

Cancer Gene Therapy-Fantasy or Foresight?

H L Kong, *FAMS, MRCP (UK), M Med (Int Med)

Abstract

Gene therapy is an exciting new method of treatment that may have far-reaching implications on the way we manage diseases in the future. Cancer has become the principal focus of this futuristic research. The breathtaking pace of gene discovery in the last two decades, coupled with the birth of recombinant DNA technology, gave rise to the concept that genes may be manipulated and used as drugs. Genetic modification of cells can be carried out in petri dishes (ex vivo) or within the living system (in vivo). In order for the therapeutic genes to exert their effect, they have to be transported into the cell nucleus where transcription takes place. Liposomes and genetically-modified viruses have been extensively used as gene vectors. The ideal vector remains elusive. It would be one that can achieve tumour-specific, sustained, and regulatable gene expression without host toxicity. As a result of the past decade of intense gene therapy research, we have learned that it is a rational scientific concept that works remarkably well in petri dishes and in laboratory animals. However, early clinical gene therapy experimentations paled in comparison. This apparent disparity between dramatic preclinical successes and the very modest clinical results of gene therapy does not in anyway nullify the concept of gene therapy. Instead, it exposes the folly of underestimating the technical complexity of gene manipulation in human diseases. Fortunately, technical hurdles such as those confronting gene therapy today are not insurmountable; they need, however, much ingenuity, resolution and time to be overcome. It is reassuring that recent advances in gene therapy provide abundant evidence that the premature infant, born of unrealistic pressure, is indeed healthy and thriving. With proper nurturing and patience, there is no doubt that, in time, it will bear fruit.

Ann Acad Med Singapore 1999; 28:409-16

Key words: Antiangiogenesis, Gene replacement, Immunotherapy, Prodrug, Vectors

Introduction

Genes define life, at least in its biological sense. The prospect of being able to manipulate human biological processes through genetic engineering understandably evokes a sense of excitement and omnipotence. Indeed, the birth of recombinant DNA technology in the 1970s immediately opened the floodgates for the successful manipulation of plant and animal genes, principally for research and agricultural purposes. It did not take a very long time before investigators proposed that human genes might similarly be engineered for therapeutic purposes. The prospect of being able to reprogramme a human being's blueprint of life is so titillating that a massive international effort to perfect the technology was unleashed a decade ago. The epic story of human gene therapy that followed relates a tale of what is arguably the most audacious medical experiment in history, the first large-scale attempt to reprogramme the genetic code of living human beings. The following account explains the rationale and the basic concepts of gene therapy, especially as it relates to cancer. It also illustrates the vast potential of gene therapy with pre-clinical and clinical examples. Finally, it diagnoses the

ailments that have plagued first-generation gene therapy, and speculates on the future of this exciting and novel therapeutic. Sceptics and scoffers would immediately discount gene therapy as a 'star-war' kind of futuristic fantasy. No matter, for this was the same sentiment that taunted the Wright Brothers when they built the first plane in 1903.

Historical Perspectives

In their landmark paper describing nucleic acids as the basis of hereditary, Avery et al¹ began the article with a prescient sentence: "Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters." These early investigators were of course thinking of plants and poultry then. However, by 1960s, farsighted investigators such as Tatum² speculated that: "treatment (of human cancers) could be achieved by modification and regulation of gene activities, or by means of gene repair or replacement. Aposhian³ proclaimed that: "if one considers the purpose of a drug to be to restore the normal function of some particular processes in the body, then

* Assistant Professor
Department of Medicine (Medical Oncology)
National University of Singapore

Address for Reprints: Dr Kong Hwai Loong, Department of Medical Oncology, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074.

DNA would be considered to be the ultimate drug.” What these bold visions lacked then were the appropriate molecular tools to achieve this lofty goal. Following the discovery of nucleic acid restriction enzymes (Arber 1962), genetic code (Nirenberg, Ochoa, Khorana 1966), DNA ligase (Gellert 1967), DNA cloning (Boyer, Cohen, Berg 1973), rapid DNA sequencing (Sanger and Barrell; Maxam and Gilbert 1977), and polymerase chain reaction (Mullis 1985), the vision of earlier investigators to manipulate human genes for therapeutic purposes became increasingly clearer.

Armed with powerful molecular tools to sculpture the genes, a growing number of investigators in the 1980s successfully transferred a number of genes into living cells in culture, as well as in laboratory animals which harboured conditions that simulated human diseases. Impressive treatment results were commonly seen. As animal models improved incrementally to resemble human diseases more closely, the impetus to cross the barrier between bench and bedside grew increasingly more cogent. In September 1990, the National Institute of Health of United States (NIH, USA) launched its first formal human gene therapy trial, using the human adenosine deaminase gene to treat two children with severe combined immunodeficiency disease (SCID).⁴ In the last 8 years since this epochal feat, there have been more than 260 human gene therapy trials approved or under review world-wide, with more than 2100 patients having been treated so far.⁵ In the same period, more than 7000 papers on various topics in gene therapy were published, precipitating the birth of 3 new international journals dedicated solely to the advancement of gene therapy.

Fundamentals of Gene Therapy

Rationale

Gene therapy, in the broadest sense, can be defined as the introduction of nucleic acids into cells for therapeutic purposes. In essence, it is the use of genes as drugs. One may conceive gene therapy as a form of ‘slow-release’ preparation, expressing the therapeutic drug over a prolonged period of time.⁶⁻⁹ Gene therapy is predicated on the understanding that the genetic blueprint of a cell defines its behaviour through the range of proteins it expresses (Fig. 1). Hereditary or acquired insults that damage the genetic content of a cell may culminate in the expression of abnormal proteins, or the lack of expression of normal proteins, either of which would dictate the cell to behave aberrantly.

Using this paradigm, the original concept of gene therapy is easy to understand—one of gene replacement. By introducing specific genes which are known to be missing or defective in the target cells, gene replacement restores normal cellular functions. A good example is the replacement of cystic fibrosis transmembrane

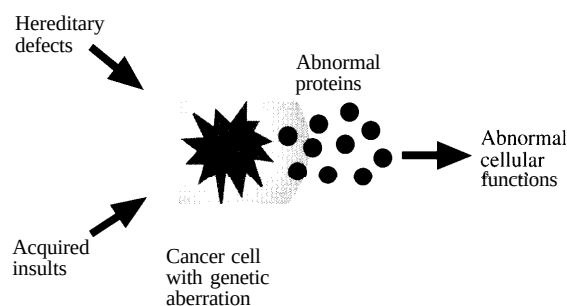


Fig. 1. Genetic basis of carcinogenesis. A wide array of hereditary and acquired factors may alter the genetic content of normal cells, culminating in the expression of abnormal proteins, which in turn lead to aberrant cellular functions that define the traits of cancer cells.

conductance regulator (CFTR) gene in subjects who suffer from cystic fibrosis.^{10,11} It is obvious that such an approach is useful mainly for monogenic diseases, and that a sufficient number of the target cells will have to be genetically modified (transduced) in order for the strategy to work. The alternative approach, which is more versatile, involves the introduction of genes into cells, not necessarily diseased cells, so that new cellular functions are created which become therapeutic to the diseased host. One could, for instance, transduce normal fibroblasts with genes to produce cytokines that inhibit the proliferation of cancer cells somewhere in the body.^{12,13}

It is important to distinguish gene therapy from gene marking and genetic enhancement. Gene marking refers to the process in which cells are genetically tagged so that their biology can be better studied; there is no direct therapeutic intent in such a manoeuvre. One outstanding example of gene marking experiment is the elucidation of the source of relapse in patients with acute myeloid leukaemia (AML) following autologous bone marrow transplantation. The bone marrow stem cells harvested from AML patients in apparent clinical remission are genetically marked. Studies have definitively shown that such harvested marrow may actually contain residual tumour cells that can contribute to leukaemia recurrence following reinfusion.¹⁴ Genetic enhancement refers to the process of conferring a desirable attribute to the host who is not diseased with respect to this trait; the intent is cosmetic or other non-medical reasons. Genetic enhancement in human is generally frowned upon. Finally, there should also be a distinction between somatic gene therapy and germline gene therapy. The latter carries with it the potential to alter the population gene pool through successive propagation of the genetic modifications, with resultant profound biological and social ramifications. It is therefore not recommended at the present time.

Methods of Gene Transfer

In general, gene transfer can be accomplished through either *ex vivo* or *in vivo* methods. In the *ex vivo* method,

the target cells are first removed from the subject and introduced into culture. They are then transduced with the therapeutic genes in petri dishes before they are returned to the subject. This method is assured of a high degree of target specificity, but is cumbersome, and suffers from the potential risk of contamination during the *ex vivo* manipulation. In contrast, the *in vivo* method involves simply the direct injection of the therapeutic genes into the body, very much akin to the way drug injections are being administered nowadays. However, the *in vivo* method is confronted with the arduous task of faithfully delivering the gene from the site of administration, through the various host barriers, to the target cells eventually. Targeting may therefore become a limiting factor to the success of *in vivo* gene transfer. The choice between *ex vivo* and *in vivo* approaches depends on the particular application.

Gene Vectors

Whether using the *ex vivo* or the *in vivo* method of gene transfer, an efficient transport mechanism is critical to ensure that the therapeutic gene is successfully translocated from cell exterior to the cell nucleus where the transcriptional machinery resides (Fig. 2). Such a gene transport vehicle is known as a gene vector. Gene vectors in use today are broadly classified into viruses and non-viral agents.^{7,9,15,16} Non-viral approaches include the injection of naked DNA, the use of gene gun to propel DNA-coated gold particles into the tissue, and the use of liposomes to facilitate DNA entry across the cell membrane.¹⁷⁻¹⁹ Non-viral approaches are relatively simpler to use, usually not limited by the gene insert capacity, and free of potentially toxic foreign proteins in the preparation. However, the transduction efficiency is often low, and the level of gene expression is also limited by the lysosomal degradation of the internalised particles.

The use of viruses as vehicles of gene transport was first conceived in the 1960s. Lederberg (1962) predicted that: "it will only be a matter of time, and perhaps not a long time, before polynucleotide sequences can be grafted by chemical procedures onto a virus DNA." Over the

millions of years, viruses have honed to perfection an incredibly complex yet efficient system to transfer their genetic content into the cells they infect. With a better understanding of which viral genes are pathogenic to humans, genetic modifications of viruses are now possible that render them non-pathogenic, but yet retain their innate ability to efficiently transfer their genetic payload.^{7,20,21} Viral vectors are now considered to be the gold standard in *in vivo* gene transfer. The list of viruses that have been manipulated to serve as gene carrier is growing steadily, including retrovirus, adenovirus, adeno-associated virus, herpes simplex virus, poxvirus, and lentivirus.²¹⁻²³ Each of these viruses has its unique biological characteristics which we can take advantage of. For instance, adenovirus exhibits a natural tropism for respiratory epithelium and liver tissue; adeno-associated virus works very well in the central nervous system and in the muscle; while herpes simplex virus has a predilection for neural tissues. Retrovirus infects only dividing cells, while adenovirus infects both dividing and non-dividing cells. Retrovirus, by virtue of its ability to integrate the therapeutic genes into host genome, is capable of prolonged gene expression, while adenoviral-mediated gene expression tends to be short. There is as yet no perfect vector. What we do have is an expanding array of gene transport specialists from which we can choose, according to the requirement of the particular gene transfer mission.

Preclinical Cancer Gene Therapy

Cancer results from uncontrolled cell proliferation, which in turn is driven by a number of genetic switches that have gone awry. Over the past two to three decades, we have witnessed an explosion in our basic understanding of these genetic switches. Given the possession of this powerful basic knowledge, coupled with the relative ineffectiveness of conventional cancer therapeutics against many malignancies, it is no surprise that cancer has naturally become the obvious target of gene therapy experimentation. The process of tumourigenesis is a cascade of molecular events, many of which represent attractive targets for genetic intervention. The major strategies in cancer gene therapy are summarised here (Table I).

- a. Gene replacement strategy is the easiest to understand. Many tumours have deleted or mutated p53 tumour suppressor gene.²⁴ Replacement of the missing p53 function through gene transfer has been demonstrated in a large number of animal experiments. In many instances, successful gene transduction is associated with an increased apoptotic rate, decreased angiogenesis, antitumour effect, and prolongation of survival.²⁵⁻²⁷ Retinoblastoma gene replacement has also been carried out successfully in experimental tumour models.²⁸

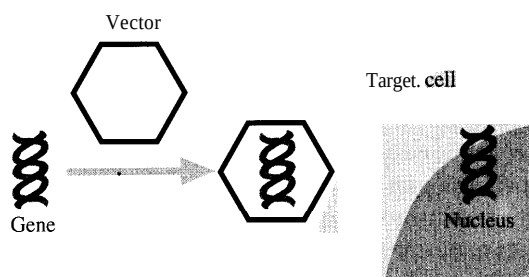


Fig. 2. Gene vector. Genetically-modified viruses and non-viral agents are used to efficiently translocate the therapeutic genes from cell exterior to the nucleus, where the transcriptional machinery resides.

TABLE I: PRINCIPAL CANCER GENE THERAPY STRATEGIES

Strategy	Description
Gene replacement	Transfer of wild-type tumour suppressor genes, such as p53 gene or retinoblastoma gene, to cells deficient in them
Anti-oncogene	Use of antisense deoxynucleotide to bind to mRNA, thereby promoting mRNA breakdown by ribonucleases; or use of ribozyme to cleave oncogene or drug resistance gene
Prodrug-activation	Conversion of relatively innocuous prodrugs to toxic metabolites within transduced cells
Multidrug resistance gene transfer	Transfer of multidrug resistance gene to normal bone marrow stem cells to protect them against myeloablative effect of chemotherapy
Genetic immunopotentialiation	Use of genes to generate tumour-specific or non-specific immunity
Pro-apoptotic gene transfer	Promotion of apoptosis (programmed cell death) in tumour cells
Gene-mediated antiangiogenesis	Transfer of genes which suppress tumour-associated angiogenesis, on the premise that tumour growth and metastasis depend heavily on neovascularisation

- b. Conversely, oncogenes can be switched off using antisense deoxynucleotides which are complementary to the messenger RNAs (mRNAs) that are coding for abnormal proteins. These oligonucleotides form RNA-DNA hybrids that are preferentially degraded by ribonucleases, thereby destroying the mRNA. Prime molecular targets for this approach include the *bcr/abl* oncogene in chronic myeloid leukaemia,^{29,30} and the *ras* oncogene which is mutated in a number of solid tumours.³¹ A related scheme involves the use of ribozymes, RNA molecules with catalytic activity, to cleave transcripts of particular oncogenes or drug resistance genes, such as *Bcl-2*.³²
- c. Another cancer gene therapy approach that is very popular is the “Trojan Horse” approach, which involves the use of prodrug-activating genes (‘suicide genes’). Cancer cells which are transduced with the suicide genes will convert relatively innocuous prodrugs to toxic metabolites within the cells; these metabolites in turn kill the very cells in which the drug activation takes place. Outstanding examples of suicide gene/prodrug combinations include: herpes simplex virus thymidine kinase (HSV-tk) gene/ganciclovir (forming toxic ganciclovir triphosphate)^{33,34} and *Escherichia coli* cytosine deaminase (CD) gene/5-fluorocytosine (forming toxic 5-fluorouracil).^{35,36} Unlike the gene replacement approach and the anti-oncogene approach which require most cancer cells within the body to be genetically modified for the strategy to be successful, suicide gene

therapy approach has a very prominent bystander antitumour effect. Gene transduction of as few as 10% of the cancer cells is associated with cell death in the remaining 90% non-transduced cells as well. This phenomenon has been attributed to the transfer of toxic metabolites through intercellular gap junctions, and/or the generation of tumour-specific immunity.^{37,38} The latter hypothesis may explain why suicide gene transfer to a tumour nodule can lead to regression of its distant metastases.

- d. The multidrug resistance gene (MDR-1) has been transferred to normal bone marrow cells, rendering them highly resistant to the ablative effect of conventional cytotoxic drugs.³⁹ This approach is distinct from the above-mentioned ones in that the target cells for gene transfer are the normal cell populations, and not the cancer cells per se.
- e. Genetic immunopotentialiation is a very promising gene therapy approach. Ample experimental and some clinical data have shown that revving up the host immunity may be accompanied by antitumour effect. Specific strategies include the introduction of cytokine genes (e.g. interleukins, interferons, granulocyte-monocyte colony stimulating factor, tumour necrosis factor) to either tumour cells or normal stromal cells such as the fibroblasts; the transfer of foreign class I MHC molecules or costimulatory molecules; and the genetic modification of dendritic cells to express tumour-specific antigens.⁴⁰⁻⁴³ The major strengths of genetic immunotherapy are its long-lasting antitumour effect, and its ability to treat disseminated tumours. More recently, the direct injection of naked DNA under the skin or into the muscle, a process also known as DNA cancer vaccination, has seen some very impressive antitumour effects in experimental models.⁴⁴
- f. Inactivation of apoptotic pathways is one of the key mechanisms responsible for tumour formation and progression. Transfer of pro-apoptotic genes such as *p53*, *bcl-xs* and *Fas* ligand has been shown to suppress tumour growth.⁴⁵
- g. Finally, a new and exciting gene therapy approach has been developed in the past 2 years. It involves the use of genes which inhibit tumour neovascularisation.^{6,46-49} Given that growth and metastasis of practically all tumours are critically dependent on the formation of new capillary network within the milieu of the tumour, the vast potential of this approach is easy to appreciate. Indeed, gene-mediated antiangiogenesis may be applicable across a broad range of tumour types.⁶ In addition, there is evidence that gene-mediated tumour antiangiogenesis effects can be made regional; one can restrict the inhibition of angiogenesis to only the organs that bear the tumours, thereby sparing physiological angiogenesis in normal organs in the body.^{6,46}

In summary, the thousands of preclinical gene therapy publications attest to the fact that gene therapy, as a proof of principle, is clearly feasible. The results in experimental systems are often very dramatic. However, it is important to realise that men are not large mice. Besides the obvious differences in size and outer appearances, there exist a host of genetic, immunological and other biological differences between the two species. For instance, laboratory mice are usually inbreds while humans are not. As such, when one attempts to predict the clinical utility of gene therapy based on *in vitro* or animal data, one has to be cognizant that such extrapolation may be fraught with pitfalls and oversimplifications. Therefore, in ascertaining the clinical usefulness of cancer gene therapy, there is no alternative to carrying out well-conducted clinical trials.

Clinical Gene Therapy Trials

The initiation of the first clinical gene therapy trial in US in 1990 paved the way for a deluge of human gene therapy activities, most of which were phase I in design, seeking to address the issue of whether gene therapy is a tenable biological concept in human beings. About 60% of all human gene therapy trials approved in US pertain to a wide array of cancers (Table II).⁵ Some of the popular cancer models in clinical trials include melanoma (by virtue of its well-known immunoresponsiveness) and primary brain tumour (by virtue of its confined nature). HSV-tk suicide gene therapy and genetic immunopotentialisation are approaches that have been most

commonly employed in clinical trials so far. In the past 8 years of human gene therapy endeavour, we have learned that it is relatively safe to introduce foreign therapeutic genes into human host, and that such gene transfer very often leads to the occurrence of the expected biological effects.⁵⁰⁻⁵³ For instance, following the introduction of cytokine genes, the relevant cytokines can be measured in the host tissues. No carcinogenesis or diseases attributable to the inadvertent replication of viruses have been reported. Further, health care workers dealing with viral vectors have not been adversely affected.²⁶

Disappointingly, however, there is very little hard evidence so far that these first-generation human gene therapy trials were able to achieve any sustained and clinically-meaningful antitumour effect. Although anecdotal accounts of impressive treatment effects have been reported, these were the exceptions rather than the rule.⁵⁰⁻⁵³ Therefore, one might be forgiven for asking: what has gone wrong? How could a highly rational and extensively-validated experimental concept flounder so haplessly in humans? The initial enthusiasm, or more aptly euphoria, of the early 1990s quickly dwindled in mid-1990s; in its place instead was a sense of disillusionment and pessimism. Fortunately, the field of gene therapy is not lacking in tenacious and resilient investigators. Through their efforts in the recent years, the reasons for the initial failures soon became apparent, and before long, the next wave of renewed efforts were already underway.

Rationalisation of Initial Failures

Given a concept as biologically rational and therapeutically exciting as gene therapy, it is easy to fall prey to over-interpretation of the dramatic results of *in vitro* and animal experiments. As a result of incessant media exposure in the past few years, the society rapidly became gene-literate, at least superficially. This led to an unhealthy and growing expectation in the early 1990s of what gene therapy can achieve in human within a very short time. The experts and laymen have both underestimated the one-upmanship of cancer and the immense complexities of successful gene therapy in human beings. Some of these complexities include:

- The short duration of gene expression with several vector systems, due to host immunity against viral vectors, and gene promoter shut-down. In the case of adenoviral vectors, it is clear that *in vivo* administration leads to the generation of humoral as well as cell-mediated host immune responses that tend to limit the duration of gene expression, and to restrict the efficacy of subsequent administrations.^{54,55} The use of less immunogenic viral vectors is therefore an important avenue of future research.
- Inability of the gene vectors to penetrate deeply into

TABLE II: SUMMARY OF CANCER GENE THERAPY TRIALS WORLD-WIDE, 1998*

Cancer	Number
Melanoma	32
Primary brain tumour	17
Prostate	14
Ovary	9
Lung	8
Breast	8
Leukaemia	7
Head and neck	6
Colon	5
Neuroblastoma	5
Renal	4
Liver (primary and secondary)	4
Lymphoma	4
Bladder	2
Mesothelioma	2
Cervix	1
Multiple myeloma	2
Germ cell tumour	1
Trials with more than 1 cancer	31
Total	162

* Hum Gene Ther 1998; 12:1803-47

the tumour bulk, resulting in only superficial (2-3 cell layer thick) gene transduction and hence lack of efficacy. It is sobering to remember that a 1 cm³ tumour contains 10⁹ tumour cells packed in a tight sphere. With currently available technology, any attempt to transduce every cell in this sphere is almost certain to fail.

- c. Inability to tackle disseminated tumours using strategies such as gene replacement and oncogene inactivation, which are essentially single-cell therapies.

Besides these technical hurdles, trial design factors are also responsible for the seemingly poor results with first-generation human gene therapy trials. Firstly, most of these trials were phase I studies, seeking to demonstrate biological effects and toxicities rather than treatment efficacy. The latter end-point is better assessed formally in late phase II and phase III studies. Secondly, most of the patients accrued in the cancer gene therapy trials had advanced, bulky, and disseminated cancers, and had been heavily pretreated with other conventional cancer therapies. It would be very difficult indeed to obtain any better result with this group of patients.

Most importantly, early enthusiasts have failed to appreciate that the perfection of any new medical technology, especially one so complex as gene therapy, will take time. The field needs time to get over the many fundamental obstacles confronting it before it can make any practical impact on the lives of our cancer patients.

Future Developments

Contrary to the negative impression given by the early clinical gene therapy trials, the future of gene therapy actually looks bright and healthy. One should not overlook the fact that gene therapy is predicated on sound logic, inspired by bold visions and farsightedness, backed by numerous experimental validations, and most importantly, driven by investigators who are determined to make it work. The latter spirit is clearly seen from the ingenious ideas and unrelenting efforts that have been displayed in the past 2 to 3 years, in a heroic attempt to improve the results of future gene therapy. Some of the newer concepts and efforts are briefly summarised here.

- a. Investigators are beginning to realise that cancer gene therapy might work better in a setting of minimal tumour burden (such as after surgical debulking of tumour), and in a relatively confined space (such as within the pleural, peritoneal or tumour cavity). Adjuvant gene therapy trials will undoubtedly be tested in the near future.
- b. Already, preclinical experiments are suggesting that combining different cancer gene therapy approaches, or combining gene therapy with more conventional cancer treatment might be synergistic.^{56,57} For instance, Chen et al⁵⁶ have shown that combining HSV-tk and interleukin gene therapy was advantageous.

Fujiwara et al⁵⁸ showed that p53 gene therapy enhanced the sensitivity of the transduced cells to cisplatin chemotherapy.

- c. New genes are being discovered almost weekly. The relentless effort of the Human Genome Project in US to decipher all 80,000 human genes by the middle of next decade will undoubtedly provide new vistas for more innovative gene therapy approaches.⁵⁹
- d. New generations of gene vectors are being designed. Indeed, vector improvement is felt to be the cornerstone of future successful gene therapy.^{50,52} Some of the innovations in this area include the use of different viral serotypes to overcome the problem of host immune responses against viral proteins ('seroswitch');⁶⁰ the use of 'stealth' viral vectors which has been stripped of as many non-essential genetic elements as possible;⁶¹ the use of adenoviral vector that produces a protein (CTLA4IgG) that inhibits host immune responses against the virus;⁶² and the use of specially-tailored vectors which is capable of selectively targeting to, replicate in, or lyse tumour tissues, but sparing normal tissues.⁶³⁻⁶⁵
- e. Newer versions of gene expression cassette are likely to have a molecular switch that can turn on or off the therapeutic gene expression at will.⁶⁶ Such regulatable promoters will reduce the likelihood of deleterious over-expression of the foreign genes. Gene-related promoter sequences that increase gene expression following exposure to radiation have also been identified.⁶⁷
- f. In view of the problem associated with the use of viral vectors, newer generations of non-viral gene delivery systems are being developed. An example is to use complexes that consist of DNA linked with protein or small polycationic molecules. Because these particles enter cells by receptor-mediated endocytosis, the incorporation of a protein coat can direct gene transfer to defined cell populations. Persistent and targeted gene expression has been noted using this approach.⁶⁸⁻⁶⁹ Another interesting non-viral approach is the use of modified filamentous bacteriophages that are conferred mammalian cell tropism through genetic modification.⁷⁰

Conclusions

Biomedical sciences are going through a time of epochal events. Genes, which hold the secret to life, are no longer unfathomable strings of alphabets. How genes are organised, how they function, and indeed, how they can be harnessed for therapeutic purposes, are under very intense scrutiny today. Nevertheless, one must not underrate the intricacies of Mother Nature or the complexities of age-old maladies such as cancers. The history of medical progress, whether in the development of antibiotics or in the perfecting of bone marrow transplants, provides ample evidence that the pace of ad-

vance is measured in decades, even generations, not in mere months or years. To demand otherwise of gene therapy is unreasonable and ultimately destructive. Today, there is a renewed and abundant sense of optimism in the field of gene therapy that, in time, the technology of gene therapy will fulfil most of its promises. A careful and unemotional examination of the progress of gene therapy in the last 2 to 3 years would suggest that the field is moving incrementally towards realising its goal of being a successful molecular therapy. It is remarkable that the short and chequered history of gene therapy in the past decade was filled with such drama and controversy. Some have felt that the concept of gene therapy is an oversold fantasy; however, a growing opinion holds that gene therapy is the ultimate showcase of human ingenuity and foresight. I would argue that gene therapy has both of these seemingly contradictory elements. This should not be surprising, if you ponder for a moment, for most major scientific advances in history have had a similar story.

Acknowledgements

The author is funded in part by the National University of Singapore Yong Loo Lin Fellowship in Medical Oncology.

REFERENCES

1. Avery O T, MacLeod C M, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Inductions of transformation by a deoxyribonucleic acid fraction isolated from pneumococcus type III. *J Exp Med* 1979; 149:297-326.
2. Tatum E L. Molecular biology, nucleic acids, and the future of medicine. *Perspect Biol Med* 1966; 10:19-32.
3. Aposhian H V. The use of DNA for gene therapy-the need, experimental approach, and implications. *Perspect Biol Med* 1970; 14:98-108.
4. Blaese R M, Culver K W, Miller A D, Carter C S, Fleishe T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* 1995; 270:475-80.
5. Human Gene Marker/Therapy Clinical Protocols. *Hum Gene Ther* 1998; 9:1803-47.
6. Kong H L, Crystal R G. Gene therapy strategies for tumor antiangiogenesis. *J Natl Cancer Inst* 1998; 90:273-86.
7. Crystal R G. Transfer of genes to humans: early lessons and obstacles to success. *Science* 1995; 270:404-10.
8. Blau H M, Springer M L. Gene therapy-a novel form of drug delivery. *N Engl J Med* 1995; 333:1204-7.
9. Mulligan R C. The basic science of gene therapy. *Science* 1993; 260:926-32.
10. Rosenfeld M A, Yoshimura K, Trapnell B C, Yoneyama K, Rosenthal E R, Dalemans W, et al. *In vivo* transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell* 1992; 68:143-55.
11. Wagner J A, Gardner I. Toward cystic fibrosis gene therapy. *Annu Rev Med* 1997; 48:203-16.
12. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 1993; 90:3539-43.
13. Restifo N P, Spiess P J, Karp S E, Mule J J, Rosenberg S A. A nonimmunogenic sarcoma transduced with the cDNA for interferon gamma elicits CD8+ T cells against the wild-type tumor: correlation with antigen presentation capability. *J Exp Med* 1992; 175:1423-31.
14. Brenner M K, Rill D R, Moen R C, Krance R A, Mirro J J, Anderson W F, et al. Gene-marking to trace origin of relapse after autologous bone-marrow transplantation. *Lancet* 1993; 341:85-6.
15. Wivel N A, Wilson J M. Methods of gene delivery. *Hematol Oncol Clin North Am* 1998; 12:483-501.
16. Prince H M. Gene transfer: a review of methods and applications. *Pathology* 1998; 30:335-47.
17. Treco D A, Selden R F. Non-viral gene therapy. *Mol Med Today* 1995; 1:314-21.
18. Hui K M, Wee J L, Oei A A, Koo W H, Ang P T, Tay S K. Immunotherapy of human cancers. *Ann Acad Med Singapore* 1996; 25:113-9.
19. Johnston S A, Tang D C. Gene gun transfection of animal cells and genetic immunization Methods *Cell Biol* 1994; 43:353-65.
20. Jolly D. Viral vector systems for gene therapy. *Cancer Gene Ther* 1994; 1:51-64.
21. Wilson J M. Adenoviruses as gene-delivery vehicles. *N Engl J Med* 1996; 334:1185-7.
22. Flotte T R, Carter B J. Adeno-associated virus vectors for gene therapy. *Gene Ther* 1995; 2:357-62.
23. Peplinski G R, Tsung K, Norton J A. Vaccinia virus for human gene therapy. *Surg Oncol Clin N Am* 1998; 7:575-88.
24. Kirsch D G, Kastan M B. Tumor-suppressor p53: implications for tumor development and prognosis. *J Clin Oncol* 1998; 16:3158-68.
25. Nielsen L L, Maneval D C. p53 tumor suppressor gene therapy for cancer. *Cancer Gene Ther* 1998; 5:52-63.
26. Roth J A, Swisher S G, Merritt J A, Lawrence D D, Kemp B L, Carrasco C H, et al. Gene therapy for non-small cell lung cancer: a preliminary report of a phase I trial of adenoviral p53 gene replacement. *Semin Oncol* 1998; 25:33-7.
27. Bouvet M, Ellis L M, Nishizaki M, Fujiwara T, Liu W, Bucana C D, et al. Adenovirus-mediated wild-type p53 gene transfer down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human colon cancer. *Cancer Res* 1998; 58:2288-92.
28. Xu H J, Zhou Y, Seigne J, Peng G S, Mixon M, Zhang C, et al. Enhanced tumor suppressor gene therapy via replication-deficient adenovirus vectors expressing an N-terminal truncated retinoblastoma protein. *Cancer Res* 1996; 15:2245-9.
29. Maran A, Waller C F, Paranjape J M, Li G, Xiao W, Zhang K, et al. 2',5'-Oligoadenylate-antisense chimeras cause RNase L to selectively degrade bcr/abl mRNA in chronic myelogenous leukemia cells. *Blood* 1998; 92:4336-43.
30. Kronenwett R, Haas R. Antisense strategies for the treatment of hematological malignancies and solid tumors. *Ann Hematol* 1998; 77:1-12.
31. Cowser L M. *In vitro* and *in vivo* activity of antisense inhibitors of ras: potential for clinical development. *Anticancer Drug Des* 1997; 12:359-71.
32. Scheid S, Heinzinger M, Waller C F, Lange W. Bcl-2 mRNA-targeted ribozymes: effects on programmed cell death in chronic myelogenous leukemia cell lines. *Ann Hematol* 1998; 76:117-25.
33. Culver K W, Ram Z, Wallbridge S, Ishii H, Oldfield E H, Blaese R M. *In vivo* gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. *Science* 1992; 256:1550-2.
34. Mullen C A, Anderson L, Woods K, Nishino M, Petropoulos D. Ganciclovir chemoablation of herpes thymidine kinase suicide gene-modified tumors produces tumor necrosis and induces systemic immune responses. *Hum Gene Ther* 1998; 9:2019-30.
35. Huber B E, Austin E A, Richards C A, Davis S T, Good S S. Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with the cytosine deaminase gene: significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. *Proc Natl Acad Sci USA* 1994; 91:8302-6.
36. Mullen C A, Coale M M, Lowe R, Blaese R M. Tumors expressing the cytosine deaminase suicide gene can be eliminated *in vivo* with 5-

- fluorocytosine and induce protective immunity to wild type tumor. *Cancer Res* 1994; 54:1503-6.
37. Freeman S M, Abboud C N, Whartenby K A, Packman C H, Koeplin D S, Moolten F L, et al. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Res* 1993; 53:5274-83.
 38. Bi W L, Parysek L M, Warnick R, Stambrook P. *In vitro* evidence that metabolic cooperation is responsible for the bystander effect observed with HSV tk retroviral gene therapy. *Hum Gene Ther* 1993; 4:725-31.
 39. Hesdorffer C, Ayello J, Ward M, Kaubisch A, Vahdat L, Balmaceda C, et al. **Phase I** trial of retroviral-mediated transfer of the human MDR1 **gene** as marrow chemoprotection in patients undergoing high-dose chemotherapy and autologous stem-cell transplantation. *J Clin Oncol* 1998; 16:165-72.
 40. Song W, Kong H L, Carpenter H, Torii H, Granstein R, Rafii S, et al. Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity. *J Exp Med* 1997; 186:1247-56.
 41. Hui K M, Ang P T, Huang L, Tay S K. Phase I study of immunotherapy of cutaneous metastases of human carcinoma using allogeneic and xenogeneic MHC DNA-liposome complexes. *Gene Ther* 1997; 4:783-90.
 42. Rosenberg S A. Karnofsky Memorial Lecture. The immunotherapy and gene therapy of cancer. *J Clin Oncol* 1992; 10:180-99.
 43. Nabel G J, Gordon D, Bishop D K, Nickoloff B J, Yang Z Y, Aruga A, et al. Immune response in human melanoma after transfer of an allogeneic class I major histocompatibility complex gene with DNA-liposome complexes. *Proc Natl Acad Sci USA* 1996; 93:15388-93.
 44. McDonnell M M, Askari F K. DNA vaccines. *N Engl J Med* 1996; 334:42-5.
 45. Shinoura N, Yoshida Y, Sadata A, Hanada K I, Yamamoto S, Kirino T, et al. Apoptosis by retrovirus- and adenovirus-mediated gene transfer of Fas ligand to glioma cells: implications for gene therapy. *Hum Gene Ther* 1998; 9:1983-93.
 46. Kong H L, Hecht D, Song W, Kovesdi I, Hackett N R, Yayon A, et al. Regional suppression of tumor growth by *in vivo* transfer of a cDNA encoding a secreted form of the extracellular domain of the flt-1 vascular endothelial growth factor receptor. *Hum Gene Ther* 1998; 9:823-33.
 47. Goldman C K, Kendall R L, Cabrera G, Soroceanu L, Heike Y, Gillespie G Y, et al. Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis, and mortality rate. *Proc Natl Acad Sci USA* 1998; 95:8795-800.
 48. Tanaka T, Manome Y, Wen P, Kufe D W, Fine H A. Viral vector-mediated transduction of a modified platelet factor 4 cDNA inhibits angiogenesis and tumor growth. *Nat Med* 1997; 3:437-42.
 49. Tanaka T, Cao Y, Folkman J, Fine H A. Viral vector-targeted antiangiogenic gene therapy utilizing an angiostatin complementary DNA. *Cancer Res* 1998; 58:3362-9.
 50. Roth J A, Cristiano R J. Gene therapy for cancer: what have we done and where are we going? *J Natl Cancer Inst* 1997; 89:21-39.
 51. Dranoff G. Cancer gene therapy: connecting basic research with clinical inquiry. *J Clin Oncol* 1998; 16:2548-56.
 52. Ross G, Erickson R, Knorr D, Motulsky A G, Parkman R, Samulski J, et al. **Gene** therapy in the United States: a five-year status report. *Hum Gene Ther* 1996; 7:1781-90.
 53. Anderson W F. Human **gene** therapy. *Nature* 1998; 392:25-30.
 54. Song W, Kong H L, Traktman I, Crystal R G. Cytotoxic T lymphocyte responses to proteins encoded by heterologous transgenes transfected *in vivo* by adenoviral vectors. *Hum Gene Ther* 1997; 8:1207-17.
 55. Yang Y, Li Q, Ertl H C, Wilson J M. Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. *J Virol* 1995; 69:2004-15.
 56. Chen S H, **Chen** X H L, Wang Y, Kosai K I, Finegold M J, Rich S S, et al. Combination gene therapy for liver metastasis of colon carcinoma *in vivo*. *Proc Natl Acad Sci USA* 1995; 92:2577-81.
 57. Dorigo O S D L, Royston I, Sobol R E, Berek J S, Fakhrai H. Combination of transforming growth factor beta antisense and interleukin-2 gene therapy in the murine ovarian teratoma model. *Gynecol Oncol* 1998; 71:204-10.
 58. Fujiwara T, Grimm E A, Mukhopadhyay T, Zhang W W, Owen-Schaub L B, Roth J A. Induction of chemosensitivity in human lung cancer cells *in vivo* by adenovirus-mediated transfer of the wild-type p53 gene. *Cancer Res* 1994; 54:2287-91.
 59. Olson M V. The human genome project. *Proc Natl Acad Sci USA* 1993; 90:4338-44.
 60. Mack C A, Song W R, Carpenter H, Wickham T J, Kovesdi I, Harvey B G, et al. Circumvention of anti-adenovirus neutralizing immunity by administration of an adenoviral vector of an alternate serotype. *Hum Gene Ther* 1997; 8:99-109.
 61. Wang Q, Finer M H. Second-generation adenovirus vectors. *Nat Med* 1996; 2:714-6.
 62. Jooss K, Turka L A, Wilson J M. Blunting of immune responses to adenoviral vectors in mouse liver and lung with CTLA4Ig. *Gene Ther* 1998; 5:309-19.
 63. Fichera A, Michelassi F, Arenas R B. Selective expression of carcinoembryonic antigen promoter in cancer cell lines: targeting strategy for gene therapy in colorectal cancer. *Dis Colon Rectum* 1998; 41:747-54.
 64. Bischoff J R, Kirn D H, Williams S A, Heise C, Horn S, Muna M, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996; 274:373-6.
 65. Goldman C K, Rogers B E, Douglas J T, Sosnowski B A, Ying W, Siegal G P, et al. Targeted **gene** delivery to Kaposi's sarcoma cells via the fibroblast growth factor receptor. *Cancer Res* 1997; 57:1447-51.
 66. Narumi K, Suzuki M, **Song** W, Moore M A, Crystal R G. Intermittent, repetitive corticosteroid-induced upregulation of platelet levels after adenovirus-mediated transfer to the liver of a chimeric glucocorticoid-responsive promoter controlling the thrombopoietin cDNA. *Blood* 1998; 92:822-33.
 67. Manome Y, Kunieda T, Wen P Y, Koga T, Kufe D W, Ohno T. Transgene expression in malignant glioma using a replication-defective adenoviral vector containing the Egr-1 promoter: activation by ionizing radiation or uptake of radioactive iododeoxyuridine. *Hum Gene Ther* 1998; 9:1409-17.
 68. Wagner E, Zatloukal K, Cotten M, Kirlappos H, Mechtler K, Curie D T, et al. Coupling of adenovirus to transferrin-polylysine/DNA complexes greatly enhances receptor-mediated gene delivery and expression of transfected genes. *Proc Natl Acad Sci USA* 1992; 89:6099-103.
 69. Simoes S, Slepishkin V, Gaspar R, Lima M C D, Duzgunes N. Gene delivery by negatively charged ternary complexes of DNA, cationic liposomes and transferrin or fusogenic peptides. *Gene Ther* 1998; 5:955-64.
 70. Larocca D, Witte A, Johnson W, Pierce G F, Baird A. Targeting bacteriophage to mammalian cell surface receptors for gene delivery. *Hum Gene Ther* 1998; 9:2393-9.