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These are heady days for gene therapy. There have been some encouraging early reports from clinical trials, and research developments continue to hint at the awesome potential of such strategies. In March, a new Institute for Human Gene Therapy will open at the University of Pennsylvania, the first of its kind in the world. And last month's decision by the US Recombinant DNA Advisory Committee (RAC) to approve three independent proposals to conduct gene therapy trials for cystic fibrosis (CF) was potentially a major landmark, for two significant reasons.

Until now, gene therapy trials have been confined to various forms of cancer and a few rare hereditary diseases — adenine deaminase deficiency, haemophilia and familial hypercholesterolaemia, (caused by a defect in the low-density lipoprotein receptor). In contrast, CF is the most common autosomal recessive disease in people of European descent (affecting about 1 in 2,500 births) and so the expectancy attached to the forthcoming studies will be enormous. Three proposals, submitted by Ron Crystal (National Institutes of Health, NIH), James Wilson (University of Michigan) and Michael Welsh (University of Iowa) in conjunction with Alan Smith (Genzyme) were approved by the RAC, and now await the formal go-ahead from the directors of the NIH and the Food and Drug Administration.

The second key facet that the new proposals share is their use, for the first time, of adenoviruses to shuttle the normal CF gene into the nasal or airway epithelial cells of patients. Such an event is all the more remarkable considering that it was less than two years ago that researchers first glimpsed the potential of replication-deficient adenoviruses for delivering genes to cells *in vivo*. At that time, Crystal and colleagues demonstrated that the α_1 antitrypsin gene could be transferred into rat lung epithelial cells. Less than a year later, Crystal's group had shown that the CF gene product could also be synthesized effectively in cotton rat airway cells which had taken up the recombinant vector. A number of groups have shown the potential of adenoviruses for directing genes into the liver, skeletal and cardiac muscle and (in papers appearing shortly in *Nature Genetics*) even the central nervous system. Although these have been limited to control genes such as *lacZ* for the most part, it is only a matter of time before specific applications are sought.

Although Crystal's research has long focused on adenoviruses, Wilson and Welsh had initially used other viruses to introduce the CF gene into cultured CF cells *in vitro* and thereby restore normal ion transport properties. This was taken at the time to indicate that gene therapy for CF was feasible, but in order to pursue this *in vivo*, the benefits of adenoviruses could not be overlooked. Their chief advantage over the more commonly used retroviruses is the ability to infect a wide range of quiescent target cells, whereas retroviruses require actively dividing, post-mitotic cells. Adenoviruses are naturally drawn to airway epithelial cells, being a common cause of upper respiratory infections in humans. Moreover, the virus is easily purified and the available data suggest that appropriately modified

virus is likely to be safe.

The adenovirus genome consists of about 36 kb of double-stranded DNA, and all three proposals call for the CF gene to be substituted for the early region 1 genes. (The recombinant virus can be grown in a cell line that complements the missing E1 gene function.) Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally in the cell, so minimizing the risks associated with insertional mutagenesis. A theoretical concern is that despite the steps taken to disable the virus to prevent replication and transformation, including deletion of the E1 region and the choice of viral serotype, complementation with latent viral DNA in the host might occur, restoring the ability to replicate. Perhaps the most reassuring evidence that this is an extremely unlikely occurrence is the fact that live adenoviral vaccines have been administered to millions of US army recruits without any apparent ill-effects, and that there has never been a reported association between adenovirus and tumorigenesis.

In early October 1992, the three teams received approval from the internal review boards of their host institutions and submitted their proposals to the RAC within days of each other. Each proposal was assessed by a team of four reviewers with the additional help of Harold Ginsberg (Columbia University), a leading authority on adenoviruses brought in as an *ad hoc* consultant. With the potential benefits of the first use of adenoviruses for gene therapy for such a common and devastating disease as CF clearly outweighing the possible drawbacks, the final votes proved to be rather anticlimactic: all three proposals were passed unanimously, with almost as much discussion focusing on questions of patient reimbursement and the wording of consent forms as the design of the studies themselves. For example, despite the small risk that transfer of the gene into the germline might occur inadvertently, the RAC balked at one suggestion that only non-fertile individuals be allowed to take part.

A total of 25 patients will be examined in the three studies. Crystal and Wilson will administer the recombinant adenovirus to one lung in 10 and 12 patients respectively, looking at the effects of increasing dosage in pairs of subjects. The Welsh study is initially more modest, applying the virus just to the nasal epithelium of three individuals, but allowing for simple measurements of biological function. The first goals are to examine the safety, biological efficacy and dose response of the treatment — no long-term clinical relief is necessarily expected from just a single administration. But the list of volunteers for these trials may be a long one — even Robert Dresing, president of the Cystic Fibrosis Foundation, has said that his affected son may well volunteer.

Meanwhile, despite mixed reports for the dozen or more current clinical trials, including some for the cancer treatments, the progress for hereditary disorders is heartening. At the December RAC meeting, Wilson presented preliminary results in the treatment of a 29-year-old woman homozygous for LDL receptor deficiency, and facing severe risks of atherosclerosis and coronary disease. Last June, some 15% of her liver was surgically removed, the cells grown in culture (on a total of 800 ten mm Petri dishes) and transfected *ex vivo* with the LDL receptor retrovirus, before being perfused back into the patient's liver. Six months later, her serum cholesterol has been lowered, her risk of heart attack (inversely proportional to the ratio of 'good' to 'bad' cholesterol) has been halved, and Wilson hopes soon to recommence drug treatments to capitalize on the improvement. The RAC needed little encouragement to grant approval to extend the studies to a further four patients. Wilson will be leaving the University of Michigan in March to become director of the new Institute of Human Gene Therapy.

If gene therapy is to become a widely available clinical reality, the ease (and cost) of administration will be as important as safety and efficacy. Retroviruses are still the vector of choice for targeting stem cells, making them of prime importance for haematological disorders, but the ability of adenoviruses to transfer genetic material to a broad variety of tissues and internal organs makes them exciting candidates for *in vivo* forms of therapy; if the safety issues have truly been resolved, then their only principal drawback may be that the period of gene expression will be tied to cell turnover, requiring repeated administrations of the virus. Nevertheless, more and more tissues and diseases are likely to be investigated using adenoviral vectors. Taken together with the CF trial data, these will soon reveal whether this new form of genetic therapy will be realized. □