AAV-mediated gene therapy for rare metabolic disorders: turning a promise into a reality

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Gene therapy is emerging as the realistic treatment option for inborn errors of metabolism (IEMs) and, with the promising safety and efficacy evidence from the proof-of-concept studies, adeno-associated virus (AAV) has become the frontrunner among viral vector candidates for these monogenic disorders. Different AAV capsids exhibit specific tissue tropisms, which can considerably increase the efficiency of gene transfer to particular organs. Here, we will discuss two distinct diseases: ornithine transcarbamylase (OTC) deficiency and Niemann–Pick disease type C, in which significant advances have been achieved in AAV-based gene therapy trials.

Inborn errors of metabolism (IEMs) are rare genetic disorders resulting in defects in biochemical and metabolic pathways affecting the metabolism of proteins, fats and carbohydrates or dysfunction of various organelles presenting as complex medical problems and affecting multiple organ systems. Although each of these conditions is individually rare, as a group, IEMs have an overall prevalence of about 1:2000 at birth. Age of presentation can vary from infancy to adulthood, with the more severe forms manifesting in early childhood associated with significant morbidity and mortality. Although developments in dietary support and pharmacological treatments with cofactors, end-product replacement, enzyme replacement therapies and chaperons have led to better outcomes, there is a lack of treatment for most IEMs. Regenerative therapies such as bone marrow and liver transplantation (LT) can change the prognosis of some of these diseases. However, it is not extensively available due to organ shortage, and it has its own risks of surgical complications and immunosuppression, and transplantation does not correct or reverse previous neurological insults. Therefore, there is still a high unmet need for safer and more effective treatment options.

Gene therapy has been emerging as a game-changer for IEMs as it is focusing on correcting the cause of the disease rather than just treating the symptoms. It is based on the transfer or editing of a genetic material to cure a disease. Gene delivery can be accomplished using an *ex vivo* or *in vivo* delivery approach via viral or non-viral vectors. *Ex vivo* gene delivery is based on transferring a gene to a stem, progenitor or differentiated cell line outside the patient's body, generally in tissue culture. After gene transfer, the cells can be selected, expanded and transplanted back into the patient. The delivery of a gene directly to a patient via direct tissue and/or systemic injection is known as *in vivo* gene delivery. Most of the current success has been attained using viral-mediated gene delivery.

Adeno-associated virus (AAV) is one of the most diligently explored viral gene therapy vehicles. AAV belongs to the parvovirus family and is not autonomously replicating. It is dependent on co-infection with other viruses, mostly adenoviruses, in order to replicate. AAVs are small, non-enveloped viruses with a single-stranded genome DNA of 4.7 kb, flanked by two inverted terminal repeats (ITRs). AAVs are endocytosed via serotype-specific receptor/co-receptor interaction at the surface of the target cell. The transgene remains as episome in the nucleus as AAVs are mostly non-integrative but integration can occur at a relatively low rate. Over the last decades, recombinant AAVs (rAAVs) were generated, which have most of the viral genome replaced with expression cassette including a promoter, gene of interest, and flanked by 'ITRs', to make them suitable for therapeutic applications. Expression cassettes are packed into different AAV capsids with different tissue tropisms. Engineered novel vectors have been created to increase AAV transduction efficiency, tissue tropism and the ability of the capsid and transgene to avoid the host immune response.

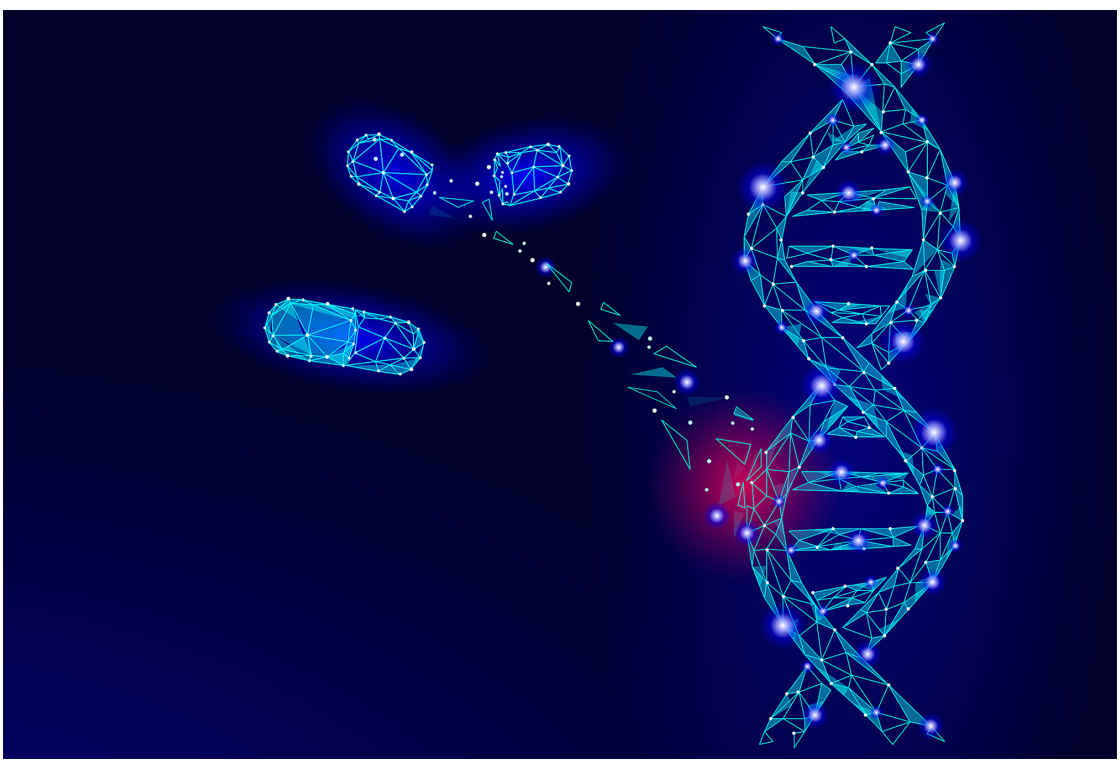
Due to the natural tropism of AAVs, most AAV gene therapy programmes focus on the liver, muscles and the central nervous system (CNS). Almost all natural AAV capsids can transduce liver efficiently following systemic administration. As most metabolic reactions happen in the hepatocyte, liver is a central organ and target for treating many IEMs. Therefore, AAVs are popular tools for liver-targeted gene therapy and there is a growing interest to treat a variety of diseases such

as haemophilia, familial hypercholesterolaemia, Crigler–Najjar syndrome and ornithine transcarbamylase (OTC) deficiency.

OTC is a mitochondrial enzyme that is essential for the excretion of ammonia through the conversion of non-toxic urea. It catalyses the synthesis of citrulline from carbamoyl phosphate and ornithine in the urea cycle. The lack of the OTC enzyme results in elevated levels of neurotoxic ammonia, which causes disease symptoms. It is the most common urea cycle disorder, inherited in an X-linked recessive manner and the prevalence was estimated to be between 1 in 14,000 and 1 in 80,000 live births. There is an extremely variable phenotypic spectrum including asymptomatic carriers, late onset and severe neonatal onset phenotypes. The standard of care includes protein restriction, ammonia scavengers and arginine/citrulline supplementation. However, this treatment is not sufficient to avoid acute hyperammonaemic episodes triggered by catabolic stress; and severe neurological damage, coma and death can still occur. Up to now, LT has been the only curative therapy; however, technical challenges, donor shortage and need for lifelong immunosuppression limit its availability. Thus, management of the disease is still challenging and there is an unmet need for curative treatment.

OTC deficiency has been a popular candidate disease target for gene therapy over the years. In 1995, a phase I pilot study (NCT00004386) using adenovirus type five vector carrying human *OTC* gene recruited partial OTC deficiency patients. The 18th participant of the trial, an 18-year-old male patient, died 4 days after receiving the gene therapy due to a severe immune reaction. This tragedy halted gene therapy trials for some time. But gene therapy efforts for OTC deficiency re-started again with AAVs in 2000s. Several AAV-mediated studies have been performed in OTC-deficient mouse models and AAV8 capsid was found to be the most effective. Single injection of AAV2/8 vector encoding *OTC* gene resulted in long-term, effective metabolic correction in adult OTC-deficient mice. However, there was only a short-term correction in the neonatal mice even with a double dose of the vector. This was associated with the loss of transgene expression in the growing liver. To overcome this challenge, there was an effort to generate engineered capsids with higher liver tropism. AAVLK03 is one of them which was shown to transduce human hepatocytes 10-fold better than AAV8 in chimera mouse–human livers. Intravenous administration of AAVLK03 vector encoding the human *OTC* gene (AAVLK03.hOTC) was reported to be safe and effective in juvenile cynomolgus monkeys.

Based on the successful proof-of-concept studies, AAV gene therapy has reached the clinical translation phase for OTC deficiency. A phase 1/2, open-label safety and dose-finding study of AAV8-mediated gene transfer of human OTC in adults with late-onset OTCD has completed recruitment (CAPtivate, NCT02991144). Preliminary results revealed that 7 out of 11 patients were responsive to



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the treatment and the longest response duration was 4 years. Long-term follow-up study planned for 6 years is on-going (NCT03636438). The phase 3 Enh3ance study is currently recruiting. On the other hand, a phase 1/2, open-label, multicentric clinical trial assessing the safety and efficacy of AAVLK03.hOTC in paediatric patients (HORACE, NCT05092685) is now at the pre-recruiting stage.

There are also a large number of AAV studies focusing on CNS. Intraparenchymal delivery is the CNS delivery route that has the advantage of direct delivery of the vector to specific target regions. Intra-cerebrospinal fluid (Intra-CSF) delivery provides the possibility of distribution over a larger area of the brain or spinal cord; however, it may cause more severe immune response. Systemic delivery is challenging for targeting the CNS even with the vectors, such as AAV9 that has some capability of passing through the blood–brain barrier. There are significant early-stage efforts to treat neurodegenerative IEMs including mainly lysosomal storage disorders (LSDs).

One of the examples of such disorders is Niemann–Pick disease type C (NPC), which is an autosomal recessive neurovisceral LSD caused by mutations in the NPC1 or NPC2 gene. NPC is characterized by defective lipid trafficking and sequestration and accumulation of endocytosed unesterified cholesterol, sphingomyelin, glycosphingolipids and sphingosine in lysosomes and late endosomes. Patients display a wide range of progressive neurological symptoms, including ataxia, cognitive decline and seizures as well as visceral manifestations such as hepatosplenomegaly, with a significantly variable disease severity and age of onset. Currently miglustat is the only licensed drug for treating NPC with a disease-modifying effect; however there are no FDA-approved therapies for NPC, thus there is a need for developing curative treatments for this debilitating, fatal disease. Several pre-clinical studies have revealed that AAV-based gene therapy has the potential to treat NPC. AAV9 is the most popular serotype as it shows high neurotropism. A single intracerebroventricular injection of an AAV9 vector encoding human *NPC1* increased survival, improved neurodegeneration, corrected biochemical pathology and advanced motor function in NPC mouse model. AAV9-mediated gene therapy was more effective than miglustat even at a low dose. Most recently, the same vector with a novel small truncated endogenous NPC1 promoter provided higher gene expression and efficacy. Another study suggested that a combined intracerebroventricular and intracisternal administration of AAV9/3 vector encoding *NPC1* gene resulted in a longer survival and better motor performance, as well as broader delivery of the vector to CNS, liver, lung and heart.

In conclusion, AAV-mediated gene therapy is promising for different types of IEMs and the number of on-going studies is rapidly increasing. Distinct tissue tropism of different AAV serotypes enables them to target different diseases. Although there are still some challenges to overcome, growing evidence confirms their safety and efficacy. Several AAV gene therapy products will most likely be approved for IEMs in the near future. ■

Conflict of Interest

BSY has no conflict of interest. PG is an academic co-founder of Bloomsbury Genetic Therapies, a UCL spinout developing a gene programme in OTC deficiency.

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