Focus

Gene therapy: Myths, pitfalls and lessons to learn

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** The author has included a glossary to explain the technical terms appeared in this article, please refer to page 6.*

Progress has been made in the discovery of new targets for improving the prevention, diagnosis and treatment of a great number of diseases. Scientists have developed novel ways to handle, manipulate and deliver biological materials including genes into the body to combat a wide array of symptoms and diseases. With more than 4,000 known genetic diseases, many of which have no satisfactory therapy, the prospect of a therapy using gene-based technology may present an attractive therapeutic option.

While the use of gene-based technology for treating diseases caused by single-gene defects seems to be the initial goal, the published literature shows that over 60% of the clinical trials approved on a global basis are designed for cancer treatment, and the United States is still leading on gene therapy research. Despite the initial claim of its potential widespread clinical use in the mid-1990s, there have been some very limited successes in using gene-based technology for clinical treatment. That said, it was reported in the press in the beginning of 2004 that the Chinese State Food and Drug Administration approved the first gene therapy product aimed at arresting the growth of tumour cells by targeting the p53 oncogene using a specific strain of adenovirus.

What is gene therapy or gene transfer?

Gene therapy is a result of recombinant DNA research. It encompasses the insertion of a "functioning" gene into the cells, and the gene product should cure or slow down the progression of a disease. The approach requires a technology that is capable of gene transfer in a variety of cells, tissues and organs. But the task of transporting genetic material into a cell with sufficient efficiency and specificity remains a challenge. There have now been distinct classes of delivery systems or vectors that can be used for gene transfer but none of them can be used universally for packaging the gene for all cell or tissue types or for all therapeutic indications. Most recently, researchers have been exploring the possibility of using "snippets" of complementary nucleotides to inhibit the product of gene expression at a messenger RNA level.

The target specificity, efficiency of delivery and expression of the therapeutic gene is of paramount importance in the design of any gene therapy protocol. Delivery vehicles used in the approved clinical studies can broadly fall into two categories:

1. Biological vectors that involve the use a micro-organism such as a virus or a bacterium in which the pathogenicity has been specifically engineered for its attenuation
2. Non-biological vectors, which utilise distinct physico-chemical properties conferred on the delivery vehicle to transfer the gene.

For site specific expression of the gene product, a tissue-specific promoter (which is a key element in starting gene expression) may be incorporated into the expression system so that the expression of the inserted gene is triggered only under the specific cellular environment. For certain diseases, in order to achieve sustained and high-level of gene expression, biological vectors such as viral vectors are the preferred choice. Unlike the non-biological vectors, which tend to produce fewer toxic or immunological problems, viral vectors, for example, because of the nature of the delivery system involved, require additional control tests aimed at ensuring the safety of gene transfer product. Emphasis has been placed on the understanding of the pathogenesis of the parental strain and its tropism or affinity for specific tissues and cell types or at what stage of the cell cycle the delivery vehicle will interact with the cells. Dose standardisation in terms of characteristics and functional gene expression following transfection are pivotal to defining the potency and safety of the gene transfer medicinal products. Even with this level of controls during in-process and product testing stages, there have been reported serious adverse events associated with certain types of viral delivery systems as discussed further below.

The first success

The first gene therapy experiment was initiated on September 14, 1990 at the National Institutes of Health for treating a rare, congenital immunodeficiency disorder called adenosine deaminase (ADA) deficiency (a sub-type of the severe combined immunodeficiency (SCID) characterised by severe and recurrent infections) under the leadership of Dr French Anderson. This disease was chosen for a number of reasons. Firstly, since the gene was cloned in 1983, there was a body of knowledge about the functionality of the gene. ADA being a "house-keeping" gene does not require tight regulation within the cells. Given the variation in enzymatic activity in normal individuals, there is a 500-fold safety margin for vector gene expression to be effective without producing adverse effects, a property not shared by many genes. Secondly, the gene delivery was mediated by using a murine retroviral vector, which can stably integrate the functional copies of the ADA gene into cultured ADA-deficient lymphocytes. Thirdly, the insertion of the normal gene into T-lymphocytes restored normal biochemical function. Fourthly, the

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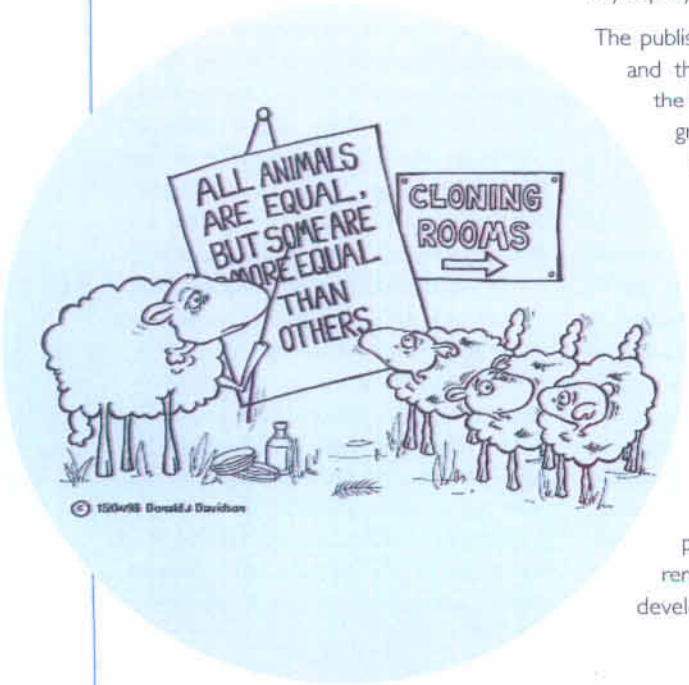
children who were enrolled in the US trials did not experience complete immunological reconstitution by other form of therapy, and therefore, gene replacement appeared to be, at that time, an attractive clinical alternative.

Using a similar viral delivery system based on a murine leukaemic retrovirus containing a functional gene encoding *yc* chain of the interleukin-2 receptor, it was reported in the early 2000s that this approach could be taken to achieve sustained correction of X-linked severe combined immunodeficiency (SCID) by *ex vivo* gene therapy. The most frequent form of SCID is due to deficiency of the common *y (yc)* chain, which is an essential element of five cytokine receptors, all of which are important for the development of T cells and natural killer (NK) cells. SCID is mostly fatal in the first year of life because of, among others, severe recurrent infections. In expert hands, live-related HLA-identical bone marrow transplantation can achieve a 90% chance of success, and with non-HLA identical matches 60-70%. In the early clinical study, the researchers set out to treat patients with the disease characterised by an early block in T and NK lymphocyte differentiation. It has been reported that the majority of the recipients showed evidence of immune reconstitution for a disease that would otherwise be fatal.



Adverse events

Acute leukaemic-like lymphoproliferative disorders

In 2002, the repeated occurrence of acute leukaemia-like lymphoproliferative disorders (ALL) in certain recipients of the experimental treatment that has been attributed to the insertion of the recombinant retrovirus at LMO-2 gene was reported by a team of clinical investigators. LMO-2 gene product belongs to a class of LIM-only protein and is located at a specific position of chromosome 11. The LMO-2 gene encodes a transcription factor that is required for normal haematopoiesis. An aberrant expression of this factor has been implicated in T cell acute lymphoblastic leukaemia. Previous studies in animals predicted that retrovirus-mediated gene transfer presented a potential but remote risk of insertional oncogenesis. Although there have been ongoing studies to delineate the underlying mechanism for the cause of these serious adverse events, reviews have been conducted by regulatory authorities including the European Committee for Medicinal Products for Human Use (CHMP) to evaluate the risks of insertional oncogenesis in retroviral gene therapy. The data reported so far in the literature seem to indicate that insertional activation of LMO-2 contributes to the risk of developing ALL disorders. There are potential cofactors that may equally contribute to such occurrence.

The published report made by the CHMP and the published literature identified the following. Firstly, it is likely that the growth advantage conferred on the transduced T cells by the therapeutic *yc* transgene may have played a role as a disease-specific risk factor. Secondly, the individuals who suffered from ALL disorder were of relatively young age that may be a predisposing risk factor to increasing the risk of an insertional oncogenesis event and hence a protocol-specific risk factor. Thirdly, the underlying pathogenesis of X-SCID may render these individuals prone to developing ALL disorder. It is noteworthy that a patient contracted chickenpox infection 30 months after gene transfer. Such a secondary event may provide a synergistic influence as the varicella zoster virus genome was detected in the T cell clone derived from this individual.

Tragic death

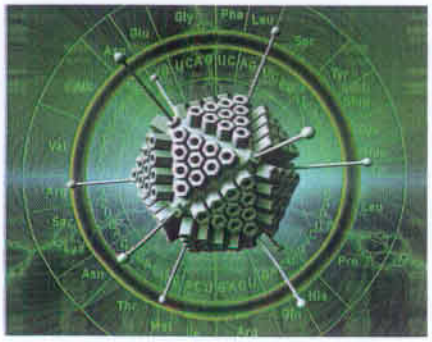
By the end of 2000, the US Food and Drug Administration stepped in to halt all eight gene therapy trials at the University of Pennsylvania. This regulatory measure resulted from the tragic death of a patient called Jesse Gelsinger, who was barely 18 years old. Gelsinger was the first patient who died during an early phase of gene therapy clinical study and his death could be directly attributed to an adenoviral vector. This particular individual suffered from a metabolic disease due to the deficiency of an enzyme, ornithine transcarbamoylase (OTC), which is required to break down ammonia. A total lack of or partial presence of this enzyme will lead to an accumulation of ammonia. The former will lead to death shortly after birth, and the latter can be controlled by drugs and dietary intake. Given the aetiology of the disease, transient production of the enzyme by adenoviral vectors could be a useful tool to correct the deficiency in the newborn and to allow implementation of a regime consisting of drug and dietary interventions. Gelsinger was in a cohort of patients with partial OTC activity who were given escalating doses of second-generation non-replicating adenoviral vectors. He was given up to 6×10^{13} viral particles containing the functional gene. Following intra-hepatic administration, he experienced fever, disseminated infiltrated coagulation without severe liver damage and acute respiratory depressive syndrome, all of which suggested some aspect of the immune system being activated.

Immediately following this, expert committees had been set up in various countries, but primarily in the US to address a number of questions pertaining to patient safety in gene therapy trials. There are a number of consensus views arising out of these reviews. Firstly, clinical monitoring is a powerful tool in enhancing the safety and

protection of trial subjects. Secondly, inclusion criteria should be carefully defined so that the enrolled patient group is appropriate, taking account of the severity of disease to be investigated, the route of administration and the duration of the study. Thirdly, requirements on informed consent should be strengthened. Fourthly, the need for accurate standardisation of dose was acknowledged so that vector potency could be compared on an inter-laboratory basis, and that has been an initiative actively pursued by gene therapy community as well as the regulatory authorities. Indeed, the International Conference on Harmonisation (ICH) has taken an interest in facilitating the validation and establishment of an interim reference material so that comparable results can be achieved in terms of infectious units: particle ratios in any given study or series of studies. Most recently, the European Pharmacopoeia Commission has developed a series of draft monographs pertaining to gene transfer medicinal products, one of which relates to the control of adenovirus vectors for human use.

European regulatory landscape

Since the adoption of the "Note for Guidance on the Quality, Pre-clinical and Clinical Aspects of Gene Transfer Medicinal Products" in April 2001, which replaces the previous guidance issued in 1995, the CHMP accepted the recommendations to constitute an Expert Group to review the current scientific and technical development in order to identify areas in the extant guidance that may require strengthening. The Expert Group first met in October 2001. All its scientific deliberations have now been published by the European Medicines Agency (EMEA) for the sake of transparency.



In the current Annex to Directive 2001/83/EC (as amended by Commission Directive 2003/63), a new category of medicinal products is created under the heading "advanced therapy medicinal products". Gene transfer produced bio-molecules is a member of this family of "advanced therapy medicinal products". According to the Annex, gene therapy medicinal product is defined as follows:

> *"...shall mean a product obtained through a set of manufacturing processes aimed at the transfer, to be performed either in vivo or ex vivo, of a prophylactic, diagnostic or therapeutic gene (ie, a piece of nucleic acid), to human/animal cells and its subsequent expression in vivo. The gene transfer involves an expression system contained in a delivery system known as a vector, which can be of viral as well as non-viral origin. The vector can also be included in a human or animal cell."*

For the purpose of regulatory submissions for a Marketing Authorisation, the Annex provides specific requirements pertaining to process control and product characteristics that largely mirror the non-binding recommendations made in the Note for Guidance. Indeed, the CPMP guidance places emphasis on the source, construction, characterisation and verification of the encoding gene sequence including its integrity and stability. All these factors directly influence the transcription and translation (that is the expression) of the inserted gene.

Furthermore, characterisation of the gene transfer vehicle is important in that it influences way the product is distributed *in vivo* and the way that the safety testing in animals and monitoring in humans are conducted. Recognising the complexity of this class of medicinal products, the Clinical Trials Directive 2001/20 creates an extended review time. In addition, written authorisation must be obtained before clinical trials can be commenced using medicinal products for gene therapy, somatic cell therapy including xenogeneic cell therapy and medicinal products containing genetically modified organisms. The Directive also places essentially a statutory ban on genetic modification of the germ-line resulting from gene transfer that is reflective of the bioethical concerns raised by the European Parliament and other bodies during the legislative development.

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Perspectives

In the past two decades, we have witnessed tremendous scientific developments in gene transfer, some of which have raised great expectations about its potential use in clinical practice. While there have been some limited successes in certain clinical studies, as illustrated above, a critical element in the evaluation of the present human gene transfer or therapy clinical protocols is the conduct of appropriate risk assessment by identifying the hazards and other relevant factors associated with a given gene transfer product that may form the rational basis for patient monitoring. It is therefore impossible to adopt a "one-size-fits-all" approach to a diversity of vectors that have been or are being developed for targeting various tissue or cell types.

In addition, the European Pharmacopoeia Commission has recently developed a number of draft monographs which may be used (as soon as they are adopted by the Commission following a period of public consultation) in conjunction of the EMEA regulatory guidance to aid research and development of gene-based therapeutic or prophylactic products. These monographs include those pertaining to the control of certain viral vectors, bacterial cells used for the manufacture of plasmid vectors and reagents used in manufacturing process of gene transfer products.

Disclaimer

The author practises regulatory and product liability law at Arnold & Porter's London office. This article does not represent the view of the firm or its clients or any advisory committees the author has previously served and currently serves.

Glossary

Acute lymphoblastic leukaemia - a type of cancer of the white blood cells

Adenovirus - a type of virus containing DNA as its genomic makeup

Cell differentiation - the process by which a cell changes from a stem (undifferentiated) cell to an adult cell, becoming modified and specialised for the performance of a specific function in the body

DNA - deoxyribonucleic acid

Genome - the complete DNA sequence of an organism containing its complete genetic information

Gene expression - a process whereby protein is synthesised from DNA through an intermediate called messenger RNA

Gene transfer - a given technique to transport genetic material into a cell

Haematopoeisis - the production of red blood cells and white blood cells in the bone marrow

HLA - human major histocompatibility complex which is a gene encoding cell surface molecules involved in antigen-specific interactions between T-lymphocytes and other cells

House keeping gene - gene that encodes enzymes required for the basic functions present in virtually all cells

Interleukin-2 - a protein made by a kind of white blood cells called T-lymphocytes when stimulated by an infection

Lymphocytes - originate from stem cells in the bone marrow. There are two populations of lymphocytes called T- and B-cells which are responsible for specific immunological responses.

Nucleotide - phosphorylated nucleotide, which consists of a purine or pyrimidine attached to ribose or deoxyribose at the 1'carbon. Each nucleotidyl residue is the basic building block of RNA and DNA.

Oncogene - a gene with the potential to cause cancer

Pathogenesis - biological process to cause a disease

Plasmid - a small closed loop of double stranded DNA containing an encoding gene that can independently replicate

Potency - a term used to define biological activities

Promoter - an essential element fix the site of initiation of switching on or off a gene and control the amount of messenger RNA and sometimes tissue specificity for gene expression

Recombinant DNA - DNA containing an inserted gene from a second source through cutting and rejoining.

Retrovirus - a type of virus which contains RNA as its genomic make-up. The virus contains an enzyme called a reverse transcriptase, an enzyme that synthesises DNA from RNA when entering into host cell

Transcription - a process whereby RNA is synthesised from DNA template

Transgene - foreign DNA

Varicella zoster virus - a typical viral rash illnesses of childhood, commonly known as chicken pox

Vector - a DNA segment capable of autonomous replication