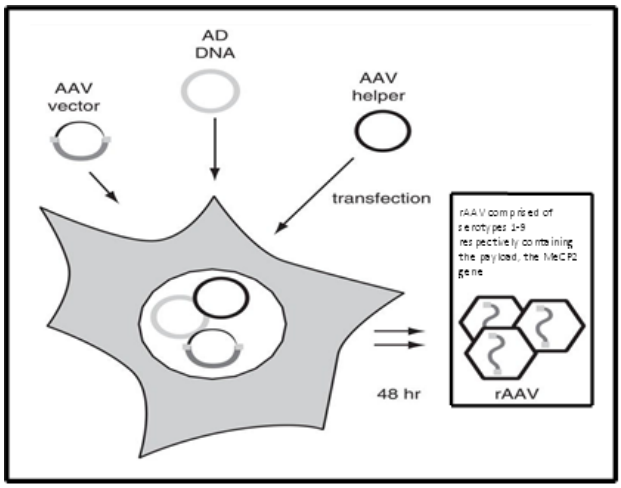
Adeno-associated virus as a delivery vehicle.

Introduction. In previous articles, we noted that gene therapy, DNA editing, RNA editing and X-chromosome reactivation, intended to correct deficiencies in the MeCP2 gene, were dependent on the delivery of the gene or active reagent to the brain and that the choice of delivery vehicle was adeno-associated virus (AAV). It was also noted that production facilities worldwide were currently committed to covid-19 vaccine production and that this might delay any proposed clinical trials to treat patients with Rett Syndrome. This short article provides some background on AAV and the difficulties in scaling up production to permit an expansion of clinical trials.

Why is AAV the preferred strategy for gene therapy? Adeno-associated viruses are a group of small, single-stranded DNA viruses (DNA is usually double-stranded) which infect humans and are unable to replicate (replication-defective) without help from other viruses, usually adenoviruses. Consequently, in nature, AAVs are only detected during co-infection with an adenovirus, hence the name AAV and classified as *Dependoviruses*, because without co-infection with an adenovirus, the AAV cannot replicate. Nevertheless, AAV are able to ***infect*** cells in the absence of a helper adenovirus. and deliver a payload. (For the purposes of this article, I shall use the MeCP2 gene as an example of the payload). Adenoviruses can cause human disease, most frequently, the common cold, although AAV themselves probably do not cause any disease. AAV is a simple virus and is composed of an outer shell called the **capsid** (the protein shell) that contains and protects the virus' DNA. There are two major reasons why AAV is the preferred vector (carrier) to deliver genes in gene therapy, i) a majority of the AAV genome can be removed and replaced with the gene of interest (i.e., MeCP2) and ii) the capsid is not highly immunogenic meaning that the immune response of recipients to the capsid protein is not strong with the result that the AAV containing the MeCP2 gene is not rapidly eliminated. In contrast, if the capsid was highly immunogenic, the AAV would most likely be eliminated by the immune response before the virus had the opportunity to deliver the gene to the target cell, in this case, neurons. An additional feature of AAV is its safety record as it has been shown to be safer than alternative viral and non-viral delivery systems.



Production of recombinant AAV. AAV for gene therapy (and the other functions noted above) are produced in the laboratory by genetic engineering and consequently, termed recombinant AAV (rAAV). Over the past decade, the production of rAAV has moved from an academic exercise to a biotechnology product, but remains a challenging exercise with high manufacturing costs. The rAAV is synthesised in specific cells which provide one of the adenovirus proteins on which the rAAV is dependent for replication; 3 other components are necessary. These are transferred into the specialised cells in the form of DNA by lipids (fats)-see the figure, a process called transfection. These different DNA preparations are i) the highly modified AAV genome containing the MeCP2 gene, termed the AAV vector, ii) the adenovirus DNA (AD DNA) containing the genes for other adenovirus helper proteins necessary for rAAV production and iii) the AAV helper DNA containing the AAV genes (including the capsid gene) which were replaced by the MeCP2 gene.

Methods to increase the efficiency of rAAV production. The overall process is inefficient because the delivery of the individual DNA components varies, so that the proportion of the specialised cells

which receive all 3 DNA molecules is lower than desirable. Furthermore, an unwanted outcome is the production of empty rAAV i.e., particles which fail to contain the MeCP2 gene payload. These empty particles have to be removed prior to clinical use, adding to the difficulty and cost of production. In addition, the lipids used to deliver the different DNA molecules are expensive and manufacturers are currently examining other methods which might be used to deliver the DNA. These include the use of other viruses which must also be replication defective to ensure that there is no interference with the synthesis of the rAAV. Replication-defective adenoviruses are being considered, essentially similar to the adenovirus vectors used to deliver the AstraZeneca and the Johnson & Johnson covid-19 vaccines.
The industry agrees that delivery of the 3 components by lipids must be superceded, but there is no clear strategy how this might be best achieved to reduce costs and increase productivity. Another major issue to be overcome is the manner in which the specialised cells, used to produce the rAAV, are cultured. At present, these cells are grown on a solid surface, similar to a petri dish, but this does not permit up-scaling in large industrial scale fermenters which requires the cells to be cultured in solution. Considerable efforts are being made to correct this to ensure a ready supply of rAAV products in future.

rAAV remains the industry choice for gene therapy and gene manipulation. Despite these difficulties and obstacles associated with industrial scale production of rAAV, AAV remains the delivery method of choice. There are approximately 9 different types of AAV, leading to the possibility of targeting specific cells in patients; AAV9 has been used in the past to target neurons in patients and AAV2 or AAV5 used to target hepatocytes in the liver. Current work with rAAV will be performed in large animal models like sheep or pigs rather than mice to better understand the increased dose requirements and any associated toxicity. The industry has been buoyed by the incredible success of Avexis/Novartis in treating patients with Spinal Muscular Dystrophy (https://rettaustralia.org.au/blog/further-studies-on-how-to-cure-rett-syndrome-re-activate-the-inactivated-x-chromosome/) and there are many companies with a direct interest in rAAV gene therapy so that progress will continue to be made.

Acknowledgements. This article was prompted by a webinar transmitted by *Genetic Engineering and Biotechnology News* (GEN) and the header figure, prepared by GEN, was downloaded from the internet. Prior to my (semi) retirement, researchers in my laboratory used AAV in vaccine studies, so that I have a specific knowledge and interest in the use of AAV for gene therapy.

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