luo J *et al*. Adenovirus-mediated dystrophin minigene transfer improves muscle strength in adult dystrophic (MDX) mice. *Gene Therapy* 1998; 5:369-79.

[89] Petrof BJ. Respiratory muscles as a target for adenovirus mediated gene therapy. *Eur Respir J* 1998; 11:492-7.

[90] Sime PJ, Xing Z, Graham FL *et al*. Adenovector-mediated gene transfer of active transforming growth factor-beta 1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 1999; 100:768-76.

[91] Sallenave J-M, Morgan K, Gauldie J, Kalsheker N. Elastase inhibitors in the lung: expression and functional relationships. In: Stockley RA (ed.). *Molecular biology of the lung Vol 1: Emphysema and infection* Basel: Birkhäuser Verlag, 1999; 69-94.

[92] Sallenave J-M, Silva A. Characterization and gene sequence of the precursor of elafin, an elastase-specific inhibitor in bronchial secretions. *Am J Respir Cell Mol Biol* 1993; 8:439-45.

[93] Sallenave J-M, Shulmann J, Crossley J *et al*. Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/elafin) in human airway epithelial cells by cytokines and neutrophilic enzymes. *Am J Respir Cell Mol Biol* 1994; 11:733-41.

[94] Simpson AJ, Porteous DJ, Haslett C, Sallenave J-M. Pulmonary epithelial cells transfected with E1-deleted adenovirus encoding human elafin cDNA are protected against neutrophil elastase. *Am J Respir Crit Care Med* 1999; 159:A239.

[95] Simpson AJ, Antonicelli F, Haslett C, Sallenave J-M. Differential LPS-induced elafin release from macrophages treated with or without E1-deleted adenovirus encoding human elafin cDNA. *Am J Respir Crit Care Med* 1999; 159:A193.

[96] Hiemstra PS, Maassen RJ, Stolk J *et al*. Antibacterial activity of antileukoprotease. *Infect Immun* 1996; 64:4520-4.

[97] McNeely TB, Dealy M, Dripps DJ *et al*. Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity *in vitro*. *J Clin Invest* 1995; 96:456-64.

[98] Jin F-Y, Nathan C, Radzioch D, Ding A. Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. *Cell* 1997; 88:417-26.

[99] Simpson AJ, Maxwell AI, Govan JRW *et al*. Elafin (elastase specific inhibitor) has antimicrobial activity against Gram positive and Gram negative respiratory pathogens. *FEBS Lett* 1999; 452:309-13.

[100] Dorin JR, Porteous DJ. Cystic fibrosis mice with disease related changes in lung and reproductive tracts. *Lancet* 1992; 340:984.

[101] Lamb JR, Tsitoura DC, Hoyne GF. Peptide mediated modulation of allergen specific immune responses. *Arb Paul Ehrlich Inst Bundesamt Sera Impstoffe Frankf A M* 1997; 91:14-20.

[102] Bergelson JM, Cunningham JA, Droguett G *et al*. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997; 275:1320-3.

[103] Qing K, Mah C, Hansen J *et al*. Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno associated virus 2. *Nature Medicine* 1999; 5:71-7.

[104] Summerford C, Bartlett JS, Samulski RJ. αVβ5 integrin: a co receptor for adeno-associated virus type 2 infection. *Nature Medicine* 1999; 5:78-82.

[105] Rogers BE, Douglas JT, Ahlem C *et al*. Use of a novel cross linking method to modify adenoviral tropism. *Gene Therapy* 1997; 4:1387-92.
[106] Dmitriev I, Krasnykh V, Miller CR *et al*. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. *J Virol* 1998; 72:9706-13.
[107] Glasser SW, Korfhagen TR, Wert SE, Whitsett JA. Transgenic models for study of pulmonary development and disease. *Am J Physiol* 1994; 267 (Lung Cell Mol Physiol 11): L489-L497.
[108] Strayer MS, Guttentag SH, Ballard PL. Targeting type II and Clara cells for adenovirus-mediated gene transfer using the surfactant protein B promoter. *Am J Respir Cell Mol Biol* 1998; 18:1-11.
[109] Kistner A, Gossen M, Zimmermann F *et al*. Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. *Proc Natl Acad Sci USA* 1996; 93:10933-8.
[110] No D, Yao T-P, Evans RM. Ecdysone-inducible gene expression in mammalian cells and transgenic mice. *Proc Natl Acad Sci USA* 1996; 93:3346-51.
[111] Parks RJ, Chen L, Anton U *et al*. A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proc Natl Acad Sci USA* 1996; 93:13565-70.
[112] Fisher KJ, Choi H, Burda J *et al*. Recombinant adenovirus deleted of all viral genes for gene therapy of cystic fibrosis. *Virology* 1996; 217:11-22.
[113] Mitani K, Graham FL, Caskey CT, Kochanek S. Rescue, propagation, and partial purification of a helper virus-dependent adenovirus vector. *Proc Natl Acad Sci USA* 1995; 92:3854-8.
[114] Scheidner G, Morral N, Parks RJ *et al*. Genomic DNA transfer with a high-capacity adenovirus vector results in improved *in vivo* gene expression and decreased toxicity. *Nature Genetics* 1998; 18:180-3.
[115] Yang Y, Trinchieri G, Wilson JM. Recombinant IL-12 prevents formation of blocking IgA antibodies to recombinant adenovirus and allows repeated gene therapy to mouse lung. *Nature Medicine* 1995; 1:890-3.
[116] Wilson C, Kay MA. Immunomodulation to enhance gene therapy. *Nature Medicine* 1995; 1:887-9.
[117] Wilson CB, Embree LJ, Schowalter D *et al*. Transient inhibition of CD28 and CD40 ligand interactions prolongs adenovirus-mediated transgene expression in the lung and facilitates expression after secondary vector administration. *J Virol* 1998; 72:7542-50.
[118] Zsengellér ZK, Boivin GP, Sawchuk SS *et al*. Anti-T cell receptor antibody prolongs transgene expression and reduces lung inflammation after adenovirus-mediated gene transfer. *Human Gene Therapy* 1997; 8:935-41.
[119] Ilan Y, Prakash R, Davidson A *et al*. Oral tolerization to adenoviral antigens permits long-term gene expression using recombinant adenoviral vectors. *J Clin Invest* 1997; 99:1098-106.
[120] Schowalter DB, Tubb JC, Liu M *et al*. Heterologous expression of adenovirus E3-gp19k in an E1a-deleted adenovirus vector inhibits MHC 1 expression *in vitro*, but does not prolong transgene expression *in vivo*. *Gene Therapy* 1997; 4:351-60.
[121] Chirmule N, Hughes JV, Gao G-P *et al*. Role of E4 in eliciting CD4 T-cell and B-cell responses to adenovirus vectors delivered to murine and nonhuman primate lungs. *J Virol* 1998; 72:6138-45.
[122] Vilquin J-T, Guérette B, Kinoshita I *et al*. FK506 immunosuppression to control the immune reactions triggered by first-generation adenovirus-mediated gene transfer. *Human Gene Therapy* 1995; 6:1391-401.
[123] Kaplan JM, Smith AE. Transient immunosuppression with deoxyspergualin improves longevity of transgene expression and ability to readminister adenoviral vector to the mouse lung. *Human Gene Therapy* 1997; 8:1095-104.
[124] Jooss K, Turka LA, Wilson JM. Blunting of immune responses to adenoviral vectors in mouse liver and lung with CTLA4Ig. *Gene Therapy* 1998; 5:309-19.
[125] Mack CA, Song W-R, Carpenter H *et al*. Circumvention of anti-adenovirus neutralizing immunity by administration of an adenoviral vector of an alternate serotype. *Human Gene Therapy* 1997; 8:99-109.
[126] Larson JE, Morrow SL, Happei L *et al*. Reversal of cystic fibrosis phenotype in mice by gene therapy *in utero*. *Lancet* 1997; 349:619-20.