Gene Therapy Technologies, Applications and Regulations: From Laboratory to Clinic

Gene Therapy Technologies, Applications and Regulations

FROM LABORATORY TO CLINIC

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Preface

There have been many new beginnings in the twentieth century regarding the treatment, prevention and diagnosis of human diseases and medical conditions. The latest, 'gene therapy', perhaps offers the greatest potential for radical new therapies. Gene therapy, as currently perceived, encompasses a set of procedures deliberately aimed at the efficient transfer of genetic material into the somatic cells of individual patients in order that the expression, or other function, of the part(s) of the genetic material designed to elicit preventive, therapeutic or diagnostic activity is fully functional and effective for its intended purpose. Originally, gene therapy was conceived as a way of correcting genetic defects in human inherited genetic disorders, of which there are now more than 4000 known. For most of these genetic disorders, there are no satisfactory treatments.

With increasing knowledge of human genes, gained largely through the Human Genome Project, it will eventually become possible to identify all of the genes and the mutations therein which underly genetic disorders. It should then be possible to contemplate replacing or compensating deleterious alleles with normal ones in those somatic cell populations most closely associated with disease manifestations. The precise correction of genetic defects is the ultimate goal of investigators developing gene therapies. The ideal strategy in inherited monogenic diseases would be to exchange a defective gene, or its damaged parts, for a normal gene by homologous recombination. However, while homologous recombination has been generally viewed as the best approach for gene correction, it is of far too low efficiency at present to be of any use. Moreover, current vector-mediated transgene delivery systems are unable to target specific sites within the human genome. They either deliver the transgene to cytoplasmic or episomal sites, where it remains unintegrated and is transiently expressed, or to random integration sites within nuclear chromosomal DNA, where it is expressed in unpredictable amounts. Therefore, at best, current gene therapies result in added normal (trans-) gene expression, which may fully or partly compensate genetic defects. It may be conceived that in somatic gene therapy of this kind the normal gene is used as a drug in the sense that it acts as the means for producing a gene product, a protein, *in situ*. The approach may be considered as only slightly different from using gene products themselves, e.g. insulin, factor VIII. However, somatic cell gene

therapy has not only the potential to ameliorate symptoms, but also appears to offer the prospect of a 'genetic cure' in sufferers of genetic disorders.

It has been quickly realised that there are wider applications of somatic gene therapy beyond treatments of genetic disorders that potentially could evolve as beneficial treatments for a whole range of human multifactorial diseases. These include cancers (malignancies), arthritis, atherosclerosis, neuropathies, autoimmunity and infectious diseases, such as human immunodeficiency virus (HIV) infections. In fact, the potential number of recipients of gene therapy in the latter category of diseases far outweighs that for the comparatively rare monogenic disorder category. Furthermore, in diseases such as cancer, there are several different metabolic and immunological targets that create a number of opportunities to design distinct and potentially efficacious gene-mediated therapies. For example, cancer cells can be induced to undergo cell suicide (apoptosis) by introduction of a normal gene, e.g. p53, to compensate a mutated one; they can be killed by conversion of a pro-drug into a toxic metabolite if the gene encoding the relevant converting enzyme is transferred to them, e.g. herpes simplex virus thymidine kinase with ganciclovir; they can be destroyed by leukocytes activated by (i) the expression of cytokine transgenes, e.g. interleukin-2, tumour necrosis factor alpha, or (ii) the expression of cell surface molecule transgenes which increase immune recognition, etc. In other diseases, in particular in HIV infections and AIDS, where there are a variety of symptomatic complications, a similar diversity of gene therapy strategies can be expected.

It should be emphasised that somatic cell gene therapy is still very much in its infancy as a treatment modality. First attempts to use gene therapy, for example for the treatment of β-thalassaemia in a clinical trial in 1980, were misguided, technologically unsound and not even ethically approved. It was another ten years before a sound, approved protocol was developed. At 12.52 p.m. on 14 September 1990, Dr Kenneth W. Culver started the infusion of genetically modified autologous lymphocytes into a four-year-old girl with adenosine deamidase (ADA) deficiency. This event, the true 'birth' of gene therapy, started the bandwagon rolling and it has been gathering speed ever since. In the short space of eight years or so there has been an exponential research effort to design and produce vectors to transfer genetic material to somatic cells and an unprecedented rush into clinical trials. It has to be said that gene therapy suffered media 'hype' and expectations were raised too high. Nevertheless, preliminary clinical studies, which have largely been phase I clinical trials to determine safety and feasibility, not efficacy, have looked promising. Furthermore, the obstacles to success, which appear formidable, are now appreciated. Scientific advances, technological improvements in vector design, and further under-

standing of pathophysiological mechanisms will be required to overcome the barriers that currently impede the successful application of gene therapy.

There are many concerns about the safe application of gene therapy. In this case, it is the nature of the therapeutic agent, i.e. potentially heritable DNA, that has raised attention and public concerns. Obviously, we, during our natural lives, endure endless assaults by potentially pathogenic and invasive microorganisms that 'load' us with their genetic material; most of it seems to be 'resisted', but some gets integrated, e.g. from retroviruses and lentiviruses. This is 'accidental', not deliberate as in gene therapy. Thus, ethical and moralistic issues are raised principally because gene therapy appears to be 'human genetic engineering'. Gene therapy involves the deliberate transfer of potentially heritable genetic material into human cells. At present, most nations have considered any intentional transfer of genetic material into germline cells to be unethical, even morally repugnant, since the 'added genes' could be transmitted to offspring, with unforeseen consequences. Therefore, the only gene therapy that has been permitted is that applied to human somatic cells, cells that are incapable of transmitting genetic material to offspring. Nevertheless, there remain several proponents for the use of germline gene therapy; arguments have been raised to the effect that it should not be completely banned as there may come a time when technological advances enable it to cure, and possibly eradicate, otherwise intractable genetic diseases. It is likely, however, that even if circumstances arose that warranted such applications, there would still be considerable opposition from those who consider the use of such technology morally wrong. One difficulty that would probably arise is how medical conditions and traits (natural variations) could be absolutely distinguished. How would baldness, for example, be considered? If germline gene therapy could prevent this late-developing condition/trait, then there might be pressure from 'sufferers' to try to avoid baldness in their offspring and future generations in their families. Would parents given the opportunity to produce 'brainier' children resist the temptation if germline gene therapy could offer this advantage? It is clear that in the future some difficult decisions will have to be made as technology progresses to the point where manipulation of the germline becomes a real feasibility.

If there are dangers and public conflicts about using germline gene therapy in the future, there are also certainly many aspects of somatic cell gene therapy as now practised that have come under the spotlight. These require our best scientific knowledge and regulatory frameworks to ensure the safe application of somatic cell gene therapies and continued public acceptance of these novel technologies and treatment modalities.

The feasibility of human somatic cell gene therapies has been enabled by pre-existing and developing technologies for preparing modified nucleic

acids and the means for transferring genetic material into cells. Currently, a number of possible ways of constructing delivery vehicles, otherwise known as vectors, are being investigated, with the ultimate goal of attaining efficient transfer of genetic material to intended target cell populations and, contingently, the appropriate level of transgene expression for resolution of the clinical condition being treated. In general, to form vectors, transgenes may either be linked into suitable plasmids and complexed with a variety of inorganic and organic chemical matrices, or be incorporated in the genomes of a number of different viruses, e.g. retroviruses, adenoviruses, adeno-associated viruses and herpes simplex viruses. Viral vectors have been, and continue to be, the primary choice of many investigators because they offer more efficient packaging and transfer of transgenes than complexed DNA, e.g. with cationic liposomes. However, despite the removal of certain viral genes to ensure the resulting viral vectors are replication-deficient except in specialised 'packaging' cell lines, there remain safety issues regarding their use. In addition, there remains much to be learnt with regard to targeting viral vectors to their intended target cell population and to the regulation of transgene expression once inside their target cell. Vectors may be used to transfer genes directly *in vivo* or to genetically modify cells in culture by an indirect *ex vivo* method. Both approaches have advantages and disadvantages. *Ex vivo* gene transfers with somatic cells that can be removed from the body and then put back once the cells are genetically altered and express the transgene product are more efficient and more readily controlled than *in vivo* methods. However, relatively few human tissues, e.g. haematopoietic, skin, endothelial and tumour cells, are amenable to the *ex vivo* gene transfer approach, since most tissue cells, e.g. from kidney, liver and brain, do not survive or grow well in culture. An alternative strategy of encapsulating genetically altered, allogeneic (or xenogeneic), cells, e.g. fibroblasts in immunoprotective polymers, and implanting these in relevant anatomical sites where they continuously secrete the transgene product without the danger of immunological rejection, may be, with further technological advances, a way of circumventing this limitation.

Potentially, there is a very wide applicability of somatic gene therapy to human diseases. Furthermore, in many diseases, a variety of gene-mediated therapeutic strategies appear of possible benefit. Therefore, there has been and is great interest from an increasing number of pharmaceutical companies seeking to explore and eventually exploit gene therapy technologies and products. Many of these technologies have originated on a small scale in R&D laboratories, but, as in the case of development and application of all other biological medicines, facilitation of technology transfer from laboratory to clinic requires appropriate quality controls and safety testing on gene therapy products (vectors and genetically modified cells), and procedures (protocols) are applied to (i) minimise patient risk, (ii) protect public health,

and (iii) maintain public confidence. As gene therapy technologies move towards major industrial commercialisation, discussions among representatives of regulatory bodies, health authorities, the medical profession, the scientific establishment and the pharmaceutical industry have taken place, and are still in progress, to develop regulatory frameworks that ensure the quality, safety, efficacy and ethical acceptability of products (vectors) and procedures (protocols). Such regulatory frameworks have to take account of the amorphous nature of gene therapy technologies and their nebulous applications and thus are decidedly complex. The aim of the regulators is, however, to ensure that products and procedures are safe and ethical to use without, at the same time, impeding the development of this emerging field of gene-mediated therapies.

This book sets out firstly to review many of the current technologies for preparing vectors for use in gene therapy protocols. Secondly, a range of medical conditions, including both inherited disorders and acquired diseases, that could potentially benefit from the application of gene-mediated therapies in somatic cells is well reviewed. The therapeutic potential of gene therapy protocols is, in many cases, compared with existing, alternative treatments. (Regrettably, cancer gene therapy could not be covered due to space limitations). Thirdly, regulatory themes are considered, including product quality and safety requirements. Finally, the transfer of technologies from laboratory to clinic is appraised with regard to the attendant requirements and facilities for (i) good laboratory practice (GLP) conditions in the R&D laboratory, (ii) large-scale production methods and good manufacturing practice (GMP) and (iii) current in-process and final product testing. The Chapters in this area provide information to those embarking on gene therapy technologies relevant to specifications of production and testing of products (and procedures) required to meet existing regulations, including quality, efficacy and safety considerations.

A panel of experts from the major industrialised countries, dealing with many aspects of human somatic gene therapy, have contributed to this book to ensure that a balanced view and global picture of the technologies, applications and regulatory requirements is presented. It is my hope that this volume will lead to the dissemination of the accumulated knowledge contained within and to the advancement of technologies and procedures pertinent to gene therapy.

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Anthony Meager
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