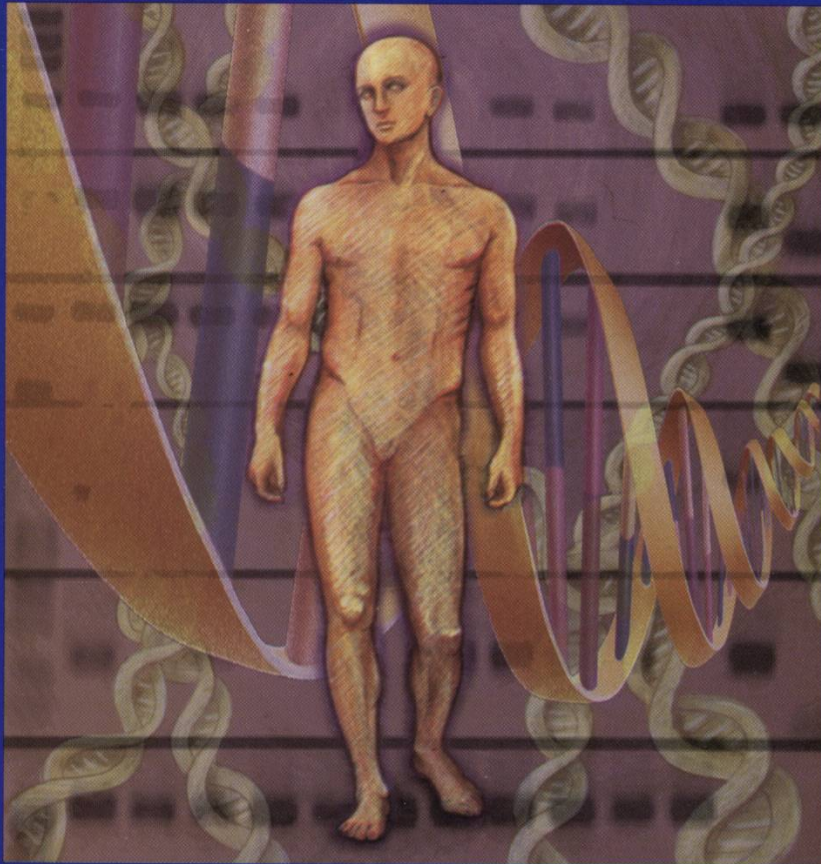


Supplement to

THE LANCET



Molecular medicine

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Supplement to *The Lancet*, July, 1999

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Gene therapy—where are we?

Alan E Smith

New technologies offer great promise in medicine, but at the same time face enormous hurdles during the early stages of development. Nowhere is this better illustrated than in recounting progress in gene therapy during the first decade of human clinical studies.

Gene therapy is the use of nucleic acids as therapeutically useful molecules (for reviews see^{1,2}). The approach has many potential applications, the most obvious of which is to correct the defects in monogenic inherited diseases such as cystic fibrosis (CF).³ However, many other applications are possible and these include gene transfer to stimulate a specific immune response,⁴ to mediate specific cell killing, to activate a pro-drug,⁵ or to produce a molecular decoy required for the replication of a virus.⁶

A vector is the vehicle used to introduce the gene into the target cell. Disabled viruses are commonly used because they can perform many of the tasks necessary to achieve successful gene transfer,⁷ such as bind to a target cell and deliver the viral genome to the nucleus for transcription. Non-viral vectors, based on plasmid DNA produced in bacteria and often complexed with lipids, are also being used since they lack foreign proteins and therefore may avoid immunological pitfalls common to engineered viral vectors—albeit at the cost of lower efficiency.⁸ The properties of commonly used vectors are summarised in panel 1.

dividing cells, ready monitoring of the transduction process, and reintroduction of a known number of transfected cells. More often, however, the vector is given *in vivo*, that is, directly to the patient (figure 1). Some of the first targets for gene therapy in human beings were bone-marrow cells, various solid tumours, lung, heart, skeletal muscle, and liver. The diseases most frequently studied have been cancer, AIDS, and CF. Figure 2 shows the gene-therapy protocols reviewed by the US Recombinant DNA Advisory Committee, and clinical protocols for all parts of the world can be accessed from this website: <http://www.wiley.co.uk/genetherapy/clinical/>

Early findings

The first therapeutic study of gene therapy was in patients with adenosine-deaminase deficiency. Mullen and colleagues⁹ used a retrovirus to introduce the gene for adenosine deaminase into lymphocytes *ex vivo*. Although the patients remained on protein therapy, the protocol appears to have had long-term clinical benefit in several patients with severe combined immune deficiency.⁹ Apart from this study, most trials of gene therapy have had an acceptable safety profile—but with limited clinical benefits. No formal phase III studies to establish clinical efficacy have been completed. Without going into details of particular clinical protocols, some generalisations are possible about the technical hurdles that have emerged

Panel 1: Characteristics of commonly used vectors

Vector	Genome	Enveloped	Insert capacity	Integration	Duration	Ease of manufacture	Comments
Viral							
Retrovirus	ssRNA	Yes	8–10 kb	Yes	Extended	Easy	Requires dividing cells
Adenovirus	dsDNA	No	7–8 kb	No	Transient	Very easy	Transduces wide range of cells especially liver
Gutted adenovirus	dsDNA	No	35 kb	No	Extended	Difficult	Improved inflammatory response and extended duration of expression
AAV	ssDNA	No	4.5 kb	unclear	Extended	Difficult at scale	Excellent duration in some tissues
Lentivirus	ssRNA	Yes	?	Yes	Extended	Difficult	Transduces non-dividing cells
Lipid based	dsDNA	No	Unlimited	No	Transient	Easy	Many lipids and formulations available
Naked DNA	dsDNA	No	Unlimited	No	Variable	Very easy	Extended duration in muscle

AAV=adeno-associated virus. ?=indicates no data available from published studies.

Few generalisations can be made as to vector selection or delivery method for any particular application, because each disease has its own specific requirements such as the target tissue and amount of gene-product required. Similarly, each vector has characteristics that may or may not be desirable for a particular application. Several early gene-therapy protocols have involved an *ex-vivo* approach, in which the gene is introduced into cells taken from the patient. This approach allows expansion of the cell population in culture, the use of vectors that need

from these and from extensive preclinical studies. These hurdles, and some of the possible approaches to resolve them, are shown in panel 2.

Hurdles and possible solutions

Identity of and access to the target cell

For many gene-therapy applications, the cell into which it is desirable to introduce the gene of interest is poorly characterised, or unknown, or not easily transfected *in vivo*. For example, although it is possible to enrich, to modify genetically, and to obtain long-term engraftment of mouse and monkey haemopoietic cells,¹⁰ this has proved much more elusive in human beings: in part, because the target progenitor cells are poorly characterised.¹¹ Further, although it is presumed that

Lancet 1999; 354 (suppl 1): 1–4

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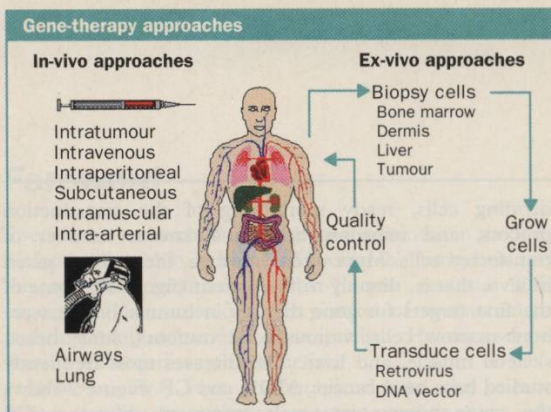


Figure 1: In-vivo and ex-vivo approaches to gene therapy

transfection of the airway epithelial cell with a gene correcting the genetic defect would benefit the patient with CF, mechanical barriers such as mucus and the ciliated surface limit the efficiency of gene transfer.

Efficacy of gene transfer

In clinical applications, by contrast to laboratory conditions, the extent of gene transfer and the level of gene expression is generally low. These shortcomings mean that high doses of viral vector¹² or of lipid/DNA complexes¹³ are usually required to achieve measurable gene transfer. High doses of any vector lead to concerns about safety and highlight the need to define the therapeutic window and avoid toxic doses of drug.

Duration of expression

The period during which a newly introduced gene is expressed is variable and differs with tissue, but is often short. For example, early-generation non-viral vectors express the gene at maximum levels for a few days.¹³ Many adenoviral vectors express the gene for only 2–3 weeks.¹⁴ By contrast, expression from adeno-associated viral vectors may not peak for several weeks, but then remain constant in some tissues for several months.¹⁵

Repeat dosing

Perhaps least surprising of all the limitations encountered during early studies of gene therapy is the reduced efficacy of repeat administrations of viral-based vectors.¹⁶ Humoral and perhaps mucosal immune responses to the incoming adenovirus vector can render subsequent administrations less effective,¹⁷ although this limitation may not apply to lipid-based vectors.

Safety

At high doses of viral vectors in vivo, inflammatory responses can be detected in animal studies. In the clinic, an adverse event was observed after bronchoscopic delivery of adenovirus¹⁸ and flu-like symptoms were seen in several patients after aerosol delivery of a lipid-based vector;¹⁹ in both studies the patients had CF. Apart from such innate immune responses, relatively few safety issues have been revealed in early clinical studies. Earlier concerns that integration of DNA sequences into the host genome would lead to oncogenic transformation—particularly with the use of retroviruses—or to germ-line transmission have subsided on the basis of extensive animal studies.^{20,21} However, great care is taken to ensure

that replication-competent virus is not present in either retrovirus or adenovirus vector preparations.²² Concerns about germ-line transmission require further study, as do the use of lentivirus-based vectors, long-term use of vectors in human beings, and administration to infants.

Clinical endpoints

Even though the applications of gene therapy attempted to date have primarily been safety studies, clinical benefit has proved surprisingly difficult to establish. In some patients with cancer, the effect of the transferred gene, as opposed to the viral vector itself, has been difficult to show unequivocally. Perhaps even more complex have been studies in CF, where changes in surrogate markers—such as transepithelial current to indicate clinical responses—have been challenging to measure in the most relevant tissues.^{14,23}

Altered expectations

Although early enthusiasm for gene therapy has been tempered by the hurdles described above, the field continues to hold much promise. New developments in vector design may overcome barriers of efficiency and duration of expression.^{24,25} Furthermore, increased understanding of the biology underlying some of the rate-limiting steps suggests that some of the barriers will be surmountable.²⁶ However, much of the excitement today is from recent developments that apply existing technology to more tractable clinical problems that may not be subject to the limitations of present vectors. For example, there are several potential applications where

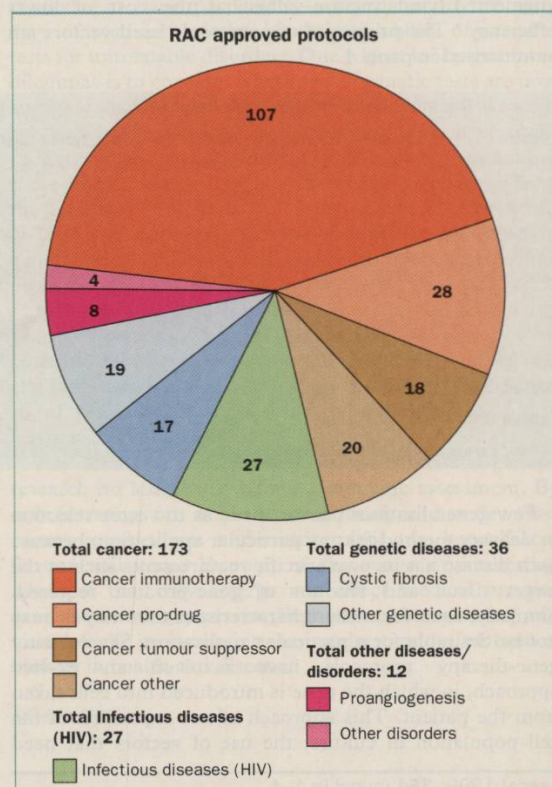


Figure 2: Summary of gene-therapy protocols reviewed by the US Recombinant DNA Advisory Committee as of February, 1999, categorised by disease. From <http://www.nih.gov/od/orca>

Panel 2: Some approaches to circumvent hurdles to successful gene transfer

Hurdle	Molecular or cellular basis	Possible approach
Identity of and access to target cell		
Target cell poorly characterised	Human pluripotent bone marrow	More basic research
	Human airway epithelium	More basic research
Target cell inaccessible	Solid metastatic tumours	Improved targeting
	Human airway epithelium	Remove mucus blanket
Efficacy of gene transfer		
Vector binding to target cell	Absence of viral receptors	Target to available receptor, formulate vector
Vector entry to target cell	Refractory tissue	Use any available receptor
Disassembly of virion/complex	Absence of entry pathway	Add endosomal sequences
Nucleic-acid transfer to nucleus	Inefficient intracellular transport	Add nuclear-localisation signals
Expression of gene	Vector transcriptionally silent	Use tissue-specific promoter
Duration of expression		
Promotor shut-down	Loss of necessary transcription factors	Use better promoter
Cells destroyed	CTL lysis of cell expressing viral or transgene proteins	Remove or reduce expression of CTL epitopes, Immune suppress, inhibit antigen presentation
Antibody to gene product	Antibody to vector encoded or therapeutic protein	Remove unnecessary sequences, tolerise
Cells turn over	Half-life of target cell	Readminister or target stem cell
Inflammatory responses		
Inflammation of target tissue	Cytokine-mediated cellular infiltration	Minimum dose
	Proinflammatory sequences in plasmid DNA	Remove proinflammatory vector sequence
		Anti-inflammatory intervention
Repeat dosing		
Repeat dose ineffective	Neutralising antibody to vector	Temporary immune suppression
		Mask viral epitopes
		Switch serotype
		Use non-viral vector
Clinical endpoints		
Poor or difficult clinical endpoints	Use surrogate markers	PCR methods to quantify mRNA and DNA
		FISH methods to locate DNA
		Disease-specific functional assays

CTL=cytotoxic T lymphocyte. FISH=fluorescent in-situ hybridisation.

expression of very modest amounts of a secreted gene product, in readily accessible cells, for a short period of time should be efficacious. One such application is the use of gene therapy to promote revascularisation of ischaemic tissue in coronary artery disease and peripheral vascular disease.^{27,28} The factors used to promote angiogenesis are very potent and have a bystander effect on adjacent tissue, such that the gene need not be expressed in large amounts, nor in every cell. In addition, such factors need to be expressed for only a limited period and the delivery vectors can easily be administered to the affected tissue with existing technology. A variety of genes and vectors have been effective in animal models of ischaemic disease and several have advanced to clinical trials. Indeed, early clinical data suggest that this may be the first unambiguously successful application of gene therapy to a common human disease.

Similarly, application of existing technology to immunotherapy of cancer has yielded encouraging preclinical data, which is currently being translated into new clinical approaches.^{4,29} Definitive data from trials in oncology and cardiovascular disease should become available within 2–3 years. To realise the full potential of the technology, however, in parallel with these clinical studies, intensive efforts must continue to develop improved vectors and to unravel the basic biology underlying gene-therapy applications and the diseases to which the technology is applied. It is also essential that the excellent collaboration between academia, industry, public and private funding agencies, and especially the regulatory authorities continues to flourish for the potential of gene therapy to be realised in a timely

fashion. Particular flexibility is necessary in the requirements for early clinical studies, in the use of surrogate markers, and the requirements for extremely rare diseases.

So where are we?

Although gene therapy is potentially a powerful clinical approach, it still lacks an unequivocal clinical success. Much was promised in the early days and to date little of that promise has been realised. This fact, however, must be seen as a failure of communication rather than a failure of technology. Novel drugs based on new technology, such as erythropoietin, can be introduced with phenomenal speed. This, however, is the exception rather than the rule and when it occurs it invariably involves a drug for an application that is well understood. More often, introduction is slower, especially when the underlying biology of the disease and of the drug are poorly understood—as was the case with interferons, interleukins, and monoclonal antibodies. Seen in this light, the question surrounding gene therapy today should be as “when” rather than “if”. Extensive preclinical work and early clinical trials have laid a solid scientific foundation. Preliminary data suggest gene therapy will be safe and have clearly defined the barriers to achieving clinical benefits in a number of indications. Optimism as to the eventual success remains high—exactly when it will happen though is harder to predict.

Acknowledgments

I thank Richard Gregory, David Meeker, and Sue Guy for help and advice in the preparation of this manuscript.

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