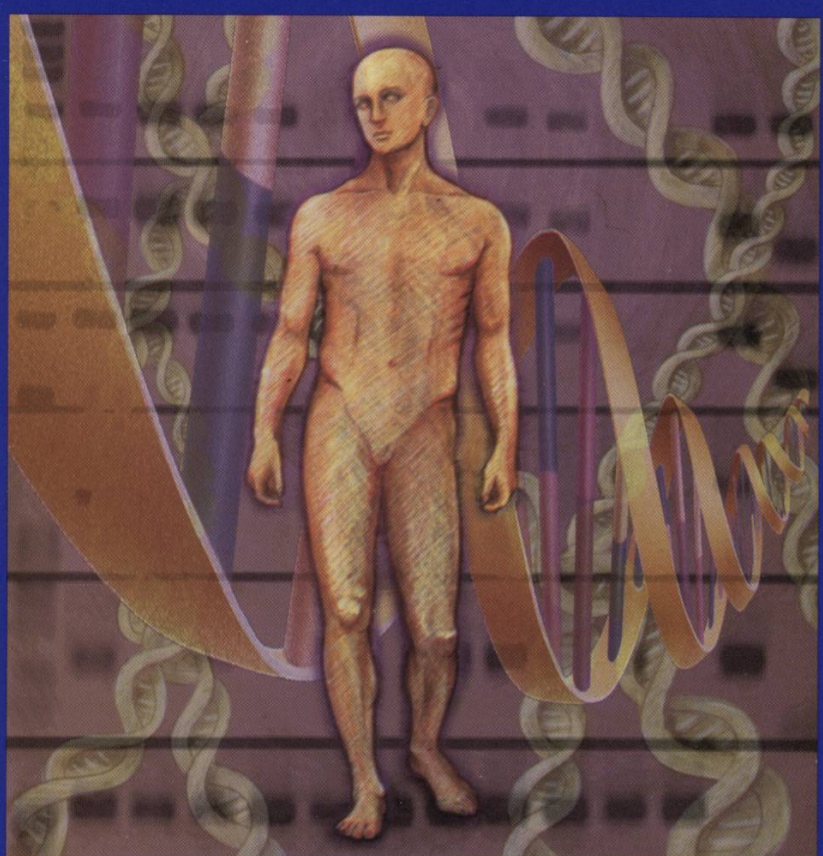
Supplement to

THE LANCET



Molecular medicine

Genzyme Ex. 2015
Sarepta Therapeutics, Inc. v. Genzyme Corporation
Page 1 of 6

Molecular medicine

Supplement to *The Lancet*, July, 1999

Contents

Foreword
Martin Bobrow

SI1 **Gene therapy—where are we?**
Alan E Smith

SI5 **The human genome project and the future of diagnostics, treatment, and prevention**
G J B van Ommen, E Bakker, J T den Dunnen

SI11 **Psychiatric genetics: progress, problems, and potential**
Michael J Owen, Alastair G Cardno

SI15 **Interpretation of results from genetic studies of multifactorial diseases**
John A Todd

SI17 **Mitochondrial DNA and disease**
P F Chinnery, D M Turnbull

SI22 **The genetics of prions—a contradiction in terms?**
Adriano Aguzzi, Sebastian Brandner

SI26 **The new biology: histopathology**
Phil Quirke, Nic Mapstone

SI32 **Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation**
Joseph P Vacanti, Robert Langer

SI35 **If I had a gene test, what would I have and who would I tell?**
Arno G Motulsky

Lancet 1999; **354** (suppl I): 1–37

Cover: Created by Valentina Candiani; based on an image supplied by Science Photo Library.

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.

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 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 colleagues[9] 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 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 I): 1–4

Genzyme Corporation, PO Box 9322, One Mountain Road, Framingham, MA 01701–9322, USA (AE Smith MD)

Correspondence to: Dr Alan E Smith

Genzyme Ex. 2015
Sarepta Therapeutics, Inc. v. Genzyme Corporation
Page 3 of 6

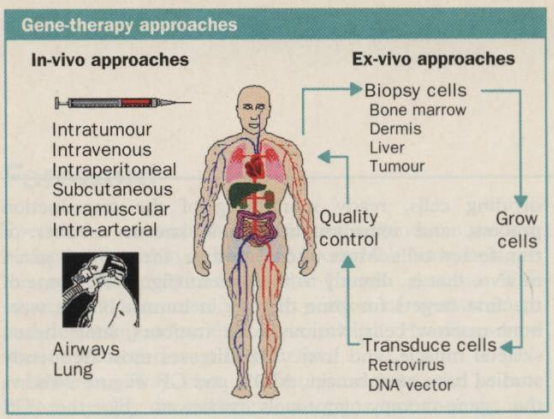


Figure 1: **In-vivo and ex-vivo approaches to gene therapy**

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 vector[12] or of lipid/DNA complexes[13] 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 admissions 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 adenovirus[18] 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

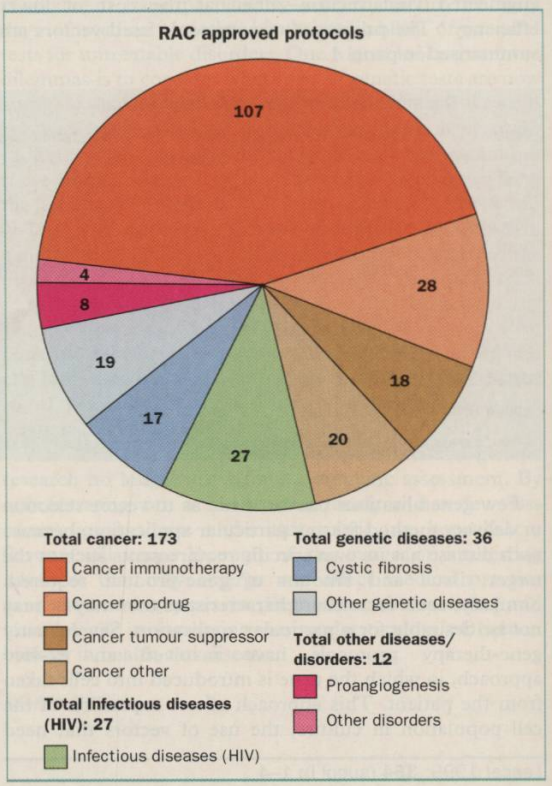


Figure 2: **Summary of gene-therapy protocols reviewed by the US Recombinant DNA Advisory Committee as of February, 1999, categorised by disease** From http://www.nih.gov/od/orda

Genzyme Ex. 2015
Sarepta Therapeutics, Inc. v. Genzyme Corporation
Page 4 of 6

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
Eficacy 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 endosomolytic sequences
Nucleic-acid transfer to nucleus	Inefficient intracellular transport	Add nuclear-localisation signals
Expression of gene	Vector transcriptionally silent	Use tissue-specific promotor
Duration of expression		
Promotor shut-down	Loss of necessary transcription factors	Use better promotor
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.

Genzyme Ex. 2015
Sarepta Therapeutics, Inc. v. Genzyme Corporation
Page 5 of 6

References

1 Verma IM. Human gene therapy. *Sci Am* 1990; **263:** 66–84.
2 Anderson WF. Human gene therapy. *Nature* 1998; **392** (suppl 6679): 25–30.
3 Boucher RC. Current status of CF gene therapy. *Trends Genet* 1996; **12:** 81–84.
4 Rosenberg SA, Zhai YF, Yang JC, et al. Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART–1 or gp100 melanoma antigens. *J Natl Cancer Inst* 1998; **90:** 1894–900.
5 Rigg A, Sikora K. Genetic prodrug activation therapy. *Mol Med Today* 1997; **3:** 359–66.
6 Lisziewicz J, Sun D, Smythe J, et al. Inhibition of human immunodeficiency virus type 1 replication by regulated expression of a polymeric Tat activation response RNA decoy as a strategy for gene therapy in AIDS. *Proc Natl Acad Sci USA* 1993; **90:** 8000–04.
7 Smith AE. Viral vectors in gene therapy. *Annu Rev Microbiol* 1995; **49:** 807–38.
8 Tseng WC, Huang L. Liposome-based gene therapy. *Pharmaceut Sci Technol Today* 1998; **1:** 206–13.
9 Mullen CA, Snitzer K, Culver KW, Morgan RA, Anderson WF, Blaese RM. Molecular analysis of T lymphocyte-directed gene therapy for adenosine deaminase deficiency: long-term expression in vivo of genes introduced with a retroviral vector. *Hum Gene Ther* 1996; **7:** 1123–29.
10 Kaptein LC, Van Beusechem VW, Riviere I, Mulligan RC, Valerio D. Long-term in vivo expression of the MFG-ADA retroviral vector in rhesus monkeys transplanted with transduced bone marrow cells. *Hum Gene Ther* 1997; **8:** 1605–10.
11 Goodell MA, Rosenzweig M, Kim H, et al. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med* 1997; **3:** 1337–45.
12 Pickles RJ, McCarty D, Matsui H, Hart PJ, Randell SH, Boucher RC. Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer. *J Virol* 1998; **72:** 6014–23.
13 Lee ER, Marshall J, Siegel CS, et al. Detailed analysis of structures and formulations of cationic lipids for efficient gene transfer to the lung. *Hum Gene Ther* 1996; **7:** 1701–17.
14 Armentano D, Zabner J, Sacks C, et al. Effect of the E4 region on the persistence of transgene expression from adenovirus vectors. *J Virol* 1997; **71:** 2408–16.
15 Fisher KJ, Jooss K, Alston J, et al. Recombinant adeno-associated virus for muscle directed gene therapy. *Nat Med* 1997; **3:** 306–12.
16 Yei S, Mittereder N, Tang K, O'Sullivan C, Trapnell BC Adenovirus-mediated gene transfer for cystic fibrosis: quantitative evaluation of repeated in vivo vector administration to the lung. *Gene Ther* 1994; **1:** 192–200.
17 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. *Hum Gene Ther* 1998; **9:** 2121–33.
18 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. *Nat Genet* 1994; **8:** 42–51.
19 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.
20 Tolstoshev P. Retroviral-mediated gene therapy—safety considerations and preclinical studies. *Bone Marrow Transpl* 1992; **9** (suppl 1): 148–50.
21 Ye X, Gao GP, Pabin C, Raper SE, Wilson JM. Evaluating the potential of germ line transmission after intravenous administration of recombinant adenovirus in the C3H mouse. *Hum Gen Ther* 1998; **9:** 2135–42.
22 Hehir KM, Armentano D, Cardoza LM, et al. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. *J Virol* 1996; **70:** 8459–67.
23 Zabner J, Couture LA, Gregory RJ, Graham SM, Smith AE, Welsh MJ. Adenovirus-mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis. *Cell* 1993; **75:** 207–16.
24 Schiedner 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. *Nat Genet* 1998; **18:** 180–83.
25 Kafri T, Blomer U, Peterson DA, Gage FH, Verma IM. Sustained expression of genes delivered directly into liver and muscle by lentiviral vectors. *Nat Genet* 1997; **17:** 314–17.
26 Scaria A, St George JA, Gregory RJ, et al. Antibody to CD40 ligand inhibits both humoral and cellular immune responses to adenoviral vectors and facilitates repeated administration to mouse airway. *Gene Ther* 1997; **4:** 611–17.
27 Losordo DW, Vale PR, Symes JF, et al. Gene therapy for myocardial angiogenesis—initial clinical results with direct myocardial injection of phVEGF(165) as sole therapy for myocardial ischemia. *Circulation* 1998; **98:** 2800–04.
28 Mack CA, Magovern CJ, Budenbender KT, et al. Salvage angiogenesis induced by adenovirus-mediated gene transfer of vascular endothelial growth factor protects against ischemic vascular occlusion. *J Vasc Surg* 1998; **27:** 699–709.
29 Simons JW, Jaffee EM, Weber CE, et al. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Res* 1997; **57:** 1537–46.

Genzyme Ex. 2015
Sarepta Therapeutics, Inc. v. Genzyme Corporation
Page 6 of 6