Editorials

Gene Therapy: How close to clinical reality?

Gene therapy may be defined as treatment by manipulation of gene(s). At present, such manipulation is permitted only in somatic cells (somatic cell gene therapy) but not in gonadal/gametic cells (germline gene therapy). In case of a genetic disease due to a defective/missing gene product, e.g. haemophilia or cystic fibrosis, the disease can be treated by inserting the 'normal' gene in cells of interest to produce the desired therapeutic protein. On the other hand, in diseases where the manifestations of a disease are due to the product of the gene itself (or dominant negative effect), e.g. mutated proto-oncogene in various cancers, the disease can be corrected by shutting off the responsible gene (by anti-sense technology or siRNA interference). A third approach is to insert a gene that confers a new property which may be beneficial in controlling the disease, e.g. a gene for immune stimulation (such as the interleukin-2 gene), or create a new metabolic path (such as herpes simplex *tk* [thymidine kinase] gene) for the treatment of cancer. Unlike human *tk*, the introduced viral *tk* metabolizes ganciclovir (prodrug) to an active anticancer drug. All the three approaches are being tried in various ongoing gene therapy trials.

The first gene therapy trial in humans was approved by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH), USA in 1989.[1] Since then, more than 1300 clinical trials (the database is available at the *Journal of Gene Medicine*'s Gene Therapy Clinical Trials Worldwide websites at *http://www.wiley.co.uk/genmed/clinical* and at *http://www.genetherapyreview.com*), using over 100 genes have been carried out all over the world till 2007.[2] The review by Edelstein *et al*. provides a good summary of the disease conditions treated, the genes and vectors used, and the phases of various clinical trials.[2] The status of research in India in this field has been reviewed recently by Mulherkar.[3]

For a better understanding of various issues involved, it is necessary to have a brief idea about the technique of gene therapy.[4] Simply, gene therapy consists of the following steps:

1. Construction of a desired transgene with appropriate promoter, regulator and integration signals
2. Gene transfer, via viral or non-viral delivery methods; which is targeted, efficient, safe and long lasting
3. Gene expression, in an adequate number of desired cells, in adequate amounts (regulated, if possible), for an adequate length of time and without any ill-effect of the gene product
4. Therapeutic correction and modification of the course of the disease with improved quality of life.

An approach that has been commonly used to achieve long-lasting expression of a therapeutic gene is to combine gene therapy with stem cell therapy, also known as stem cell gene therapy. In this procedure, the bone marrow of the individual to be treated is aspirated to obtain CD34 positive autologous haematopoietic stem cells (HSC). These cells are first enriched and expanded *in vitro*, and then transduced with a normal gene that is defective in the condition to be treated, using a suitable vector. The vector

commonly used is gamma retrovirus (that has been rendered replication-incompetent) because of its capacity to stably integrate into the cell genome. The transduced stem cells, with or without selection, are then transplanted back into the patient. This procedure is called *ex vivo* gene therapy since the transduction is done outside the body.

Experience over the past 20 years has shown that all the above steps can be achieved in humans, though safety and efficacy issues have not yet been resolved completely. Of the 1309 trials reviewed by Edelstein *et al*.,[2] only 32 were in phase III. Most of the others had not crossed phase I or phase II. It was only in the year 2000 that a breakthrough was achieved in the treatment of X-linked severe combined immunodeficiency disease (SCID-X1) due to cytokine receptor gene (γc) deficiency, producing a gamma chain subunit of several cytokine receptors (IL-2, 4, 7, 9, 15 and 21 R). In 2000, the group of Cavazzana-Calvo and Fischer from Paris reported complete immunological reconstitution in 2 children with SCID-X1 following transplantation of genetically corrected autologous CD34+ stem cells.[5,6] Both patients showed a remarkable immunological recovery within 3 months of a gene-modified autologous HSC transplantation. Ten children were recruited in the trial between 1999 and 2002. Nine of the children showed emergence of gene-corrected T and NK lymphocytes, and in 7 of them the immunological reconstitution has been almost full.[7] After 7.5 years, these children are living normally. Another group of 10 children with SCID-X1 has been successfully treated by the London group.[8,9] Unfortunately, the success has been marred by development of leukaemia due to insertional mutagenesis in 5 of the 20 children who had been treated—4 in the Paris group and 1 in the London group; of whom 1 has succumbed to the disease.[10–13]

A recent report of the long term success of gene therapy in 8 of 10 patients with SCID due to adenosine deaminase (ADA) deficiency has rekindled the hope that gene therapy might have arrived.[14] These patients have remained clinically and immunologically well without ADA replacement at 1.8–8.0 (median 4.1) years following infusion of autologous CD34+ bone marrow stem cells, transduced with the ADA gene, using a gamma retroviral vector (the European Medicines Agency has designated the product as an orphan drug). The other 2 patients are alive but had to restart ADA therapy, one at 0.4 years and the other at 4.5 years after the gene therapy. In 5 responders, gamma globulin replacement has been stopped as their immunoglobulin levels and response to antigens/vaccines has become adequate. Six children have started going to school regularly. The success of the Italian group compared with previous efforts in this area has been attributed to (i) stopping ADA replacement therapy 3 weeks before infusion of transduced cells, providing selection pressure for corrected cells to proliferate, (ii) use of non-myeloablative conditioning for HSCT, and (iii) use of adequate numbers of infused transduced stem cells.[15] There was no adverse vector- or gene-related side-effect in these patients[16] unlike the SCID-X1 patients treated earlier with an almost similar protocol. While this can be considered to be a dream come true, this correction has to persist for a long time, and reasons have to be found for the lack of insertional oncogenesis in these patients.

The occurrence of insertional oncogenesis has raised serious concerns regarding the safety of viral vectors, particularly gamma retroviruses.[13] Four patients of SCID-X1 treated at Paris developed T-cell acute leukaemia; 2 were reported in 2003 and 2 in 2008 after 30–68 months of successful gene therapy.[10,11] Three of the 4 patients had insertion of the vector 5´ to *LMO2* proto-oncogene (one had a second insertion near *BMI1* also), and the fourth patient had an insertion near *CCND2*. Additional genetic abnormalities were seen in all the patients. Integration of vector 5´ to the gene was associated with overexpression of related cellular oncogenes, but by itself was not sufficient for leukaemogenesis. The role of the transduced gene and also the nature of the underlying disease have been implicated. Incidentally, 1 of the 10 patients treated in London also developed acute leukaemia, and a similar insertion near the *LMO2* gene was also seen in this patient.[12] On the other hand, none of the 10 patients of ADA–SCID treated with gene therapy developed any T cell clonal proliferation despite being treated by a similar gamma retrovirus vector, and its insertion was also near the

LMO2 and *CCND2* gene in some patients.[16] Due to this reason, the trial in Paris has been put on hold and scientists are back to the bench to redesign safer methods of introducing a desired gene in the target cells.[2,12,17] In addition, the choice appears to be shifting from retroviruses to adenoviruses as viral vectors for gene therapy. But then, we cannot forget the death of Jesse Gelsinger in 1999 caused by an overwhelming systemic inflammatory reaction to the adenoviral vector used for his treatment.[18] (One more patient died of arthritis on 24 July 2007 after receiving the second dose of an experimental therapeutic gene. The US Food and Drugs Administration [FDA] did not attribute the death to gene therapy.[19])

So, shall we celebrate the success or procrastinate due to the hazards? It would be prudent to choose the middle path. The success of gene therapy in immune deficiency states may be a special situation due to proliferative advantage of genetically corrected cells,[20] but for other indications the challenge of targeted, safe, efficient, long term transduction of transgenes still needs to be overcome.[15] The results of ongoing phase III clinical trials of gene therapy are expected to come in the next 12–18 months, which may provide a more realistic assessment of the status of gene therapy. The State Food and Drug Administration of China has licensed the manufacture and marketing of adenovirus-p53 injection (trademarked as Gendicine) for treatment of head and neck squamous cell carcinoma in 2003.[21,22] Five products in phase III trials are (i) adenoviral (Ad) HSV *tk* for glioblastoma, (ii) Ad-p53 for head and neck cancer and Li–Fraumeni syndrome in the USA, (iii) allogeneic prostate cancer cell line transduced with granulocyte macrophage colony stimulating factor (GM-CSF) as prostate cancer vaccine, (iv) tumour cells transduced with TGF-ƀ for non-small cell lung cancer, and (v) intratumoral injection of major histocompatibility complex (MHC) antigen in a non-viral vector for melanoma.[23]

The success of gene therapy in SCID, both in SCID–ADA and SCID-X1, has been glaring.[7,9,14] Even though a number of children did develop a leukaemia-like illness, a majority of them have been able to lead a quality of life which would have been otherwise impossible. Considering the hopelessness of the alternatives, scientists and pharmaceutical companies are looking for whatever gains are presently possible, with an eye on continued development in the future. Considerable effort is being made to improve gene delivery tools, both viral and non-viral.[13,15,20] A group of scientists have been able to target insertion of the phenylalanine hydroxylase gene to the desired site.[24] Attempts are also being made to swap the abnormal gene by a normal gene by homologous recombination, or reversing the mutation in the defective gene.[15] Thus, while there have been setbacks, and no definite time-frame may be laid, the future of gene therapy still remains bright. If not tomorrow, gene therapy in the clinic may be there the day after tomorrow.

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Lessons from the Challenges of Polio Eradication in India

In 1988, India resolved to eliminate the transmission of wild polioviruses (WPVs) by the year 2000, but failed; in 2002, the target was revised to 2005.[1] However, the endemic transmission of WPV types 1 and 3 continues even in 2009.[2] India's problems on the polio eradication front are symptomatic of the defects in her health system.[3] The only other countries with uninterrupted endemic transmission of WPVs are Pakistan, Afghanistan and Nigeria, all with weak health systems.

Readers may recall the debate on the proposed health system reforms between two rival political party candidates in the recent election for President of the USA. In India, the political party that wins a majority forms the government, which implements the party's policy on health system. I am unaware whether any political party addresses issues related to the maladies (and the possible remedies) of our health system. So, there is no reform to implement though, in my opinion, reform is urgently required in the health sector.

The inordinate delay in polio eradication suggests systemic deficiencies in our health infrastructure, especially regarding the organization of the health system and the sharing of responsibilities between the Centre and the states.[3]

The health system in the context of polio eradication

India's health system should consist of two arms: 'medical care'—focusing on individuals, particularly after they fall ill, for restoration to health; and 'public health'—focusing on the community and the environment for breaking the chains of transmission of infections to control endemic diseases and outbreaks. Medical care for rural communities is predominantly in the public sector but remains minimalist in spite