Gene and cell therapy in India

Rita Mulherkar

Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai 410 210, India

Non-communicable diseases like cardiovascular diseases, diabetes and cancer are increasing in developing countries including India. Novel treatment modalities like gene therapy and cell therapy are attractive for such diseases where current therapies have failed. Although the clinical potential of these therapies has been demonstrated in the West, their use still remains in experimental stages. In India a number of groups are carrying out basic research on gene delivery vector development and strategies for cancer gene therapy, and development of adult and embryonic stem cells. With the establishment of human embryonic stem cell (hESC) lines in a few centres, India is now geared to become one of the major contributors to the emerging field of hESC. This review will cover some of the published and unpublished work with potential to be translated into clinical trials, and discuss the possible hurdles in carrying out clinical trials in the country.

Keywords: Clinical trials, gene and cell therapy, human embryonic stem cells, viral vectors.

GENE and cell-based therapies are exciting and promising new avenues, especially for the treatment of incurable diseases. Gene therapy clinical trials started in the US more than two decades ago. The first gene therapy trial was for the treatment of brain tumours in 1980 which began at National Cancer Institute (NCI), USA[1]. Since then, till 2008, there have been 1472 gene therapy clinical trials approved or ongoing all over the world, of which more than 65% are for cancer (source – www.wiley.co.uk). A miniscule number of these trials is being carried out in developing countries. China, however, has emerged as a world leader with the first gene therapy product licensed. Based on recombinant adenoviral p53, called Genedicine, the injectable drug has been approved in China for the treatment of head and neck cancer. Due to more stringent safety and efficacy regulations, although a number of gene therapy products are in the pipeline in the US and Europe, no drug has been approved as yet. Nonetheless, gene therapy still promises to be the medicine of the future. Recently, long-term outcome of gene therapy for severe combined immunodeficiency due to adenosine deaminase deficiency, combined with reduced-intensity conditioning has been shown to be promising, safe and effective by a group in Italy[2].

e-mail: rmulherkar@actrec.gov.in

Biotechnology and recombinant DNA technology for health sciences are areas where India is surging ahead. Basic research in cancer gene therapy received attention more than two decades ago; however, recently, the focus of the Government funding bodies in India has changed from cancer gene therapy to cell therapy. Since the last few years a large number of groups working on cell therapy have mushroomed. In the West, stem cell-based trials are few and are regulated to ensure proper ethical and scientific use. A few isolated cell-based therapies have been reported from India. Although according to Vale and Dell[3], India's footprint in life sciences is relatively small, there are a few groups which are carrying out state-of-the-art basic research in the area of gene and cell-based therapy. Some of this work has the potential to be translated into clinical trials.

Gene therapy in India

Gene therapy is emerging as a new modality of treatment with a great potential. Although initially gene therapy held promise to replace defective genes and cure monogenic disorders, it has met with limited success in clinical trials for genetic disorders. One of the major problems is regulated and long-term expression of the therapeutic gene. Cancer on the other hand, is a disease where numerous strategies can be used to kill the tumour cell. From the reports on numerous human clinical trials, gene therapy has shown promise for cancer, especially in combination with conventional modalities of treatment. In India, there are few groups working in the field focusing mainly on vector development and preclinical studies in cancer gene therapy. However, overall there is little interest in gene therapy translational research in the country. There is no regulatory framework for gene therapy clinical trials in India, making it difficult for those interested in carrying out phase 1 clinical trials. Most of the preclinical studies have remained at the bench level.

Development of viral and non-viral vectors in India

Developing safe and efficient gene-delivery vectors has been the crux of all research for scientists working in the field. Viral vectors, including adenoviral and retroviral vectors are the most preferred vectors for delivering genes. All early gene therapy trials from 1980 to 1994 were based on the use of retroviruses. Till 2004, Moloney

Murine Leukaemia Virus-based retroviral vectors were the leading ones used in clinical trials[4]. The decline in the use of retroviral vectors and growth of adenoviruses began in 2004 after reports of clinical studies in patients with brain tumour demonstrated that retroviral gene therapy failed due to poor transduction efficiency *in vivo*[5,6]. Many investigators who had begun their work with retroviral vectors, subsequently switched to adenoviral vectors. Recently, however, there is a renewed interest in retroviruses, where replication-competent retroviruses are being used in cancer gene therapy[7].

Using the pro-drug activation strategy, Mulherkar's group at the Advanced Centre for Treatment, Research and Education for Cancer (ACTREC), Mumbai, began using retroviral vectors carrying Herpes simplex virus thymidine kinase (HSV-tk) gene in a xenograft nude mouse model for head and neck squamous cell carcinoma (HNSCC)[8]. Although promising results were obtained in the mouse model, they switched to adenoviral vectors due to reports of low transduction efficiency of retroviruses *in vivo* in human clinical trials. They have been working on increasing infectivity and tumour cell kill using the Ad-HSV-tk system. Published data from Mulherkar's laboratory demonstrate that increased infectivity and transgene expression could be achieved with adenoviral vectors in the presence of the histone deacetylase (HDAC) inhibitor – valproic acid (VPA)[9].

Sarkar's group at the University of Delhi has been interested in transfecting hepatocytes and found that murine leukaemia virus was inefficient in transducing hepatocytes. Hence they developed vectors based on Sendai virus. In order to provide specificity of transfection they have used hepatocyte-specific ligands as the transfection vehicle[10]. Galactose-terminated asialoglycoproteins, such as asialofetuin or asialo-orosomucoid, have been conjugated with a polycation to serve as a vehicle to transfer the DNA by endocytosis via the hepatocyte-specific asialoglycoprotein receptor (ASGR). In order to avoid degradation of molecules transferred by this pathway in the lysosome, the researchers have used F-protein of Sendai virus as a hepatocyte-specific ligand. F-protein has a high affinity for ASGR, and its fusogenic activity leads to delivery of the cargo to the cytosol, rather than to the endosomes[10]. The authors have also combined the power of virosomal and nanoparticulate drug delivery systems in designing a novel and efficient hybrid vector to transfer a model drug inside the cytosol of liver cells in culture[11].

Viral vectors based on Epstein Barr Virus (EBV), a member of the gamma herpes virus family, primarily infecting B lymphocytes, have been developed and demonstrated to be efficient in an *in vitro* system by Banerjee's group at the Saha Institute for Nuclear Physics, Kolkata[12]. EBV vector that has the unique capacity to carry doubly modified p27 gene along with the BCR-ABL siRNA expression construct has been reported by this group[13]. The HSV-tk suicide gene has also been incorporated in the vector, which promotes apoptosis in a BCR-ABL-independent pathway. The authors propose that such a multi-gene delivery strategy for BCR-ABL$^+$ CML cells by targeting not only the fusion transcript, but also the downstream signalling would be ideal to overcome drug resistance in the acute phase of CML[13].

Lentiviral vectors have been shown to exhibit a high efficiency of gene delivery in both dividing and post-mitotic cells[14]. There is at least one clinical trial protocol in which the investigator proposes to use gutless lentiviral vector carrying anti-sense sequences against human immunodeficiency virus (HIV) envelope gene and transduce CD4$^+$ T-cells *ex vivo*[15]. Indian HIV type 2 (HIV-2) isolate-derived third-generation lentiviral vector has been prepared and tested by Mukhopadhyaya's group at ACTREC[16]. It has novel and versatile multiple cloning sites that can also facilitate single-step sub-cloning of a PCR-amplified transgene cassette by T/A cloning strategy. Efficiency of the vector was functionally demonstrated by development of a transgenic enhanced green fluorescence protein expressing cell line[16]. The lentiviral vector has also been used to deliver short hairpin RNAs (shRNA) *in vivo* in Balb/c mice with excellent efficiency[17].

Naked plasmid DNA injection into tissues has been shown to be safe, although less efficient than viral vectors. There were 270 gene therapy clinical trials worldwide using plasmid DNA in 2008 (www.wiley.co.uk/genmed/clinical). In the HNSCC xenograft nude mouse model studies, Ambade and Mulherkar[18] have reported that intramuscular injection of IL-2 plasmid DNA results in tumour regression. Circulating levels of IL-2 were observed till day seven after injection, which could activate NK cells as well as adoptively transferred T cells in the nude mice model[18]. In the West, in order to improve gene transfer efficiency of naked DNA into tissues, electroporation has been carried out successfully to transfer DNA-based vaccines and therapeutic plasmids[19]. A phase 1 clinical trial protocol for electroporating IL-12 plasmid DNA into metastatic melanoma has been published recently[20].

Preclinical cancer gene therapy studies in India

HNSCC being the major cancer in India and also due to easy accessibility of these cancers, especially oral cancers, Mulherkar's group chose to work on them. They have established a HNSCC cell line from a patient's tumour, called NT8e (ref. 21). The cell line grows as a monolayer *in vitro* and as a transplantable tumour in immuno-compromised mice *in vivo*, and has been used as a model for preclinical studies. Initially a recombinant, replication-incompetent retrovirus carrying thymidine kinase gene from HSV-tk was constructed, from which a virus producing cell line (VPC) was generated. The VPC

was injected intra-tumourally and was found to efficiently bring about tumour regression in the xenograft nude mouse model[8]. The VPC was also administered along with naked plasmid IL-2 injected intra-muscularly and was found to be effective[22]. Based on the preclinical studies, a phase 1 clinical trial protocol was written and presented before an ad hoc committee at the national level. The protocol was not viewed favourably since VPC of mouse origin was to be injected intra-tumourally into human patients. However, during that time there were reports that retroviruses were unable to efficiently transduce human tumour cells *in vivo*[5,6] and that adenoviruses were more efficient[23]. Hence the group constructed a recombinant adenovirus carrying HSV-tk for preclinical studies. Improved transgene expression and viral infectivity have been seen in the presence of VPA[9]. This work has the potential to be translated into a phase 1 clinical trial for HNSCC patients.

Mulherkar's group has also started work on non-viral vectors to target shRNA to sensitize tumour cells to chemotherapy and radiotherapy. Cyclin D1 down regulation using shRNA was found to increase sensitivity to the drug cisplatin and ATM down regulation resulted in sensitization of tumour cells to radiation (unpublished data). Non-viral vectors based on modified polyethylenimine are being tested to target and deliver shRNA into tumour cells.

Another interesting work in the area of cancer gene therapy has been carried out in Somasundaram's laboratory of the Indian Institute of Science, Bangalore. His group has developed a replication-deficient recombinant adenovirus expressing p73 (Ad-p73) and demonstrated its utility as a potential cancer gene therapy reagent[24–27]. Unlike p53 which gets degraded during HPV infection, p73 is not affected. Hence the authors propose that Ad-p73, which has a great therapeutic potential, could be used as a gene therapy agent against HPV-positive cervical cancers[25–27].

Why are there no gene therapy clinical trials in India?

Significant progress in the field of gene therapy has been made and a lot has been learnt from human clinical trials worldwide. Although there have been grave setbacks with a few unexpected serious adverse events, the trials have given promising results and provide hope for some of the untreatable diseases like SCID[2] and cancer[28–30]. In spite of the large number of gene therapy clinical trials going on worldwide, India is uncertain whether to give priority to this technology. A critical mass of scientists and clinicians knowledgeable in the field to discuss the future of gene therapy in this country is lacking. To address current hurdles in gene therapy, India needs to increase integration between researchers and clinicians. In addition, it needs to foster high-quality education and training, and increase public awareness about gene therapy.

One major problem is the prohibitive cost involved in preparing gene therapy reagents which require cGMP conditions. The European Union Clinical Trials Directive has laid down stringent regulations for gene therapy[31], making it difficult for academic gene therapy trials without funds. The US FDA however, has decided to take a flexible approach to early clinical trials of cell and gene therapies and does not require full cGMP manufacturing[31]. In the absence of regulatory guidelines, Indian scientists are uncertain about how to prepare clinical-grade reagents for clinical trials and often refer to the guidelines from other countries.

Recognizing this problem in the US, the National Institutes of Health (NIH) established the National Gene Vector Laboratories (NGVLs) in 1995, to provide centralized resources for the production and distribution of clinical-grade gene vectors. Three institutions – Indiana University, Baylor College of Medicine and City of Hope house NGVL vector-production facilities, each specializing in the development of different types of gene vectors. Two additional laboratories located at the University of Florida in Gainesville and at the Southern Research Institute in Birmingham, Alabama performed preclinical toxicology testing of vectors, a frequent prerequisite for human studies. In May 2008, the National Gene Vector Biorepository (NGVB) was instituted. The goal of the NGVB is to provide gene therapy investigators with a variety of services that can enhance their research.

There are hardly any laboratories or biotechnology companies catering to gene therapy services in India. Actis Biologics, Mumbai is perhaps the only Indian company which has developed its own gene therapy programme. Academic research institutions find it difficult to bear the cost of such trials. Scientists in India do not have the finances and support to float start-up companies. The Government therefore, has to support academic gene therapy clinical trials and come up with a regulatory framework on early academic gene therapy clinical trials. The regulators in India are still not geared to the international standards. There is no mechanism to facilitate researchers to undertake clinical trials, which involve taking some risk, balanced with potential benefit to the patients and the society. Unless India starts its own gene therapy clinical trials and has its own guidelines in place, it will have to depend on the rest of the world for this potentially promising therapy.

Cell therapy in India

Cell-based therapy, also known as regenerative medicine, is another exciting new therapy which has the potential to repair or replace diseased tissues, or as cancer vaccines. The cells of human origin could be somatic – either

autologous or allergenic, adult stem cells (ASCs) or embryo-derived stem cells or stem cells from umbilical cord blood. Commercial products based on cell therapy are available in the West for skin ulcers and sports injury to the knee cartilage[32,33]. There are a few reports of somatic cell therapy trials, and also on adult stem cell and embryonic stem cell research in India. With the potential promise of using stem cells for non-communicable diseases, India has now started harnessing stem cell research to address its health needs[34].

Somatic cell therapy

Somatic cells used in therapy include autologous and allergenic somatic cells. One of the cell types being used widely for cancer vaccination is dendritic cell (DC). DCs are antigen-presenting cells which present tumour antigens to T-lymphocytes. They can be loaded with tumour antigens *ex vivo* and re-infused into the patient. Rajkumar's group at Cancer Institute, Chennai is working on a project on DC vaccine, sponsored by the Department of Biotechnology (DBT), Government of India. The group has developed and characterized DCs from peripheral blood mononuclear cells using GM-CSF and IL4 (T. Rajkumar, pers. commun.). The immature DCs have been primed with a patient's tumour lysate and then allowed to mature in the presence of IL1β and TNF-α. After functional characterization, they conducted a phase 1 trial using three arms – saline only, unprimed mature DCs and primed mature DCs. Twelve patients who had completed the study showed no major toxicity. No objective clinical responses were seen, but immune response such as DTH response, CD3-gamma interferon intracellular assays and proliferation assays were positive in some of the patients (unpublished data).

Adult stem cells from adult tissues for regenerative medicine

ASCs which have the capacity of self-renewal and differentiation into adult cell types hold promise for diseases like neurological disorders, cancer, cardiovascular diseases and diabetes. Identification and isolation of these rare cells is a challenging task. ASCs have been derived from different tissues such as adipose tissue, pancreas and liver, as well as from umbilical cord blood and bone marrow.

Hardikar's group has been working on understanding the potential of tissue-resident human pancreatic progenitor cells for cell replacement therapy in diabetes[35,36]. The islet-derived mesenchymal cells are believed to be better committed to differentiate into insulin-producing cells[37]. The group has tested the potential of such insulin-producing cells in restoring normal glucose concentrations in diabetic mice and is planning to initiate studies in monkeys (A. Hardikar, pers. commun.). The group is working on improving the protocols to achieve increase in insulin expression in these cells.

Vemuganti's group has reported isolation and characterization of limbal stem cells from human limbal biopsies[38]. These cells were positive for mesenchymal markers and showed differentiation into adipocytes and osteocytes. The same group has also demonstrated that limbal tissues can be cultured on various types of scaffolds to create a sheet of limbal–corneal epithelium for research and clinical transplantation[39]. The group has treated 125 patients using stem cells from the patients' limbus (http://www.lvpei.org/research/cornea_injuries.html). Presently their research is focused on producing total cornea using stem cell technology.

Clinical trials with bone marrow-derived stem cells

Stem cells isolated from adult bone marrow have the potential to differentiate into different cell types[40]. Bone marrow transplantation (BMT) is an approved cell-based life-saving treatment for many incurable diseases. There are only a few centres carrying out BMT in the developing countries. BMT started in India in 1983 at the Tata Memorial Hospital, Mumbai and soon after in 1986 at Christian Medical College and Hospital, Vellore[41]. Although BMT follows blood-transfusion guidelines, the use of bone marrow as source of ASCs in clinical trials in India is governed by the National Apex Committee for Stem Cell Research and Therapy (SCRT) constituted by the Indian Council of Medical Research (ICMR)[42]. There are centres in the country which offer stem cell therapy without a strong scientific basis or proper safety and efficacy tests. This has been criticized by senior clinicians[43].

A few clinical trials have been published in India using stem cells from various sources. Mehta *et al.*[44] have used stem cells in a heterogeneous group of medically untreatable neurological disorders. A total of 180 patients, including paediatric patients, underwent subarachnoid placement of stem cells. The stem cells used were adipose-derived mesenchymal stem cells, hESC-derived hematopoietic stem cells and autologous bone marrow-derived hematopoietic stem cells, although no technical details have been given. There was no clinically comparable control group in the study. Khan *et al.*[45–47] have demonstrated efficacy of autologous BMT for treatment of chronic liver failure and hepatic progenitor cells to manage hyperbilirubinemia in a two-year-old with Crigler–Najjar Syndrome. Trivedi *et al.*[48] have reported the use of human adipose-tissue-derived, insulin-making mesenchymal stem cells transfused with cultured bone marrow in five insulinopenic diabetes mellitus patients. The adipose tissue and bone marrow aspirates were collected from the same individual who was related to the patient. No data on the follow-up status of these patients are available.

There are Indian companies with foreign collaborations which are carrying out clinical trials with stem cells for different diseases. Nichi-In Centre for Regenerative Medicine is an Indo-Japan collaboration which provides autologous NK cell-based immuno-cell therapy for cancer. It has been providing stem cell isolation, enrichment and expansion services to partner hospitals all over India. It has carried out clinical trials using stem cells from the patients' own bone marrow for liver cirrhosis. Stempeutics is another company floated by a hospital run by Manipal Education and Medical Group, Bengaluru, which is offering regenerative medicine to patients using mesenchymal stem cells derived from their own bone marrow.

Umbilical cord blood-based stem cells in clinical trials

Umbilical cord blood is a rich source of stem cells. The stem cells show multi-lineage differentiation potential and differentiate into adipogenic, chondrogenic, osteogenic and neuronal lineages when cultured with lineage-specific differentiation medium, thus making it a good source for regenerative medicine. There are Indian companies which have established umbilical cord blood banks and are now developing stem cell-based therapies linked with health problems in India[34]. Chennai-based LifeCell, which has a cord blood stem cell banking facility, has launched TRICell, which is a stem cell centre for stem cell transplants and research. Reliance Life Sciences also has set up a cord blood bank in Mumbai and has an active stem cell programme. Cryo Stemcell is another cord and cord blood bank located in Bengaluru, which has conducted trials for Buerger's disease caused due to limb ischaemia using stem cells. It has also conducted clinical trials for liver cirrhosis with a hospital in Hyderabad[47].

Human embryonic stem cell research in India

Human embryonic stem cell (hESC) is a valuable tool to study development and is a potential source of stem cells for regenerative medicine. Their use in cell replacement therapy is being debated due to numerous ethical and safety issues[49,50]. Embryonic stem cell (ESC) research has a strong backing from the Government funding bodies such as DBT, ICMR, etc. and is on the rise in India. There are more than 70 papers published on ESC from India. This review is restricted to hESCs generated in the country.

The ICMR has permitted establishing ESC lines from spare embryos provided the spare embryos are obtained in an ethically acceptable manner. One of the first well-characterized hESC lines from the Indian subcontinent was reported by Mandal *et al.*[51]. These cells were grown on mouse embryonic fibroblasts (MEF) as feeder layer. Because they were grown on xenogenic cells, their use for human clinical trials may be limited. Saxena *et al.*[52] have reported the derivation of FGF2-expressing germ layer-derived human fibroblast cells from embryoid bodies. These feeders could support hESCs as efficiently as that on MEF. Kumar *et al.*[53] have described the derivation of two hESC cell lines using frozen and fresh slow-growing surplus embryos, obtained from collaborating IVF clinics, on in-house-derived human feeder layers. The ESCs have been characterized with respect to markers and were also found to form teratomas *in vivo* in SCID mice[53]. Two hESC lines have been derived by Inamdar's group at the Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru from low quality, discarded embryos[54]. These are now accepted for deposition and distribution by the UK Stem Cell Bank[34].

India is now geared to become one of the major contributors to the emerging field of hESC and is set to harness this technology for therapy. DBT (under the Ministry of Science and Technology) and ICMR have jointly formulated draft guidelines for stem cell research. For implementation and monitoring of the guidelines two separate bodies – Institutional and National SCRT Committees have been proposed[42]. However, additional guidelines are required to address the risk of *in vivo* teratocarcinoma formation associated with ESCs. Strict adherence and compliance with biosafety and regulatory issues is warranted. Quality standards followed in India should not be different from rest of the world.

Conclusion

Gene therapy is a promising new modality of treatment with at least five products for cancer in phase III clinical trials[55], and three experimental biologics drugs approved by the Chinese State Food and Drug Administration[56]. Considering the number of ongoing clinical trials and the success of some of them, gene therapy preclinical and clinical studies in India have to be encouraged. Cell therapies, especially ASCs and hESCs, have received a great deal of attention with regulatory guidelines in place[42]. However, keeping in mind that a combination of gene and cell therapies will be most effective, India has to gear up to gene therapy product development and clinical trials. The Government of India has entrusted DBT with the responsibility of setting up the National Biotechnology Regulatory Authority, which would be an independent, autonomous and professional body to provide a mechanism for biosafety clearance of genetically modified products and processes (www.dbtindia.nic.in). Hopefully, this body will offer guidelines for gene and cell therapy clinical trials and look into gene therapy as an important technology in India.

1. Oldfield, E. H., Ram, Z., Culver, K. W., Blaese, R. M., DeVroom, H. L. and Anderson, W. F., Gene therapy for the treatment of

brain tumors using intra-tumoral transduction with the thymidine kinase gene and intravenous ganciclovir. *Hum. Gene Ther.*, 1993, **4**(1), 39–69.
2. Aiuti, A. *et al.*, Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N. Engl. J. Med.*, 2009, **360**(5), 447–458.
3. Vale, R. D. and Dell, K., The biological sciences in India: Aiming high for the future. *J. Cell Biol.*, 2009, **184**(3), 342–353.
4. Edelstein, M. L., Abedi, M. R., Wixon, J. and Edelstein, R. M., Gene therapy clinical trials worldwide 1989–2004 – an overview. *J. Gene Med.*, 2004, **6**(6), 597–602.
5. Rainov, N. G. and Ren, H., Clinical trials with retrovirus mediated gene therapy – what have we learned? *J. Neurooncol.*, 2003, **65**(3), 227–236.
6. Rainov, N. G. and Ren, H., Gene therapy for human malignant brain tumors. *Cancer J.*, 2003, **9**(3), 180–188.
7. Tai, C. K. and Kasahara, N., Replication-competent retrovirus vectors for cancer gene therapy. *Front. Biosci.*, 2008, **13**, 3083–3095.
8. Wagle, A. S., Joshi, G. V., Naresh, K. N. and Mulherkar, R., Preclinical studies for gene therapy of head and neck cancers using the HSV-tk/GCV strategy. *Indian J. Biotechnol.*, 2005, **4**, 82–87.
9. Kothari, V., Joshi, G., Nama, S., Somasundaram, K. and Mulherkar, R., HDAC inhibitor valproic acid enhances tumor cell kill in adenovirus-HSVtk mediated suicide gene therapy in HNSCC xenograft mouse model. *Int. J. Cancer*, 2010, **126**(3), 733–742.
10. Sarkar, D. P., Ramani, K. and Tyagi, S. K., Targeted gene delivery by virosomes. *Methods Mol. Biol.*, 2002, **199**, 163–173.
11. Jana, S. S., Bharali, D. J., Mani, P., Maitra, A., Gupta, C. M. and Sarkar, D. P., Targeted cytosolic delivery of hydrogel nanoparticles into HepG2 cells through engineered Sendai viral envelopes. *FEBS Lett.*, 2002, **515**(1–3), 184–188.
12. Basu, U. and Banerjee, S., An engineered EBV vector expressing human factor VIII and von Willebrand factor in cultured B-cells. *J. Gene Med.*, 2004, **6**(7), 760–768.
13. Sengupta, A., Banerjee, D., Chandra, S. and Banerjee, S., Gene therapy for BCR-ABL$^+$ human CML with dual phosphorylation resistant p27Kip1 and stable RNA interference using an EBV vector. *J. Gene Med.*, 2006, **8**(10), 1251–1261.
14. Galimi, F. and Verma, I. M., Opportunities for the use of lentiviral vectors in human gene therapy. *Curr. Top. Microbiol. Immunol.*, 2002, **261**, 245–254.
15. MacGregor, R. R., Clinical protocol. A phase 1 open-label clinical trial of the safety and tolerability of single escalating doses of autologous CD4 T cells transduced with VRX496 in HIV-positive subjects. *Hum. Gene Ther.*, 2001, **12**(16), 2028–2029.
16. Santhosh, C. V., Tamhane, M. C., Kamat, R. H., Patel, V. V. and Mukhopadhyaya, R., A lentiviral vector with novel multiple cloning sites: stable transgene expression *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.*, 2008, **371**(3), 546–550.
17. Rub, A. *et al.*, Cholesterol depletion associated with Leishmania major infection alters macrophage CD40 signalosome composition and effector function. *Nature Immunol.*, 2009, **10**(3), 273–280.
18. Ambade, A., Joshi, G. V. and Mulherkar, R., Adoptive T cell transfer augments IL-2 mediated tumour regression in a HNSCC xenograft nude mouse model. *Cancer Lett.*, 2008, **272**(2), 316–324.
19. Bodles-Brakhop, A. M., Heller, R. and Draghia-Akli, R., Electroporation for the delivery of DNA-based vaccines and immunotherapeutics: current clinical developments. *Mol. Ther.*, 2009, **4**, 585–592.
20. Daud, A. I. *et al.*, Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. *J. Clin. Oncol.*, 2008, **26**(36), 5896–5903.
21. Mulherkar, R. *et al.*, Establishment of a human squamous cell carcinoma cell line of the upper aero-digestive tract. *Cancer Lett.*, 1997, **118**(1), 115–121.
22. Ambade, A., Joshi, G. V. and Mulherkar, R., Suicide gene therapy in combination with immunotherapy leads to significant anti-tumour immune response and tumour regression in a xenograft mouse model for head and neck squamous cell carcinoma. *Indian J. Med. Res.*, 2010, **132**, 415–422.
23. Puumalainen, A. M. *et al.*, Beta-galactosidase gene transfer to human malignant glioma *in vivo* using replication-deficient retroviruses and adenoviruses. *Hum. Gene Ther.*, 1998, **9**(12), 1769–1774.
24. Das, S., El-Deiry, W. S. and Somasundaram, K., Regulation of the p53 homolog p73 by adenoviral oncogene E1A. *J. Biol. Chem.*, 2003, **278**(20), 18313–18320.
25. Das, S., El-Deiry, W. S. and Somasundaram, K., Efficient growth inhibition of HPV 16 E6-expressing cells by an adenovirus-expressing p53 homologue p73beta. *Oncogene*, 2003, **22**(52), 8394–8402.
26. Das, S., Nama, S., Antony, S. and Somasundaram, K., p73 beta-expressing recombinant adenovirus: a potential anticancer agent. *Cancer Gene Ther.*, 2005, **12**(4), 417–426.
27. Das, S. and Somasundaram, K., Therapeutic potential of an adenovirus expressing p73 beta, a p53 homologue, against human papilloma virus positive cervical cancer *in vitro* and *in vivo*. *Cancer Biol. Ther.*, 2006, **5**(2), 210–217.
28. Senzer, N. and Nemunaitis, J., A review of contusugene ladenovec (Advexin) p53 therapy. *Curr. Opin. Mol. Ther.*, 2009, **11**(1), 54–61.
29. Senzer, N. *et al.*, p53 therapy in a patient with Li-Fraumeni syndrome. *Mol. Cancer Ther.*, 2007, **6**(5), 1478–1482.
30. Freytag, S. O. *et al.*, Five-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy for treatment of prostate cancer. *Mol. Ther.*, 2007, **15**(3), 636–642.
31. Seymour, L. W., The future of gene therapy in the UK. *Trends Biotechnol.*, 2006, **24**(8), 347–349.
32. Mason, C. and Dunnill, P., A brief definition of regenerative medicine. *Regen. Med.*, 2008, **3**(1), 1–5.
33. Kemp, P., History of regenerative medicine: looking backwards to move forwards. *Regen. Med.*, 2006, **1**(5), 653–669.
34. Lander, B., Thorsteinsdottir, H., Singer, P. A. and Daar, A. S., Harnessing stem cells for health needs in India. *Cell Stem Cell*, 2008, **3**(1), 11–15.
35. Hardikar, A. A., Lees, J. G., Sidhu, K. S., Colvin, E. and Tuch, B. E., Stem-cell therapy for diabetes cure: how close are we? *Curr. Stem Cell Res. Ther.*, 2006, **1**(3), 425–436.
36. Joglekar, M. V., Parekh, V. S. and Hardikar, A. A., New pancreas from old: microregulators of pancreas regeneration. *Trends Endocrinol. Metab.*, 2007, **18**(10), 393–400.
37. Gershengorn, M. C., Geras-Raaka, E., Hardikar, A. A. and Raaka, B. M., Are better islet cell precursors generated by epithelial-to-mesenchymal transition? *Cell Cycle*, 2005, **4**(3), 380–382.
38. Polisetty, N., Fatima, A., Madhira, S. L., Sangwan, V. S. and Vemuganti, G. K., Mesenchymal cells from limbal stroma of human eye. *Mol. Vis.*, 2008, **14**, 431–442.
39. Dravida, S. *et al.*, A biomimetic scaffold for culturing limbal stem cells: a promising alternative for clinical transplantation. *J. Tissue Eng. Regen. Med.*, 2008, **2**(5), 263–271.
40. Jiang, Y. *et al.*, Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, 2002, **418**(6893), 41–49.
41. Chandy, M., Stem cell transplantation in India. *Bone Marrow Transplant (Suppl. 1)*, 2008, **42**, S81–S84.
42. Agarwal, S. S., Regulating stem cell research and therapy. *Indian J. Med. Res.*, 2006, **124**(3), 225–228.
43. Pandya, S., Stem cell transplantation in India: tall claims, questionable ethics. *Indian J. Med. Ethics*, 2008, **V**(1), 5.
44. Mehta, T., Feroz, A., Thakkar, U., Vanikar, A., Shah, V. and Trivedi, H., Subarachnoid placement of stem cells in neurological disorders. *Transplant. Proc.*, 2008, **40**(4), 1145–1147.
45. Khan, A. A. *et al.*, Treatment of Crigler–Najjar Syndrome type 1 by hepatic progenitor cell transplantation: a simple procedure for

management of hyperbilirubinemia. *Transplant. Proc.*, 2008, **40**(4), 1148–1150.
46. Khan, A. A. *et al.*, Management of hyperbilirubinemia in biliary atresia by hepatic progenitor cell transplantation through hepatic artery: a case report. *Transplant. Proc.*, 2008, **40**(4), 1153–1155.
47. Khan, A. A. *et al.*, Safety and efficacy of autologous bone marrow stem cell transplantation through hepatic artery for the treatment of chronic liver failure: a preliminary study. *Transplant. Proc.*, 2008, **40**(4), 1140–1144.
48. Trivedi, H. L. *et al.*, Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin. *Transplant. Proc.*, 2008, **40**(4), 1135–1139.
49. Deb, K. D., Jayaprakash, A. D., Sharma, V. and Totey, S., Embryonic stem cells: from markers to market. *Rejuv. Res.*, 2008, **11**(1), 19–37.
50. Deb, K. D. and Sarda, K., Human embryonic stem cells: preclinical perspectives. *J. Transl. Med.*, 2008, **6**, 7.
51. Mandal, A. *et al.*, Characterization and *in vitro* differentiation potential of a new human embryonic stem cell line, ReliCellhES1. *Differentiation*, 2006, **74**(2–3), 81–90.
52. Saxena, S., Hanwate, M., Deb, K., Sharma, V. and Totey, S., FGF2 secreting human fibroblast feeder cells: a novel culture system for human embryonic stem cells. *Mol. Reprod. Dev.*, 2008, **75**(10), 1523–1532.
53. Kumar, N. *et al.*, Derivation and characterization of two genetically unique human embryonic stem cell lines on in-house-derived human feeders. *Stem Cells Dev.*, 2009, **3**, 435–445.
54. Inamdar, M., Venu, P., Srinivas, M. S., Rao, K. and VijayRaghavan, K., Derivation and characterization of two sibling human embryonic stem cell lines from discarded Grade III embryos. *Stem Cells Dev.*, 2009, **3**, 423–433.
55. Guinn, B. A. and Mulherkar, R., International progress in cancer gene therapy. *Cancer Gene Ther.*, 2008, **15**(12), 765–775.
56. Jia, H. and Kling, J., China offers alternative gateway for experimental drugs. *Nature Biotechnol.*, 2006, **24**(2), 117–118.

Received 10 May 2010; accepted 12 October 2010