GENE THERAPY: SUCCESS OR FAILURE?

Dr Jim Vadolas *

The Greek Conference supports the Cell and Gene Therapy Research Group, at The Murdoch Childrens Research Institute, The Group is headed by Dr Jim Vadolas and at Kos 2007, Dr Vadolas provided a further report as to the progress of the work undertaken by his Group and in addition spoke to his PowerPoint presentation.

Introduction

In February 2001, two research groups Headed by Francis Collins at the National Human Genome Research Institute and Craig Venter at Celera announced the near completion of the Human Genome Project: the DNA[1] sequence of 3 billion or so DNA base pairs that make up the 22 somatic chromosomes, and the X and Y chromosomes.

The sequence of the human genome provides the first holistic view of our genetic heritage. The Human Genome Project has provided access to the thousands of genes and their products (i.e., RNA[2] and proteins) which function in a complicated and orchestrated manner that creates the mystery of life.

Importantly, the human genome is being used to help us understand the genetic basis of disease and allow us to develop better therapeutic strategies with fewer side effects. Most genetic diseases are the direct result of a mutation in one gene. However, one of the most difficult problems ahead is to find out how genes contribute to diseases that have a complex pattern of inheritance, such as in the cases of diabetes, asthma, cancer and mental illness.

In all these cases, no one single gene has the "yes/no" power to say whether a person has a disease or not. It is likely that more than one mutation is required before the disease is manifest, and a number of genes may each make a subtle contribution to a person's susceptibility to a disease.

Gene Therapy

Gene therapy holds great promise for the effective therapy of many genetic diseases. The term “gene therapy” is used to describe the insertion of normal DNA directly into patient-derived cells to correct a genetic defect. This treatment is able to

replace,

alter, or

supplement

a gene that is absent or abnormal in key target cells (ie bone marrow-derived stem cells) with the greatest efficiency. Scientists have focused increasingly on using viral systems to overcome this challenge.

The efficient mechanisms of integration of retroviral and lentiviral DNA into the genome of host cells has therefore made these vectors the systems of choice for the development of many gene therapy vectors. The first gene therapy trial started in 1990 with the famous treatment of adenosine deaminase deficiency by French Anderson and colleagues. Although this trial was considered for many years as a failure because the transferred gene was not expressed over a long period of time, it paved the way for further clinical trials.

According to the database dedicated to registered clinical trials[3] there have been 1,309 registered clinical trials (to December 2007) with over 3,500 treated patients. The majority of trials have been in the treatment of cancers, while the clinical trails designed to investigate the treatment of inherited monogenic disorders amount to less than 20%. Surprisingly, 18 years after the first clinical trial only 2.5% of the trials have actually reached their Phase III.

The reason for the slow rate of progress reflects the many technical hurdles that still exist in gene therapy.

Despite the apparent lack of progress, there have been a series of significant preclinical and clinical landmark results in gene therapy. In 1995, R G Crystal and colleagues reported the administration of a gene therapy viral vector, termed adenovirus, containing the human gene (CFTR) that can potentially be used to treat individuals with cystic fibrosis.

This was a Phase I study, in patients, investigating the safety, toxicity and biological effects.

This study was made famous because it reported the first adverse event in gene therapy. One patient developed flu-like symptoms following inhalation of experimental recombinant adenovirus.

In subsequent developments:

1. In 1997, Jeffery Isner and colleague reported the successful treatment of critical limb ischemia by transient expression of vascular endothelial growth factor (VEGF) via local intramuscular injection. The over expression of VEGF from plasmid DNA was sufficient to induce therapeutic angiogenesis in selected patients and save the affected limb from amputation.

2. In 1999, RNA/DNA oligonucleotide designed to promote endogenous repair of genomic DNA was used to permanently correct the Crigler Njjar Syndrom in the Gunn rat liver. The chimeric oligonucleotide was either complexed with polyethylenimine or encapsulated in anionic liposomes (administered intra veinously), and targeted to the hepatocyte via the asialoglycoprotein receptor.

   Correction of the genetic defect in the Gunn rat restored enzyme expression and bilirubin conjugating activity, with improvement in the metabolic abnormality.

3. Also in 1999, while the field of gene therapy was beginning to give hope to many patients suffering from severe genetic disorders, the most adverse event then reported in gene therapy occurred.

   The first patient to die from a reaction to a gene therapy took place at the University of Pennsylvania's Institute of Human Gene Therapy in Philadelphia. Jesse Gelsinger died four days after receiving the experimental recombinant adenoviruses containing the corrective gene for ornithine transcarbamylase deficiency (OTCD).

   It is not clear exactly why the gene therapy treatment caused Jesse Gelsinger's death, but it appears that his immune system launched an attack on the adenoviral vector, which resulted in organ failures.

4. Following in the shadows of the disastrous Philadelphia trial, in 2000 Mark Kay and colleagues reported the successful gene transfer and expression of human coagulation factor IX in haemophilia B patients. The team packaged the gene for Factor IX, into a defective adeno-associated virus (AAV). They then delivered the recombinant virus intra muscularly into patients who lacked Factor IX. Normally, these hemophilia patients required regular injections of Factor IX to prevent uncontrolled bleeding but following the therapy required a significantly reduced number of Factor IX injections.

5. Among the major reported successes in the field gene therapy, occurred in 2000 when Alain Fischer, Mariana Cavazzana-Calvo and colleagues at the Necker Hospital for Sick Children in Paris reported the successful treatment of several patients with X-SCID (an inherited form of immunodeficiency). After more than 20 years of intense research and many setbacks the usefulness of gene therapy for the treatment of inherited conditions was clearly demonstrated.

Gene therapy had finally come of age.

However, the impressive results were tempered when in 2002 it was discovered that 3 of the 10 X-SCID patients treated by Alain Fischer and colleagues, developed a leukaemia-like disorder three years after retroviral gene therapy. The leukaemia-like condition occurred as the result of the integrated gene therapy vector caused the non-regulated and over expression of certain genes caused by inserstional oncogenesis (genotoxicity). While the leukaemia was promptly and completely managed with chemotherapy, this study highlighted the risks associated with gene therapy.

The main concern with the use of retroviral vectors is the random integration into the host genome.

For many years, it was perceived that such events were thought to be extremely low. However, the adverse events in the Paris gene therapy trial clearly highlighted that the perceived low risk of insertional oncogenesis associated with retroviral gene therapy was severely underestimated.

Despite these adverse events, encouraging results have continued to emerge from several gene therapy trials. The use of lentiviral vectors to obtain high-level expression of therapeutic genes has been demonstrated in several animal models leading to the correction of the haemoglobin disorders, sickle cell anaemia, and β-thalassaemia in mice.

Adrain Thrasher MD from the Institute of Child Health and Greater Ormond Street recently reported no serious adverse events in ten children treated with gene therapy for the inherited immunodeficiency X-SCID, 6 years following infusion of genetically modified cells. Clinical responses were excellent and are all leading normal lives at home.

Similarly, researcher Alessandro Aiuti, MD, from the San Raffaele Telethon Institute for Gene Therapy, in Milan, Italy, recently reported a trial of 12 children (aged 7 months to 5.5 years) with SCID caused by a defective gene for adenosine deaminase (ADA).

All of the children treated with gene therapy are currently healthy, thriving and living normal lives with no adverse events related to the gene transfer. All of the infants with longer follow-up had completely reconstituted immune system and no need for treatment.

This progress, coupled with developments in the ability to select and expand genetically modified stem cells *in vitro* and *in vivo*, has advanced the possibility of gene therapy for several genetic disorders in the near future. However, given the benefits that some patients have already derived from this gene therapy strategy it is important to consider risk factors that may potentially lead to the failure of gene

therapy trials and hopefully avoid any adverse events. While the risk for inserstional oncogenesis might vary significantly with different gene therapy constructs and diseases, the observations from the Paris gene therapy trial underlines the need for extreme caution in pursuing these approaches.

Mindful of the limitations and potential problems of viral gene delivery, our work at the Cell and Gene Therapy group, Murdoch Childrens Research Institute over the last ten years has focused on establishing a comprehensive and coherent approach to therapy with intact genomic loci, using thalassaemia, as models for haematological diseases.

Our key objective is to demonstrate that using the latest knowledge and resources from the Human Genome Project, we can develop safe and effective "genomic therapies" for common diseases.

Dr Sara Howden, a PhD student within the Murdoch Chlidrens Research Institute developed a gene targeting strategy whereby the gene therapy vector can be introduced into a designated chromosomal region thus avoiding the problems associated with random integration and inserstional oncogenesis.

Dr Howden's paper describes this technology in detail, entitled: "Transient expression of mRNA encoding AAV-derived Rep protein facilitates targeted integration of a 21 kb plasmid into the AAVS1 site". This investigation has recently published in the *Journal of Gene Medicine*[4] and for which her published extract reads:

Background

There is a risk of insertional mutagenesis when techniques that facilitate random integration of exogenous DNA into the human genome are used for gene therapy. Wild-type adeno-associated virus (AAV) integrates preferentially into a specific site on human chromosome 19 (AAVS1). This is mediated by the interaction of the viral Rep68/78 proteins with Rep-binding elements in the AAV genome and AAVS1. This specificity is often lost when AAV is used as a gene therapy vector due to removal of the sequences coding for Rep.

Methods

Messenger RNA coding for the Rep68/78 proteins was prepared *in vitro* and co-transfected with a 21 kb DNA plasmid containing the P5 integration efficiency element (P5IEE) from AAV. Single cells were seeded in plates to establish clonal cell lines that were subsequently analysed by dual colour fluorescent *in situ* hybridisation (FISH) to determine whether site-specific plasmid integration had occurred on chromosome 19.

Results

The co-transfection of plasmid DNA with Rep68/78 mRNA gave a 2.5-fold increase in DNA integration when compared to transfection of cells with plasmid DNA alone. Rep68/78 mRNA expression facilitated site-specific plasmid integration to chromosome 19 in 30% (14/44) of all analysed integration sites, while no targeted integration events were observed following transfection of cells with plasmid DNA alone.

Conclusions

These results demonstrate that transient expression of Rep protein using transfected mRNA facilitates site-specific integration of plasmid DNA. This approach allows expression of Rep for only a short time, and may circumvent the toxicity and chromosome instability associated with long-term expression of Rep

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Our own experience in this field has produce novel gene therapy strategies, which allow us to target therapeutic genes into specific sites in our genome and thus avoid genotoxicity. Our studies have lead us to believe that the development of "genomic therapies" based on the targeted site-specific chromosome integration is a strategy that should provide safe and effective therapy not only for our target disease, but also for more common inherited conditions.

Finally, it is clearly that gene therapy is a promising therapeutic option and gaining momentum once again.

There are many patients eager to see it work but with any experimental procedure unexpected results can sometimes hold back the field for several year. However, I am optimistic and, indeed, anticipate in the not too distant future multi-centre gene therapy clinical trials will be initiated worldwide.

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* Dr Jim Vadolas - Cell and Gene Therapy Research Group, The Murdoch Childrens Research Institute, The University of Melbourne, Department of Paediatrics, Royal Children's Hospital, Melbourne, Australia.

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[1] Deoxyribonucleic acid
[2] related nucleic acid
[3] http://www.wiley.co.uk/genmed/clinical/
[4] The Journal of Gene Medicine [2007] Vol 10; Issue 1, pp 42-50; at www3.interscience.wiley.com/