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Retinal Gene Therapy

Introduction: Since most causes of incurable blindness have an underlying genetic basis and frequently affect cells of the retina, retinal gene therapy is a therapeutic approach that has great potential to revolutionise our current management of a number of retinal diseases over forthcoming decades. In its most basic concept, gene therapy is a process whereby the disease or pathological process is modified at the genetic level by treatment with nucleotides (DNA or RNA) rather than by proteins or other drugs. Using DNA has a particular advantage because if the DNA can be stabilised in the nucleus it could potentially remain indefinitely, providing a permanent genetic modification following one single treatment.

Gene therapy could in theory be administered with nucleotides only, but these soluble molecules would have difficulty penetrating the cell membrane and would undergo rapid degeneration after entry into the cytoplasm of the host cell. A viral vector is commonly used in order to get the DNA into the nucleus more effectively. This is the capsule of a virus capable of infecting a specific cell type which has had all wild type genetic material removed – in place of the viral genes, therapeutic genes are inserted through the process of viral cloning. Some viruses (e.g. lentiviruses) carry an RNA template which is used to reverse transcribe double-stranded DNA directly into the host genome. Other viruses, such as adenovirus carrying double-stranded DNA, do not integrate into the host genome. The virus or vector is described as being enveloped if it contains cell membrane around its protein shell and this property is associated with an increased risk of immune reaction. The gene size is also a limiting factor for some vectors and critically the ABCA4 (Stargardt) gene at 6.7 kilobase (kb) is too large for adeno-associated viral (AAV) vectors, but will fit into lentivirus.

Table 1: characteristics of viral vectors used in human retinal gene therapy clinical trials.
Key – ds/ss = double/single stranded; kB = kilobase (1,000 nucleotides of DNA)

Viral vector type	Viral Genome	Genomic insertion	Envelope	Maximum gene size	Immune reactions	Clinical trials
Adenovirus	dsDNA	No	No	45kb	Yes	2
Lentivirus	dsRNA	Yes	Yes	9kb	Yes	soon
Adeno-associated virus (AAV)	ssDNA	Minimal	No	5kb	Minimal	6

Background to retinal gene therapy: The retina is a very attractive site for gene therapy for several reasons. In the first instance the cells of the retina mostly do not divide which is a safety benefit because uncontrolled genetic modification of proliferating cells brings with it an increased risk of malignancy. This fact has been observed in gene therapy trials to treat haematological disorders.[1] Also, compared to other organs such as the liver, the dose of viral vector particles required to target the retina is several log units lower which reduces the risk of immune reactions.[2] Finally, the target area for delivery of viral vectors is enclosed within the eye (even more so in the subretinal space) and systemic dissemination of viral vector is extremely low, which further reduces the risk of systemic immune reactions.[2]

The first clinical trial of retinal gene therapy used adenovirus expressing thymidine kinase to target retinoblastoma tumour cells following intravitreal injection.[3] There were no observed side-effects following this treatment, however, the design of this small pilot study made it very difficult to assess any definite efficacy. Subsequently, adenovirus was also used to express pigment epithelium-derived factor (PEDF), a naturally occurring anti-angiogenic protein, following intravitreal delivery in

patients with advanced neurovascular age-related macular degeneration.[4] This was also safely tolerated but the trial was not large enough to prove unequivocally any treatment effect. Following this, in 2007, gene therapy trials using AAV were started in several centres to treat Leber's Congenital Amaurous (LCA) caused by mutations in the isomerise enzyme RPE65.[5-7] The LCA trials had a great advantage over previous adenoviral clinical trials, because RPE65 is an important enzyme in the visual cycle and hence the success of gene transfer could be assessed by an improvement in vision in the treated eye compared to the control eye. Indeed, in one study, the pupil response was significantly enhanced in the study eye compared to the fellow eye which provided objective evidence of efficacy and, in another study, ectopic administration of the viral vector led to a shift in foveal fixation to the centre of the region where the viral vector had been injected.[8-9]

Significantly, both of these effects have been sustained and long-lasting, confirming predictions that a single dose of AAV gene therapy would provide an indefinite therapeutic effect. In 2010 a commercial study led by Genzyme started using AAV to deliver sFLT1 which inhibits neovascularisation (NCT01024998). More recently a non-commercial multicentre gene therapy clinical trial using AAV to treat choroideraemia has also started in the UK (NCT01461213).
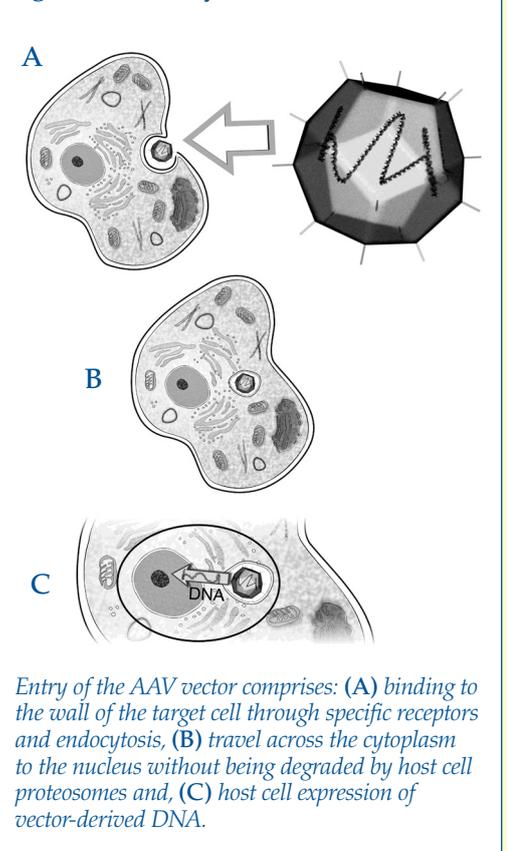
Life cycle of the AAV vector: In order to be effective, the viral vector needs to penetrate the cell wall, travel to the nucleus and express its gene within the DNA of the host cell (Figure 1). In the case of AAV, the small particle size and low immunogenicity facilitates exposure of many viral particles to a single cell. The protein capsule around the vector can be modified to give it specific infectivity for certain cell types. For instance AAV serotype 2 (AAV2) targets retinal pigment epithelium (RPE) highly effectively, but AAV8 has much better infectivity photoreceptor cells.[10] When travelling across the cytoplasm viral particles are subject to degradation by number of host mechanisms. In the case of AAV, binding of proteosomes to the viral capsule identify it for degradation. Recent work has shown that modification of tyrosine residues on the AAV capsule can help viral particles evade this cell-based defence mechanism and hence increase infectivity of the vector.[11] Finally the vector enters the nucleus which is the probable site of removal of the capsule and stabilisation of the viral single-stranded DNA following reverse strand synthesis by the host cell DNA polymerase. The double-stranded DNA is then believed to form a circle, which remains stable close to the host DNA where it can be transcribed indefinitely. The circularisation is facilitated by a specific palindromic viral sequence known as an inverted terminal repeat (ITR), which is the only original part of the AAV sequence that remains on the transgene. The ITRs take up some of the limited space but they are essential for packaging of the DNA during vector production.

The gene will, in addition to the therapeutic gene, require a number of other regulatory sequences. The therapeutic gene is typically the coding sequence of DNA excluding any introns. Splicing of RNA is, however, an important part of gene expression and in particular may be associated with export of RNA through the nuclear pores into the cytoplasm where translation can take place. Therefore in some cases a small splicing reaction will be included at some point within the vector genome even though it takes up valuable space and is not technically necessary. In order for the RNA to be translated efficiently a modified sequence just upstream of the first amino acid coding position is also required to facilitate the binding of ribosomes, also termed "Kozak" consensus. The gene will need to be switched on and this is achieved with a promoter which can be active in all cells or may be selective in targeting only specific cells (a promoter is a DNA sequence that is recognised by the host cell as a point at which a gene starts). The end of transcription is signalled by another sequence downstream of the gene known as a poly-adenylation (Poly-A) signal, which triggers the addition of multiple adenosine (A) nucleotides to the RNA transcript stabilising it for export into the cytoplasm where it is translated into protein. The palindromic ITR sequences fold over to create double-stranded DNA "caps" at either end of the initial single-stranded DNA vector genome but may also recombine at each end to circularise the DNA after second strand synthesis, or link together one or more double-stranded AAV genomes to form a large circular sequence.

Conclusions: The excellent safety profile of AAV and ability to monitor the effects so effectively using the fellow eye as a control means that retinal gene therapy will now most likely develop more rapidly than gene therapy for other diseases over the next decade. The main limiting factor for AAV is its small gene carrying capacity, which is too small for genes such as ABCA4 (Stargardt disease) and Usherin-2A (Usher Syndrome). Nevertheless there are many small gene recessive diseases suitable for AAV clinical trials to keep clinicians busy at the current time and almost certainly a large safe gene vector will emerge in future. The possibility of using AAV gene therapy to control angiogenesis following potentially one single intraocular injection is a particularly exciting concept.

Figure 1 – Life cycle of the AAV vector

A

B

C

DNA

Entry of the AAV vector comprises: **(A)** *binding to the wall of the target cell through specific receptors and endocytosis,* **(B)** *travel across the cytoplasm to the nucleus without being degraded by host cell proteosomes and,* **(C)** *host cell expression of vector-derived DNA.*

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