CELL & GENE THERAPY INSIGHTS

CHALLENGES & ADVANCES IN VIRAL VECTORS

SPOTLIGHT

EDITORIAL

Clinical translation of viral vectors for gene therapies and beyond

Gerhard Bauer and Mohamed Abou-El-Enein

Gene therapy has, after years of setbacks, returned as a highly advantageous novel therapy for the treatment of severely debilitating diseases for which there were no treatment options.

Clinical applications of viral vector-mediated gene therapy will soon be approaching their third decade of use. The initial idea of re-engineering naturally arising viruses for efficient integrating and non-integrating gene delivery strategies was groundbreaking [1]. A huge amount of effort went into basic research in the 1980s to make murine oncoretroviruses replication incompetent, adapt them to efficiently transduce human cells and then reliably integrate their payload into the genome. At the same time, adenoviruses were re-engineered to transport genetic information into target cells without gene integration. Soon afterwards, adenoviral vectors were used for human *in vivo* gene therapy applications, while culture technologies for human T cells and human hematopoietic progenitor cells were developed to allow for retroviral vector transductions *ex vivo* [2]. At that time, while used under appropriate transduction conditions and transferring a well-designed gene, oncoretroviral vectors based on the murine moloney leukemia virus could be utilized in clinical testing. Unfortunately, the *in vivo* application of a first-generation adenoviral vector in a clinical trial of gene therapy for ornithine transcarbamylase (OTC) deficiency ended with the death of a young patient in the year 2000, due to a systemic, uncontrollable inflammatory reaction [3]. Similarly, the oncoretroviral mediated clinical trial of hematopoietic stem cell gene therapy for X-linked

severe combined immunodeficiency (SCID) in France caused several patients to develop leukemia, due to insertional toxicity [4]. By carefully evaluating these severe drawbacks, soon a clearer understanding of the safety issues involved in viral-mediated gene therapy was achieved [5]. Safer adenoviral vectors were developed that would not cause systemic inflammatory reactions, and for retroviral vectors, tighter control of transduction conditions and inserted copy numbers, combined with integration site analyses with a better understanding of the integration pattern in transduced cells lowered the inherent risk of insertional toxicities. A major improvement in integrating vector gene therapy was achieved when HIV-based vectors became available, offering a safer integration pattern [6]. The further development and application of adeno associated viral vectors (AAV) also offered significant improvement in non-integrating *in vivo* gene therapy, since these vectors showed extremely high safety with almost no toxicities, particularly in gene delivery to the CNS [7].

Soon after, gene therapy clinical trials, particularly using AAV and lentiviral vectors, established clear clinical benefits in many patients [8]. Lentiviral vectors have shown therapeutic efficacy in the treatment of ADA SCID [9], adrenoleukodystrophy [10] and Wiskott–Aldrich syndrome [11]. AAV vectors have been successfully used for treatments of Leber's congenital amaurosis 2 [12], a rare inherited eye disease with a mutation in the *RPE65* gene, choroideremia [13], an X-linked recessive retinal disease, and hemophilia B [14]. Additionally, an AAV vector, Glybera® (uniQure), has been approved as the first gene therapy application in Europe, for the treatment of lipoprotein lipase deficiency. Subsequently, other gene therapy products entered the EU market [15]; an oncolytic herpes virus 1 (HSV-1), Imlygic® (Amgen), for the treatment of melanoma, Strimvelis® (GlaxoSmithKline), autologous hematopoietic stem cells (HSCs) transduced with a retroviral vector transferring the ADA gene to treat children with ADA SCID, and most recently, Zalmoxis® (MolMed SpA), allogeneic T cells for the treatment of high-risk hematologic malignancies (in conjunction with stem cell transplantation), transduced with a retroviral vector transferring a herpes simplex virus-1 (HSV-1) thymidine kinase. In the USA, no marketing approval for gene therapy vectors has yet been given; however, several successful gene therapy products, including transduced stem cell products, are in the pipeline for marketing approval.

While Europe has made excellent progress in approving gene therapy vectors and certain cellular gene therapies as marketed products, the USA has made great strides in pioneering a new gene therapy technology in the fight against cancer – chimeric antigen receptor T cells (CAR-T cells) [16]. Autologous patient T cells are genetically engineered using retro- or lenti-viral vectors, transferring the genetic information for a new T-cell receptor that can recognize a specific antigen on cancer cells, bind to it and enable the T cells to elicit cytotoxicity on the target cells. Remarkable cancer remissions, particularly in leukemias, could be demonstrated [17], and significant amounts of funding have been directed into the clinical development of this technology. The drawbacks, however, are sometimes

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systemic toxicities caused by cytokine storms, which still need to be monitored, controlled and made manageable. To manufacture the gene-modified cellular product, autologous T-cell expansions and transductions need to be performed in a controlled environment, which is currently only possible in specialized centers [18]. However, closed-system culture technologies are under development that will, in the future, allow the generation of CAR-T cells at a much wider scale, and also in areas where Good Manufacturing Practice (GMP) laboratories are not available.

Currently, the transduction of HSCs with integrating vectors, particularly lentiviral vectors for clinical applications is the most complicated procedure, requiring several highly technical steps [19]. To treat ADA SCID, for instance, the patient's bone marrow or mobilized peripheral blood stem cells are harvested, $CD34^+$ hematopoietic stem and progenitor cells are isolated and cultured in conditions that allow efficient transduction, but at the same time, will not diminish the cells' long-term engraftment potential. The transduced $CD34^+$ cells are tested extensively prior to the infusion into the recipient. Product safety is imperative, with tests for sterility, endotoxin, mycoplasma and integrated copy number performed. It is vital that as few therapeutic gene copies will be integrated per genome to limit integrational toxicity with any possibility of leukemia generation. The cells will be administered with myeloablation or reduction, to allow for efficient engraftment of the gene corrected cells. It took the field almost 20 years to perfect this protocol. Only a few specialized centers in the world can perform this procedure. Maybe when this application becomes more mainstream, more medical centers will be equipped with specialized facilities and highly trained personnel, able to perform it.

Scaling up of the manufacturing process from laboratory scale to clinical scale is not trivial, both in vector and cellular manufacturing.

The translation of laboratory research into all these aforementioned clinical applications with therapeutic efficacies, although having progressed significantly, has always been challenging and required the development of unique knowledge and costly manufacturing procedures [20]. Often, both vector manufacturing and cellular manufacturing is required, and both need to be performed under GMP conditions. These controlled manufacturing procedures must be approved by the appropriate regional regulatory agencies and carried out in properly equipped facilities with equally properly trained personnel. Scaling up of the manufacturing process from laboratory scale to clinical scale is not trivial, both in vector and cellular manufacturing. Vector purity, particularly for direct *in vivo* administration is of utmost importance, and vector titer (with good packaging efficacy and little interference from empty particles) is directly responsible for good clinical efficacy. In the past, retroviral vector could be manufactured using stable producer cells. This production method cannot easily be applied to lentiviral vector or AAV vector manufacturing. Transient plasmid transfection into certified human producer cells is the most common

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vector production method, currently. Transfection efficiency is over 90% in most protocols; however, vector particle yield is largely dependent on the properties of the packaged therapeutic gene, with a high degree of variability. Vector purification strategies are also wide ranging, from spin filtration, tangential flow, gradient ultracentrifugation to chromatography methods. Vector certification tests include sterility, endotoxin, mycoplasma, replication competent vector, plasmid DNA, host cell DNA and proteins, other tests may also be necessary; again, the most important aspect of the manufactured vector lot is its safety.

What lies ahead in the future? Most likely, new gene editing technologies with zinc finger nucleases, transcription activator-like effector nucleases and clustered regularly interspaced short palindromic repeat-associated systems will be widely employed in clinical testing for gene therapies [21]. Instead of inserting a new functional gene into cells at random and leaving the old one behind, the non-functional gene will be replaced with the new functional gene, in the correct locus. This will remove the danger of random insertional toxicity with upregulation of oncogenes in the vicinity of the new gene. *In vivo* gene editing is also being developed; however, any adverse events associated with this novel technology, particularly unwanted genetic editing of germ line cells and off target effects will need to be studied carefully, prior to its implementation in clinical testing.

Gene therapy has, after years of setbacks, returned as a highly advantageous novel therapy for the treatment of severely debilitating diseases for which there were no treatment options. As marketed therapeutic products, gene therapies will add an arsenal of new options to provide life-saving clinical benefits to many patients worldwide.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

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