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Gene therapy is a medical treatment that treats or prevents a disease by altering a patient's genome. This involves replacing, silencing or inserting a gene into the genome.

Adeno-associated virus (AAV) gene therapy uses a small viral vector to deliver genetic material to target cells, which then use it to produce the functional proteins they were otherwise missing.

The challenge is that AAV vectors are recognised as foreign by the body's immune system. **Neutralising antibodies (NAbs)** can block the vector before it reaches its target cells. **Cytotoxic T cells** can destroy cells that have successfully transduced. Overcoming the immune response is therefore central to increasing effectiveness.

Leber congenital amaurosis (LCA)

is an inherited retinal dystrophy (IRD): diseases involving the dysfunction of light-sensitive cells in the retina. It is caused by an autosomal recessive mutation in one of several genes that leads to blindness starting in early infancy. LCA is one of the earliest and most severe forms of IRD and accounts for 20% of childhood blindness. Clinical presentations vary and include: visual loss, sensory nystagmus and absent electrical signals on electroretinogram. The most common mutated gene is *CEP290*, but the current and only treatment, **Luxturna**, uses an AAV vector to target the *RPE65* gene. [3]



Duchenne Muscular Dystrophy (DMD) is a fatal X-linked genetic disorder affecting 1 in 5,000 boys. It causes muscle wasting from an early age. It is caused by a mutation in the dystrophin gene, an anchoring protein that links the actin filaments in muscle cells with the cell membrane.

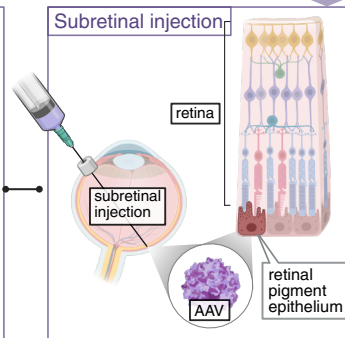
The only gene therapy, **Elevydys**, uses AAVs to deliver a shortened but functional 'micro-dystrophin' gene to muscle cells.



is an X-linked genetic disorder caused by a defect in the gene coding for clotting factor VIII (FVIII). Clotting factors are proteins in the blood produced by the liver that activate to form clots to prevent excessive bleeding. Patients with haemophilia experience excessive bleeding: from small cuts, gums and nosebleeds. Life-threatening internal bleeding is common among haemophiliacs. A new gene therapy, **Roctavian**, was approved by the FDA in 2023 and uses an AAV to deliver a fully functional FVIII to liver cells.[5]

Immune privilege is when local inflammatory responses are restricted in sensitive tissues (e.g. CNS, eye, placenta, testes) to prevent tissue damage. AAV therapies for the eye exploit this immune privilege to minimise vector clearance and immune rejection. Luxturna is delivered specifically into the subretinal space, which is an area of high immune privilege, this reduces the risk of immune rejection.

However, subretinal delivery of Luxturna risks AAV leakage into the vitreous space, which is now understood to have less immune privilege than previously thought, which increases the risk of immune rejection. A recent study found the solution to be a post-injection intravitreal lavage to wash out leaked vector from the vitreous humor. [2]



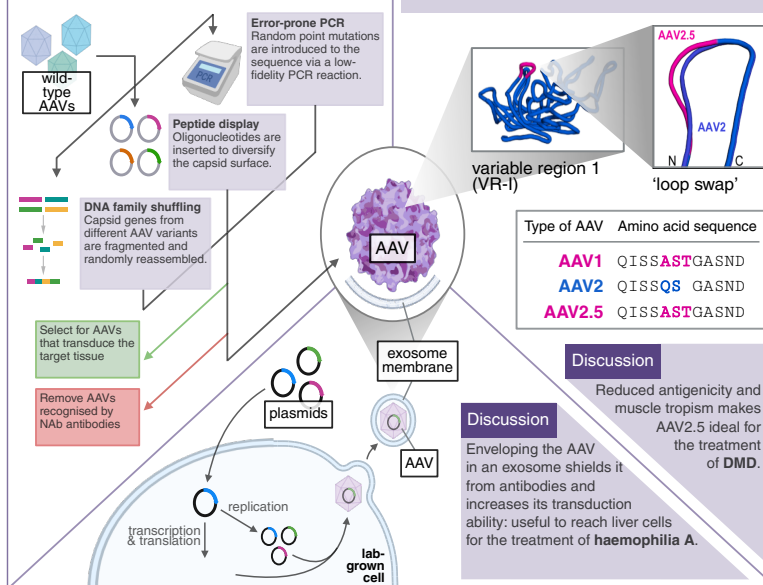
Luxturna is limited to repairing *RPE65*-defective LCA. The most common mutated gene, *CEP290*, is too large (7.4 kilobases) for an AAV vector to carry. Current research is exploring repairing genes directly on-site rather than replacing it with a functional gene, including the use of *in vivo* CRISPR gene editing.

The diagram illustrates the process of AAV infusion. A human figure is shown with an IV infusion bag connected to a catheter in the arm. A central circle labeled 'AAV' shows viral particles. Arrows indicate the distribution of AAV: one arrow points to 'muscle' and another points to 'liver', both labeled 'blood'.

The current gene therapy for DMD is a gene for micro-dystrophin and not the entire gene. New research is exploring delivering the entire DMD gene in two parts so to further increase treatment efficacy. [4]

Studies show that with roctavian gene therapy, expression of FVIII decreases, therefore decreasing effectiveness over the long term Current research is being done to achieve a more modest initial inc in fVIII expression but maintaining levels of expression year-on-year. [5]

A diverse set of AAVs is produced, then selected for ability to transduce tissue and evade NABs. Advantage is that no structural knowledge of AAVs is needed.



The diagram illustrates the immune response to AAV and the effects of various drugs. The top part shows the process of endocytosis of an AAV particle by a dendritic cell, followed by antigen processing and presentation to a naive CD4⁺ T cell. The T cell then interacts with a B cell, which produces antibodies. The bottom part shows the effects of various drugs on the immune response. Rituximab induces apoptosis in B cells. Bortezomib induces apoptosis in plasma cells. Corticosteroids inhibit the IL-2 receptor and intracellular signaling pathways. Calcineurin inhibitors (like cyclosporine) and rapamycin (sirolimus) inhibit the calcineurin/NFAT pathway. mTOR inhibitors (like rapamycin) inhibit the mTOR pathway. These drugs are used to suppress the immune response to AAV.

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