Translational Benefits of Gene Therapy to Date

M Mary McMenamin

Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, OX1 3QX, United Kingdom.

Correspondence to: M Mary McMenamin
Tel: 44-1865 272419
E-mail: mary.mcmenamin@dpag.ox.ac.uk

Received February 16 2011; accepted March 10, 2011.

E-mail: 2008cocr@gmail.com
Tel (Fax): 86-22-2352 2919

ABSTRACT Gene therapy is now a reality with a number of early phase clinical trials having been completed and several currently in progress. In spite of some early setbacks substantial progress has been made with treatment of several different diseases using a variety of delivery vectors and transgenes. Indeed for some diseases gene therapy is now the treatment of choice, in particular the inherited immune deficiencies. Treatment of ocular diseases and cancer are also showing great promise. Immune responses and insertional mutagenesis still pose problems but refinement of delivery systems and an increased understanding of oncogene activation should ensure that more successful protocols will emerge in the near future. Continuous progress suggests that a wider range of diseases can be treated with gene therapy in the future.

KEY WORDS: gene therapy, neoplasm, clinical trial, immunology.

Copyright © 2011 by Tianjin Medical University Cancer Institute & Hospital and Springer

Introduction

Gene therapy can be defined as the introduction, replacement or removal of genetic material into an individual for therapeutic purposes. Basically it involves the use of DNA/RNA as a drug and is a natural evolution in pharmaceutical technology with the potential means to treat a variety of medical disorders. The technology of gene therapy, namely gene transfer, has also proved very beneficial for gene functional studies, as assessment of gene function can be best ascertained by either over-expression or knock-down strategies. However, the goals of gene therapy and gene functional studies are rather different; for gene therapy the challenge is to develop an efficient and safe drug delivery system.

All gene therapy currently being undertaken involves the delivery of new genetic material to somatic cells. Such genetic modification cannot be passed to offspring and to that extent it is basically similar to treating any disease by supplying a missing biological function e.g. treating diabetes by giving a patient insulin. The goal with gene therapy is permanent or long term correction of the deficiency. The basic technology of germline gene therapy, while prohibited for therapeutic purposes in humans is being successfully applied in animal experiments for the investigation of gene function and is proving
to be exceptionally valuable in dissecting the genetic controls of development and in creating models for the study of human diseases. Somatic cell gene therapy can be accomplished by *ex vivo* gene transfer to cells that had been removed from the patient. The cells are genetically modified in culture and subsequently returned to the patient’s body. Alternatively, the vector carrying the functional sequence can be directly injected into the patient to achieve *in vivo* gene transfer.

Genes or functional sequences can be used not only to replace or repair a defective gene that is leading to disease (e.g. cystic fibrosis or haemophilia) but to provide a new or changed function to a cell (e.g. program an immune cell to attack cancer) or to produce elevated levels of an existing product (e.g. a rate limiting enzyme in Parkinson’s Disease). Alternatively therapy can involve knockdown strategies and with the recent explosion in RNA interference (RNAi) technology this strategy is becoming increasingly popular[1]. So although gene therapy holds obvious potential for genetic diseases e.g. cystic fibrosis[2] other more complex diseases such as cancer[3,4], heart disease[5], infectious diseases e.g. HIV[6,7], neurological diseases[8,9] e.g. Parkinson’s[10,11] and Alzheimer’s[12,13] disease are also amenable to treatment by gene therapy. The requirements for successful gene therapy are (i) transfer of the gene or sequences with high efficiency, (ii) extended duration of gene expression possibly involving physiological regulation (although transient expression may be more appropriate for some diseases such as cancer) and (iii) safety. Genes can be introduced into a cell by either viral[14,15], or non-viral methodologies[16-18]. The non-viral methods in use, while less toxic than viral vectors are for the most part less efficient[19]. The principal viral vectors in use for gene therapy include retroviruses in particular lentiviruses eg HIV, SIV, DNA viruses, Herpes virus, Adenovirus and Adeno-associated virus. 

**Current Trials**

It is now almost 30 years since Friedmann and Roblin published a paper in Science titled “Gene therapy for human genetic disease”[20] and there have been several ups and downs in the intervening years. However, since 1990, more than 1500 clinical trials have been approved worldwide utilizing viral and non-viral vectors and targeting various organs, cell types, and diseases[21]. There have been successes as well as setbacks with immune responses and insertional mutagenesis posing as the main hurdles. An overwhelming innate immune response to an intravenously delivered adenoviral vector caused the death of a patient with a rare metabolic disorder in 1999[22] but in 2009 Science considered the “return of gene therapy” as one of the major scientific breakthroughs of that year.

**Immune deficiencies**

The first gene therapy clinical trials were initiated in 1990 and involved ex vivo gene transfer to umbilical cord blood cells or to autologous lymphocytes of children with severe combined immune deficiency (SCID) due to mutations in the adenosine deaminase (ADA) gene[23-25]. These early studies were inefficient and did not involve transduction of hematopoietic stem cells (HSC), thereby limiting the extent and duration of gene transfer.

In 2000, the first clearly successful gene therapy was reported. *Ex vivo* retroviral gene transfer of the γc cytokine receptor common sub-unit into autologous HSC cells cured boys with X-linked SCID[26]. However, 5 children (4 in France and one in the UK) in trials developed leukaemia[27]. This was caused by integration of the gene near the LMO2 T cell oncogene[27]. It is likely that the combination of the growth advantage of the gene-corrected T cells and the activation of LMO2 was responsible for this outcome. Retroviral vectors integrate randomly into the host genome, but show a preference for transcriptionally active genes, and they contain sequences that are prone to activating nearby genes encoded by the host chromosome.

Unlike the experience with X-SCID, retroviral transduction of HSC for adenosine deaminase deficiency (ADA)-SCID have shown high therapeutic efficacy without the development of leukaemia[28,29]. More than 30 ADA-SCID patients have now been treated with gene therapy worldwide, with successful outcome in most cases, consisting of progressive immune reconstitution, efficient systemic detoxification, and long-term multilineage engraftment[30]. Eight of ten children have essentially been cured in an Italian trial. For the past ten years, these patients no longer require enzyme replacement therapy and they live normal lives, attending school instead of living as “bubble” boys or girls.

**β-thalassemia**

Results from an ongoing French gene therapy trial for β-thalassemia using a lentivirus are proving successful. One treated patient has not required red blood cell transfusions for three years now[31]. Gene transfer was performed ex vivo to HSCs. However, a concern has also emerged with this trial as in spite of very low-efficiency of gene transfer, approximately 10% of the patient’s blood cells contain viral integrants in the HMGA2 gene, apparently resulting in increased gene expression and a growth advantage. Thus far, this does not seem to have caused adverse effects in the patient. These events linked to changes in endogenous gene expression upon viral integration were unexpected, and therefore underscore that much remains to be learned about the effects of insertion of viral sequences and therapeutic expression cassettes into host genomes. In the long-run, development of site-specific integration systems is desirable.
X-linked adrenoleukodystrophy

Nevertheless, the successful use of lentiviral gene transfer to HSCs was recently reported for X-linked adrenoleukodystrophy (ADL), a devastating lipid storage disorder in boys that results in demyelination of neurons in the brain. Gene transfer halted the progressive brain damage in two seven year old patients[32]. It is possible that benefits from the use of integrating vectors in many cases and diseases will outweigh risks associated with insertional mutagenesis, in particular as vectors are being developed with reduced impact on cellular gene expression and as these risks are being better understood. Furthermore, non-integrating lentiviral vectors have also been developed recently, but their long-term safety and efficacy remains to be evaluated[33].

It is possible that benefits from the use of integrating vectors will outweigh risks associated with insertional mutagenesis. Vectors are being developed with reduced impact on cellular gene expression and as these risks are being better understood. In addition non-integrating lentiviral vectors have also been developed recently, but their long-term safety and efficacy remains to be evaluated[33].

Haemophilia B

With AAV vectors numerous investigations have yielded impressive results on the correction of various genetic diseases in mice, dog, non-human primates and other animal models[34]. For example, factor IX gene transfer resulted in ten years of stable correction of haemophilia B in canines. It was also shown to induce immune tolerance to factor IX and several other therapeutic proteins[35,36]. However, similar therapeutic expression in humans was not achieved. This has been shown to be due to the presence of pre-existing antibodies against the capsid of the AAV2 serotype. In addition the viral capsid of AAV2 can generate a CD8+ T cell response to the viral capsid[37]. Use of different serotypes is being explored to potentially circumvent this problem. AAV vector capsids are being engineered to be more resistant to neutralizing antibodies in the human population to transduce target cells more efficiently to show enhanced tropism to specific tissues.

Neurodegenerative Diseases

Parkinson’s Disease

There are currently three different gene therapy trials for Parkinson’s Disease (PD) ongoing. In one an AAV2 vector expressing glutamic acid decarboxylase (GAD) which synthesizes gamma-Aminobutyric acid (GABA) was transferred into the substantia nigra (STN)[38,39]. The results from a Phase II study for this trial have shown that patients significantly improved the tremors, rigidity and other motor skill problems that are hallmark of the illness[40]. In a second a vector expressing neurturin (NTN) a growth factor similar to glial-derived neurotrophic factor, in the putamen a phase I trial was promising but a phase II trial showed no efficacy[41,42]. In a third trial a tricistronic lentiviral vector encoding all genes required for dopamine synthesis has been successfully tested in nonhuman primates. The three genes are tyrosine hydroxylase (TH), aromatic-L-amino acid decarboxylase (AADC) which converts and GTP cyclohydrolase I (GTPCHI) guanosine 5'-triphosphate cyclohydrolase. Nine patients received intrastriatal injections into the striatum which restored extracellular concentrations of dopamine and corrected the motor deficits for 12 months without associated dyskinesia[43]. Safety and tolerability endpoints have been sustained for more than two years. Two-year Phase I/II data indicate long-term efficacy at the lowest dose level. There have been improvements in “ON” time and quality of life, with stable or reduced L-DOPA, in all cohorts to date.

Alzheimer’s Disease

One therapeutic target for Alzheimer’s Disease (AD) is the cholinergic system, specifically in the basal forebrain. Neurotrophins, such as nerve growth factor (NGF), have potential to be neuroprotective and therefore, administration of NGF to cholinergic neurons has been undertaken to provide symptomatic benefit and modify the disease course. A Phase I trial involving 10 subjects receiving bilateral injections of AAV2-NGF to the nucleus basalis of Meynert (NBM) has now been successfully completed revealing an excellent safety profile and a phase II trial now initiated[44].

Motor Neuron Disease

Recent advances and findings from preclinical studies in animal models for motor neuron disease (MND) provide optimism that gene therapy might be an effective therapeutic strategy for treating MND[45]. This has involved attempts to provide neuroprotection for motor neurons by administration of the growth factors IGF1, GDNF or VEGF. Delivery has been problematic but there have been different approaches. Using an AAV vector, retrograde delivery of IGF1 by intramuscular injection has been shown to prolong life and delay disease progression, even when delivered at the time of overt disease symptoms[46]. VEGF in an AAV vector also delivered by intramuscular, intraspinal or intrathecal delivery and has been shown to prolong survival[47].

Ocular Diseases

Recent success in delivering vision to a canine model of a severe, early-onset blinding disease, Leber congenital amaurosis (LCA) demonstrates that AAV2 is capable of delivering a corrective gene to the target retinal cells. Subretinal administration of a vector expressing RPE65 (retinal pigment epithelium-specific 65 kD protein) led to gain of light sensitivity and, in some cases, of vision, in patients with Leber’s congenital amaurosis (LCA)[48].
Proof of light sensitivity was documented at the site of retinal gene transfer. Patients gained vision as evidenced by behaviour correlates such as the ability to walk through a maze. A one-year follow-up showed no diminution in any of the clinical parameters, indicating long-term rescue of vision as assessed by psychophysical, behavioural and molecular biological studies.

A subsequent trial in children showed that the extent of visual improvement is age-dependent and earlier intervention, when degeneration of photoreceptors has progressed less, results in substantial gains in ambulatory vision\(^{[50]}\). Four children (8-11 years old) are now able to play sports and attend school without the use of learning aids\(^{[51]}\).

The initial results from the first three gene therapy trials to use AAV vectors to treat an inherited retinal degeneration have now been published\(^{[52,53]}\). These trials have demonstrated no significant vector-related side effects and provided evidence of successful gene transfer with improved vision in several patients\(^{[53]}\).

**Cancer**

Cancer gene therapy accounts for two-thirds of the current ongoing gene therapy trials. Viruses which require replicating cells for genomic integration, can be used to advantage in treating cancer, where cellular division is hyperactive. Gene therapy strategies for cancer that have been explored include (i) suicide gene therapy, (ii) the use of oncolytic vectors, (iii) cytokine gene transfer, (iv) inhibition of activated oncogenes by antisense mechanisms and (v) transfer of tumour suppressor genes\(^{[54]}\).

Traditionally, suicide gene therapy is one of the most commonly used strategies in treating cancer\(^{[55]}\). It is based upon the principle that tumour cells can be selectively transduced with an enzyme, often thymidine kinase which enables them to selectively metabolise a non-toxic prodrug into a toxic prodrug, causing cellular death. Thymidine kinase expressed in Herpes Simplex virus (HSV), activates ganciclovir into a cytotoxic species. Suicide therapy has a strategy advantage in that it causes death of surrounding cells, through the bystander effect where the toxic drug is transffected to surrounding cell type through gap junctions and other cellular connections. It has been suggested that only 10% of cells need to be transfected to cause 100% cell death. In a Phase I clinical trial a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor was trialed in 26 patients to determine the safety profile of the virus, look for evidence of biological activity, and identify a dosing schedule for later studies. Nineteen of 26 patient post-treatment biopsies contained residual tumour of which 14 showed tumour necrosis of breast, head and neck and gastrointestinal cancers, and malignant melanoma. These patients had failed prior therapy, suggesting that there are good prospects for this gene therapy strategy\(^{[56]}\).

It has long been recognised that some viruses are natural oncolytics\(^{[57]}\). Adenoviruses where first used in to treat cervical cancer of 30 patients, and showed 26 caused tumour necrosis. The idea behind this approach is that the vector replicates in the cancer cell, lyses the infected cell and releases progeny virus. These in turn infect neighboring cells in the tumor, and start a new lytic cycle. In theory, such vectors should spread through and eliminate a tumor.

Oncolytic Adenoviral vectors carrying four different mutations have been tested in human clinical trials\(^{[58]}\). All four vectors caused little toxicity, even when administered intravenously at dose levels of 1 \times 10^{13} virus particles. However, their efficacy as single agents was limited; they produced better results when used in combination with chemotherapy. One (ONYX-015) was the most extensively studied vector among the four with numerous phase I and phase II clinical studies, but it did not proceed through phase III clinical trials in the United States. However, the development of a very similar vector (named H101) occurred in China, and H101 has been approved as an anticancer drug there\(^{[59]}\).

Suicide and oncolytic therapy have been trialled together\(^{[60]}\). Replication-competent adenoviruses carrying the HSVtk gene, driven by endogenous E3 promoter were used. Inclusion of the HSVtk gene in a replicating adenovirus did not augment antitumor efficacy when used with treated with ganciclovir, suggesting that this combination of treatments may nor work well together. The first combinatorial therapy trial was a Phase I clinical trial of fusion-Ad5 expressing HSV-TK in place of E1 region, to reduce toxicity intra tumour injection, followed by 1–2 weeks of 5-fluorocytosine (5-FC) and ganciclovir. In a dose escalating trial half of the patients exhibited significant (> 25%) declines in serum prostate surface antigen, mild side effects\(^{[61]}\).

An important lesson learned from preclinical and clinical research in cancer gene therapy is that efficient transduction of the cancer cells in the tumour is essential for efficacious treatment. One problem is that the interaction of adenoviruses with blood cells and plasma proteins thwart efficient tumour cell transduction. New technologies are however now being developed to ensure efficient vector delivery into tumours\(^{[62,63]}\). One approach potentially leading to improvements in delivery of adenoviral vectors for cancer gene therapy involves chemical coating of the vector particles. Genetically modified adenoviruses with altered host range (“targeted viruses”) have been generated by engineering new polypeptide ligands in the capsids of the particles, yielding viruses that preferentially infect specific cell or tissue types. In parallel, by engineering mutations at known receptor-binding sites in the capsid, “detargeted”
vectors have been generated to reduce transduction of nontarget tissues\(^{[64]}\).

Overall, the viral vectors used in the clinical studies reported so far have been well tolerated and safe. Robust technology platforms have been established and many of the problems have been identified. New platforms for preclinical evaluation of new oncolytic vectors are available\(^{[65]}\). Combining the technologies and building on new insights from both cancer biology and virology will facilitate the generation of new vectors that will, it is hoped, be as safe as current vectors, but more efficacious.

**Conclusion**

Gene therapy has applications across many fields of medicine. Progress has been slow but important observations have emerged from ongoing trials. Key technical issues of insertional mutagenesis and immune mediated rejection still need to be resolved and to that end existing protocols are being modified to overcome identified hurdles. Improvement in vector design that increases the duration of expression and precision targeting that reduces immunogenicity and toxicity should improve gene expression. More successful protocols are expected to emerge in the near future. During the next decade technological developments, particularly in the areas of gene delivery will be critical for the successful clinical practice of gene therapy.

**Conflict of interest statement**

No potential conflicts of interest were disclosed.

**References**

33. Monse H, Laufs S, Kuate S, et al. Viral determinants of integration site preferences of simian immuno-


