

Moving gene therapies from the lab to the hospital bed: The adeno-associated virus as a promising gene therapy vector to treat disease

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Abstract

Gene therapy is a treatment method repairing mutated or deleted genes to correct genetic disorders. With many monogenic diseases lacking effective therapeutic approaches, gene therapy offers an exciting avenue to resolve the underlying genetic abnormality. Further, gene therapy could also address complex multifactorial diseases by targeting a critical pathway. Regardless of the therapeutic goal, an important factor to consider is the challenging step of efficiently and precisely delivering genes of interest to cells of interest. These vectors must ideally evade immune response as they infect cells of interest and be able to infect a wide spectrum of target cells. One such vector that has demonstrated much promise is the adeno-associated virus (AAV). The AAV was discovered over 50 years ago and has since been studied extensively for the treatment of many diseases. Since the first AAV clinical trial started in 1996 for the treatment of cystic fibrosis, hundreds of trials have examined AAV-based therapies for the treatment of hemophilia, rheumatoid arthritis, Duchenne's muscular dystrophy, Leber's congenital amaurosis, lipoprotein lipase deficiency, and many other genetic disorders. With the first AAV drug approved in the last four years, we may soon gain insight into the feasibility of this type of gene therapy product. This review discusses the basic structure and design of AAV vectors and reviews recent advances in AAV technology enabling these therapies to reach the clinic.

There are currently over six hundred clinical trials registered in the United States National Institutes of Health studying "Gene Therapy."¹ Broadly speaking, gene therapy is a treatment method to replace or repair mutated or deleted genes to correct genetic disorders (e.g., repairing mutations in clotting factors to correct hemophilia). Importantly, the possibilities for gene therapy extend to complex pathologies such as autoimmune disorders by providing genes to suppress local autoimmune attack,² or to selectively target cancer cells to suppress oncogenesis or hone immune cells.³ With these seemingly endless therapeutic possibilities, it is important to consider the delivery methods of gene therapy. These vectors must ideally act as an undetected "Trojan Horse," capable of evading all immune reaction and selectively targeting only cells of interest. The vector that has come closest to meeting these criteria, is the adeno-associated virus (AAV).

The adeno-associated virus

The AAV was first discovered in 1965 in Pittsburgh.⁴ Though initially thought of as an impurity of the adenovirus preparation, it was discovered that it was a parvovirus that was thereafter described as "adeno-associated." Since then, many unique features have been discovered that make the AAV highly suited to use as a clinical gene therapy vector. First and foremost, the AAV is considered non-pathogenic as it causes little to no immune response.⁵ In fact, most of the population has been infected by wild-type AAV without any obvious or common symptoms.⁶ Second, there are many AAV serotypes with unique tropism for a variety of tissues⁷ and rational capsid modifications can result in improved selectivity and even change its immunological profile.⁸ Third, the AAV's structure suits a gene therapy vector as a non-enveloped single-stranded DNA virus. The wild-type genome encodes replication and capsid proteins and is flanked by two inverted terminal repeats (ITRs).⁹ Though the wild-type single-stranded AAV takes upwards of four months to initiate gene

expression,¹⁰ modification to remove a component of the 3' ITR can allow packaging as a self-complementary double-stranded virus capable of initiating gene expression less than one week after infection.^{11,12} This does come at the expense of limiting packaging to ~2.5 kb, though as only the ITR is necessary for packaging, the remaining ~2.3 kb can be engineered with a suitable promoter and gene of interest. Finally, the AAV genome structure is highly stable, enabling prolonged transgene expression with reports of residual expression up to four years after therapy in a human patient.¹³ Taken together, non-pathogenicity and fast yet prolonged expression have combined to result in the AAV becoming one of the most studied gene therapy vectors.

For AAV to be viable in the clinic, another important consideration is its manufacture. Most importantly, the AAV is replication-deficient and is hence sub-classified as a "dependovirus". This means that the AAV depends on the functions of a helper virus, such as an adenovirus or herpesvirus to replicate in a host mammalian cell.¹⁴ Clinically, this means that the AAV cannot autonomously replicate in a host but also means that large-scale manufacturing is challenging and costly. Production of a pure recombinant AAV lacking any wild-type virus impurities and produced without the use of adenovirus (hence avoiding adenovirus impurities), has conventionally been done by a triple plasmid transfection system.¹⁵ Adherent HEK293 cells are transfected with the construct of interest, a plasmid containing the AAV-specific replication and capsid genes, and a third plasmid expressing the essential adenovirus genes.⁷ Importantly, the use of adherent cell cultures requiring serum-supplemented media greatly limits production of virus meeting current acceptable manufacturing protocols,¹⁶ but there are other technologies,¹⁷ and some recent advances have allowed serum-free suspension cultures, thereby improving production efficiency.¹⁸ With these promising advancements, scalability at bearable cost continues to improve and lead to greater opportunity for an AAV therapy to reach patients globally.

Despite these successes, as the AAV has gained greater attention, researchers have begun to discover previously unappreciated risks and

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challenges to using the AAV itself. To date, there have been limited reports of an association between AAV infection and spontaneous abortion.¹⁹ Additionally, a recent paper suggested that a certain serotype of AAV (AAV2) may be linked to hepatocellular carcinoma.²⁰ Importantly, these findings have since been heavily challenged,^{21–23} though given the frequent presence of the wild-type 3' ITR in these cancers, the wild-type AAV may pose a small risk.²² This may not be a problem for modified AAV vectors since clinical AAVs use only 145 bp (the 5' ITR) from the wild-type genome.

Alongside potential pathogenicity, research has found the presence of AAV-neutralizing antibodies in humans.²⁴ This was unexpected based on animal preclinical studies but may explain the acute liver damage resulting in short-term elevation of liver enzymes following liver AAV infection.²⁵ Nonetheless, there are many strategies to avoid this roadblock such as improving vector efficiency to reduce doses needed or transient immune suppression before AAV administration.²⁶ The research community has seen many successful clinical trials for many monogenic diseases—more than 120 published clinical trials using AAV vectors have failed to find severe side effects.

Clinical research using the AAV

In 1996, the first AAV reached the clinic in a phase I clinical trial for the treatment of the monogenic disease cystic fibrosis.²⁷ Since then, AAVs have been studied to treat hemophilia B, rheumatoid arthritis, Duchenne's muscular dystrophy, Leber's congenital amaurosis, lipoprotein lipase deficiency, and many other diseases.²⁸ Among the most promising research includes treatment for hemophilia B, a disease characterized by impaired blood clotting due to insufficient factor IX.²⁵ In ongoing phase I/II clinical trials, there have been reports of patients being free of multiple weekly factor IX infusions for over a year after a single AAV injection, with these patients maintaining factor IX levels sufficient for normal clotting times comparable to healthy counterparts.²⁹ Another disease with abundant promising AAV clinical research is Leber's congenital amaurosis, which causes childhood blindness. Early clinical trials showed improved visual acuity just weeks after replacement of the mutated gene (RPE65) by AAV.³⁰ Since, this work has advanced to a stage III clinical trial³¹ and the biotech company, Spark Therapeutics, has released incredible findings: having treated 29 patients, all demonstrate profound improvements in light sensitivity and eye mobility and Spark Therapeutics report that there have been no “product-related serious adverse events.”³² Through all these promising findings on AAV-based therapies, the trailblazer into clinical approval is treatment for congenital metabolic disorder lipoprotein lipase deficiency (LPLD) that reached clinical approval in Europe just four years ago.

Alipogene tiparvovec (Glybera®) was first recommended for approval in 2012.³³ This was the result of a long process requiring four reviews by the Committee on Human Medicinal Products.³⁴ Though large datasets of clinical outcomes are yet to be released, a few key lessons have already been learned from the first AAV approved therapy. First, much like Glybera®, future gene therapies will also need to face the challenge of identifying a sufficient population for a phase III clinical trial for rare diseases and the associated struggles when taking small phase III trials to review boards for final clinical approval. Furthermore, the cost associated with multiple appeals for drug administrations is often prohibitive and was only successful for Glybera® thanks to private donors. Even after approval, health care systems will be faced with the extreme cost associated with such a

therapy—after much speculation and many estimates,³⁵ the final cost landed on \$1.4 million USD for the one-time treatment.³⁶ But for a disease with severe cost in quality and quantity of life without an effective treatment, an immediately expensive gene therapy product may in fact be cost-effective and bearable to users with extended payment plans.³⁷

Conclusion

Gene therapy is a promising avenue for clinical research to treat both rare monogenic diseases and common multifactorial diseases. In this review, the promising characteristics of the AAV as a gene therapy vector have been discussed, alongside important details of virus production. Clinicians are now able to prescribe AAV-based therapies and could become involved in the development of future therapies. An understanding of the limitations and risks associated with current production technologies provides a realistic perspective on future development and use of these medications for Canadian patients and the Canadian healthcare system as a whole.

With the first AAV-based drug approved for clinical use in Europe, the challenging path to the clinic has been clarified. By having a realistic perspective on the clinical challenges facing AAV-based therapies from manufacturing to immunology, it is hoped that future research will consider that to be acceptable to health agencies and public health providers, AAV treatments will need to offer major improvements in quantity and quality of life for patients, and cost-saving outcomes for private and public healthcare. With this consideration, it is possible that AAV gene therapies could one day be part of the standard of care for rare and common diseases. Promising results from AAV clinical trials treating Leber's congenital amaurosis and hemophilia make it likely that Canadian physicians need to be prepared to evaluate AAV therapies as a treatment option for their patients in the not-so-distant future.

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