Gene therapy delivery tools poised for success in ocular gene therapy

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<td>Broad tropism for different cell types/tissues</td>
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**Table of vectors used**

The gene that needs to be delivered and the target tissue. The broad range of different serotypes, some of which have been converted into gene therapy vectors, are currently being tested for their ability to transduce the retina.

### Turning genes on only where they are required

Choosing a suitable capsid type represents just one way to direct or target gene expression in specific cell types. Another way to direct gene expression following subretinal administration or vitreal injection is to engineer the gene of interest with cell type specific promoters – sequences of DNA upstream of the gene that regulate gene expression. For example, expression of a given transgene may be restricted to a specific cell type such as the retinal pigment epithelia (RPE) or ganglion cells by using gene promoters specific to transcripts expressed in those cell types. Alternatively, using the rhodopsin or rhodopsin kinase promoter limits expression of a transgene to rod photoreceptors. This capability provides additional comfort in terms of regulatory safety concerns to insure that a given transgene is expressed only in the target cell.

Dr Aurichio showed examples of how AAV can be used to correct defects in some animal models including severe retinal diseases where most of the genes are expressed in photoreceptors and require gene replacement as a therapeutic strategy. For example in recessive RP, brought about by a homozygous mutation of the subunits of the phosphodiesterase (sPDE) gene, delivery of a normal sPDE gene has brought about functional rescue from the disease state.

In other ocular applications, for example in dominant disease, RNAi can knock down the mutant rhodopsin by 70 per cent leaving the wild type rhodopsin gene untouched. Gene therapy may also be applied to animal models of ocular neovascularisation such as retinopathy of prematurity (ROP) and AMD. One model of ROP that induces neovascularisation exposes newborn mice to high levels of oxygen and then returns animals to normal oxygen levels at which point an AAV carrying an angiogenic gene can be introduced to block neovascularisation.

### Lentiviral vectors provide an alternative delivery tool

A very different viral vector used for gene therapy applications was presented by Dr Kam Balaggan MRCOphth, specialist registrar at Moorfields Eye Hospital and research fellow in the Division of Molecular Therapy, Institute of Ophthalmology. London headed by Prof Robin Ali. Lentiviral vectors, unlike adenovirus and AAV, are RNA viruses with a capacity to efficiently transduce both dividing and non-dividing cells and are associated with minimal intraocular immune responses.

The principal advantages of lentiviral vectors are that they accommodate a large carrying capacity and mediate high levels of transgene expression. For certain applications they can deliver genes too large for AAV to carry while also having predictable kinetics of expression, typically expressing at a very early stage following administration. New early all the different types of lentiviral vectors described to date can efficiently target ocular RPE cells with some possessing the capacity to also transduce photoreceptors and other neurosensory retinal cells with varying efficiency after conventional subretinal delivery.

Lentiviral vectors may be derived from primate sources such as HIV and the simian immunodeficiency virus (SIV) or from non-primate sources such as horse (equine infectious anaemia virus-EIAV), cat (feline immunodeficiency virus-FIV) and cow (bovine immunodeficiency virus-BIV). The principal advantage of such derivatives is that their use further minimises any potentially deleterious consequences given that their wild type forms are known to be non-pathogenic in humans. Lentiviral vectors integrate efficiently into the host cell genome and so the transgene may propagate to all daughter cells following cell division.

The wild type HIV genome, typically around 9,700 base pairs in size, can be engineered into a gene therapy vector because most of the pathogenic components of these viruses can be safely removed without causing any diminution in either viral titre or activity. To create vector tools the viral genome may be split across three separate plasmids, coding for all the essential sequences necessary for transcription and integration of the viral genome, insertion of the gene of interest and a back bone with as little as 550 base pairs (down from the original 9,700 base pairs) so very little of the original pathogenic vector remains.

As two of the plasmids do not have any encapsidation sequence they do not get incorporated into the viral particle but function purely to provide the structural proteins and enzymes necessary for correct functioning of the lentiviral particle.

The natural tropism for wild type HIV-1 is the CD4+ lymphocyte. However, it is possible to expand this affinity beyond CD4+ cells by removing the original protein encoding the wild type viral envelope and replacing with a gene encoding the envelope protein from a different virus.

Dr Balaggan and colleagues have tested a number of re-engineered vectors for delivery of genes to the retina in animal...
models, using green fluorescent protein as a marker gene to track transduction and expression efficiencies. Using the EIAV vector Dr Balaggan’s research group have stably and efficiently transduced retinal pigment epithelial cells in animal models resulting in high levels of GFP expression within just three days of subretinal administration and persisting for at least 16 months.

The implications of maintaining transgene expression for 16 months following a single injection in itself illustrates the attractiveness of gene therapy because such an approach could be used for the long-term delivery of therapeutic proteins. For example in exudative AMD, genes encoding anti-VEGF proteins could be delivered resulting in sustained VEGF targeting after just a single injection, in contrast to the repeated delivery that is required of current pharmacological anti-VEGF agents including Lucentis and Avastin. As it is likely that life-long administration of pharmacological agents will be required to sustain therapeutic efficacy, the advantages of a gene-based approach for patients with exudative AMD could be immense.

**Clinical evaluation of lentiviral vector tools**

Use of viral vectors in clinical gene therapy has generally yielded few notable successes aside from two trials based on providing gene based therapies for SCID (severe combined immune deficiency), a disease of both cellular and humoral immunity, a form of which may be caused by a mutation in the IL2RG gene which encodes the alpha chain of the interleukin-2 receptor.

Research teams in France, and later in the UK, successfully used an integrating retroviral vector to deliver a correct functional copy of the IL2RG gene to autologous bone marrow cells which were then transplanted back into patients resulting in a dramatic recovery. Following treatment, patients produced normal numbers of T cells, did not need to live in specialised environments and responded well to childhood immunisation. This was a significant milestone in the history of gene therapy.

However, even in advance of the SCID trials, there were concerns that apparent random integration of exogenous DNA into host cells could, in theory, activate a proto-oncogene or deactivate a tumour suppressor gene. This concern became a reality when four patients from one of the trials developed T cell leukaemia thought in at least two patients to arise from the vector genome inserting near to a proto-oncogene (LMO2) which is known to induce T cell leukaemia formation.

To address the issue of insertional mutagenesis, a new generation of non-integrating lentiviral vectors has been developed and are currently undergoing testing. These “integration-deficient” lentiviral vectors are expected to substantially reduce the risk of insertional mutagenesis in addition to reducing the risk of inadvertent germ line transmission, for applications involving post-mitotic or essentially post-mitotic cells, such as those in the eye.

**Non-integrating lentivirus show equivalent efficiencies**

Dr Balaggan’s group in collaboration with colleagues from Prof Adrian Thrasher’s group at the Institute of Child Health, London have developed and tested self-inactivating advanced vectors carrying a single substitution mutation in one position of the gene encoding the integrase enzyme of HIV which catalyses the integration of the double-stranded DNA into the host cell chromosome. The result of this alteration reduces the integration frequency of HIV vector by 10,000 fold in vitro.

**“The implications of maintaining transgene expression for 16 months following a single injection in itself illustrates the attractiveness of gene therapy because such an approach could be used for the long-term delivery of therapeutic proteins”**

To test the feasibility of a gene therapy approach to choroidal neovascularisation Dr Balaggan and colleagues have used EIAV vectors to deliver angiostatic genes (endostatin and angiostatin) originally characterised for their tumour suppression characteristics now known to have multiple mechanisms of action including the inhibition of VEGF, and also imparting a positive effect on endothelial cell apoptosis. Animal studies showed that administration of the therapeutic genes inhibited angiogenesis in the order of 50-60 per cent. An additional efficacy measure of permeability was recorded by calculating the proportional degree of increased leakage from early phases to latter phases resulting in a reduction of approximately 25 per cent. In the therapeutic groups the size of the lesion is substantially smaller than that in the control groups as is the degree of leakage between early and late phases.

Dr Balaggan’s group also evaluated the efficacy of the newer non-integrating HIV1 vectors, in models of retinitis pigmentosa secondary to RPE65 defects. Analysis of two models of early onset retinal dystrophy, the RPE65 mutation which accounts for about 10 per cent of LCA cases and the MERTK deficiency modelled in the RCS rat, substantial rescue was observed in function in the RPE65 deficient mouse in the order of 300-500 per cent increase in ‘b’ wave amplitudes over fellow un-injected eyes. Similarly, in the MERTK deficient rat, preservation of function at six to eight weeks was recorded compared with un-injected eyes where no rescue was seen with control vectors. These technologies and pre-clinical results provide added confidence for the use of lentiviral vectors in gene therapy applications for a range of ocular diseases.

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