MEETING REPORT

Gene therapy*

The vast majority of rare genetic disorders are currently without any cure, causing misery to an estimated 70 million individuals in our country. Gene therapy offers a potential platform technology to find cures for most genetic disorders, particularly those due to defects in a single gene. Moreover, gene therapy holds treatment potential in many cancers and other disorders, such as peripheral artery diseases and arthritis. In many diseases, it offers the only hope towards a cure.

Currently, more than 1000 clinical trials involving gene therapy are underway on a range of diseases. Already two gene therapy treatments, one for osteoarthritis and the other for cancer have been approved in South Korea and USA respectively. Moreover, studies carried out in animal models, and limited patient trials clearly show the potential of gene therapy in the treatment of genetic disorders. Unfortunately, no gene therapy trial has been registered in India so far. A number of laboratories across the country are engaged in gene therapy-related research, and there is a need to pool in our resources and efforts to achieve tangible success for the benefit of patients. To this end, a discussion meeting was organized recently. In addition to research scientists working in this area, the meeting was attended by industry representatives and doctors treating patients with rare genetic disorders.

Subrata Sinha (National Brain Research Centre, Manesar) in his inaugural talk described his work on targeting cancer cells using the oncofetal antigen placental alkaline phosphatase (PAP) through the Sendai virus-based virosome. This system for targeted delivery has been pioneered in India by Debi Sarkar (IISER, Mohali) and has great potential as a gene/drug delivery system for direct delivery to cytosol. He described the use of Sendai virosome for targeting cancer cells through PAP. This antigen is expressed on the cell surface in a number of cancer cell types, and has low shedding, making it a suitable targeting molecule. His group used phage display library of human ScFv clones to select for PAP cross-reacting clones. A fusion construct of the selected ScFv clones was made with Sendai virus F-protein integrated into a virosome for doxorubicin delivery to the cancer cells. The same strategy was used to selectively deliver genes capable of converting prodrug to active drug in cancer cells. Hence the virosome technology is versatile and appears to be an efficient delivery system that could be targeted to specific tissues.

Sarkar's group has used this technology in gene therapy with the Gunn rat model for a human disorder caused due to lack of uridine diphosphoglucuronate glucuronosyl transferase 1A1 (UGT1A1) activity in hepatocytes. The team delivered the UGT1A1 gene along with the sleeping beauty transposon which promoted integration of the human UGT1A1 into the host genome.

Subrata Banerjee (Saha Institute of Nuclear Physics, Kolkata) described the Epstein Barr Virus (EBV)-based episomal vector which has the advantage of a large genome (172 kb) and can accommodate genes of large size that are difficult in the adeno-associated vectors. In cell lines infected with EBV virions, the DNA remains episomal for long periods. This obviates the dangers associated with chromosomal integration at undesirable genomic sites. The flip side of EBV-based vectors is the paucity of animal models that can be used for their infection and further development as gene delivery systems.

Sivaprakash Ramalingam (IGIB, New Delhi) talked about the various technologies available for targeted genome engineering, including gene-editing nucleases like TALENS, and RNA-guided methodology like CRISPR/Cas9. In his experience with hemoglobinopathies, especially sickle cell anaemia, TALEN gave high specificity and low off-target risk, although it is more cumbersome to establish in the laboratory than CRISPR/Cas9.

The talks by Dwaipayan Sen (VIT University, Vellore) and Arkasubhra Ghosh (GROW Lab, Narayana Nethralaya Foundation, Bengaluru) described the use of recombinant adeno-associated viral (rAAV) vectors. Sen described his work with hemophilia, for which the current treatment option is supplementation with factor VIII/IX. It is expensive, and about 30% of patients develop neutralizing antibodies against the factors, making it necessary to develop other treatment options like gene therapy. Immune response against the rAAV vectors is also a problem, and efforts are on to reduce its immunogenicity. The seropositivity of Indian population against rAAV vectors is expected to be high. Sen pointed out that the transduction efficiency with rAAV vectors could be enhanced by preventing its proteasomal degradation. He has used mutants of different AAV serotypes, defective in phosphorylation to escape proteasome, which not only enhances vector transduction efficiency, but also significantly reduces host immune response.

Ghosh enumerated the advantages of rAAV vectors, including their ability to target nondividing cells, their high level of gene expression and excellent stability. However, these vectors are expensive to produce and have a small capacity for DNA that can be accommodated (~5 kb). He showed that longer genes could be delivered in a split format, since rAAV genomes concatemerize in the cell by homologous recombination. The two halves of a large gene can be cloned in separate vectors which can be co-transfected in the same cell. The large gene can then be reconstructed intracellularly by vector concatemerization. He tried this approach for DMD and obtained hybrid mini dystrophin. His group has studied the systemic delivery of rAAV vectors to different tissues in neonatal and adult mice. They have also tried local injection in adult and neonatal dogs, and found the delivery to muscle cells lower in adult animals than neonates. Ghosh is now setting up a GMP facility for vector production at Narayana Nethralaya.

Rupesh Dash (ILS, Bhubaneshwar) works on oral squamous cell carcinoma,

*A report on discussion meeting on 'Gene Therapy for Curing Rare Genetic Disorders' organized on 16 September 2017 at School of Life Sciences, Jawaharlal Nehru University, New Delhi. The meeting was co-sponsored by World Without GNE Myopathy (NGO working on rare genetic disorders).

which is the number one cancer in Indian males. He described possible therapies like use of cancer terminator viruses that selectively replicate and destroy cancer cells. He also plans to use microbubble-based gene delivery, and AAV vector-based delivery of tumour suppressor genes in oral cancer.

Sujata Mohanty (AIIMS, New Delhi) talked about the use of stem cells in developing gene therapy for rare genetic disorders. She heads the DBT Centre for Excellence in Stem Cell Research and has set up a CGMP facility for handling stem cells of therapeutic quality. Her group has cryopreserved many mesenchymal stem cell lines, following the ICMR guidelines. Quality control is done by karyotyping and marker testing by flow cytometry. This state-of-the-art facility set up by her dedicated efforts will be invaluable for research and therapeutic developments using gene therapy.

The talks by scientists were followed by presentations from industry representatives. Sumathy (Bharat Biotech, Hyderabad) presented the priority areas of her company, which is the leading manufacturer of a large number of vaccines, and is also engaged in clinical trials of many of them. The company has a large GMP manufacturing capability and is knowledgeable about the regulatory procedures. These assets could be tapped in future endeavours for gene therapy.

Ravinder Makkar (Sanofi Genzyme) pointed out that his company was the first to come out with enzyme therapy for lysosomal storage disorders and supply the enzymes to Indian patients. It is concerned about the lack of clarity in the roadmap ahead, uncertainty in regulatory guidelines, and cost of technology development that are hampering new technology developments in India.

Bhaskar Jyoti Sonowal (Shire) apprised about this Irish company which has entered the Indian market only a year ago. The company is interested in genetic disorders like haemophilia, and is involved in phase 1/2 clinical trial for Factor VIII. However, it could not recruit any Indian patients for the trial as the mechanism for fast-track recruitment of patients is not in place in India. Hence guidelines need to be streamlined for the benefit of patients.

After the presentation of the talks highlighting current level of expertise in the country, a panel discussion on 'Developing gene therapy for rare genetic disorders in India: How to go forward' was held. The discussion was chaired and moderated by Madhulika Kabra (AIIMS, New Delhi). The panelists included Sheffali Gulati (AIIMS, New Delhi), Ratna Puri (Sir Ganga Ram Hospital, New Delhi) and Sudha Bhattacharya (Jawaharlal Nehru University, New Delhi). All panellists emphasized the importance of gene therapy for ultimately curing patients with rare genetic disorders, as most of the treatments currently available for a few diseases are expensive and require to be taken throughout life. However, they pointed out a number of obstacles that need to be crossed before it becomes feasible to carry out a gene therapy trial in India. These include setting up of integrated multidisciplinary clinical care centres, simplified regulatory guidelines with possibility to carry out exploratory studies, funding support for generating preclinical data and for clinical trials, and GMP production facilities for making vectors and stem cells. The panellists also emphasized the importance of patient groups in this endeavour.

In conclusion, the participants felt that India needs to have a National Mission on Gene Therapy that will allow development of relevant technologies, infrastructure for implementation and time-bound execution plan.

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MEETING REPORT

River rejuvenation*

India is in the midst of a water crisis and is now considered to be a water-scarce country. The Indian Himalayan and Peninsular rivers are fast depleting. To address the water crisis in India, initiatives to rejuvenate Indian rivers have been taken up by both Government and non-Government organizations.

Identifying the type of distortions and planning to revive the natural status is the concept of river rejuvenation under the 'Art of Living' banner. The initiative has been supported by the Government and other corporates. The conference highlighted methodologies used to rejuvenate rivers in Karnataka, Maharashtra, Tamil Nadu and Kerala. This initiative is now being extended to other states in India.

The aspect related to flow of a river and its rejuvenation was highlighted by K. Subramanya (formerly at Indian Institute of Technology, Kanpur). In the flow of a stream, a combination of surface water and groundwater keeps it running. The groundwater discharges into the stream when its surface water level falls below the water table. During monsoon, run-off from the catchment area drains as streams join the river and generates the flow of the river. Post-monsoon, the run-off tapers and the base flow or contribution of groundwater into the stream increases and feeds into the river for the rest of the year.

Subhajyoti Das (formerly with Central Ground Water Board, Bhubaneswar) provided an overview of endangered rivers in India. Environment and ecological systems are essential to sustain the river flow and water quality. Plants and trees are essential for run-off generation and infiltration of water. Roots of trees and plants help in keeping the soil porous for infiltration and also help in arresting soil erosion. Rivers also sustain aquatic plants, fishes and other aquatic life-forms which help in keeping them pure. Das explained that reduced river flow due to creation of dams, diversion of water, discharge of untreated sewage, overexploitation of groundwater, decreasing base

*A report on the River Rejuvenation conference that was held by the Art of Living Foundation on 5 and 6 December 2017 at Bengaluru.