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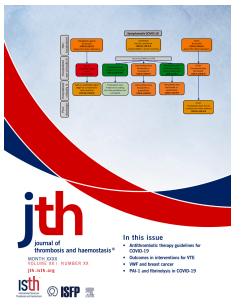

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The Hepatic Odyssey of AAV: From Gene Delivery to Long-Term Outcomes

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Abstract

Adeno-associated virus (AAV) vectors are the most developed approach for in-vivo gene therapy. This treatment has resulted in therapeutically meaningful treatment responses for a range of monogenic inherited disorders, including hemophilia. Understanding the core stages in the lifecycle of AAV supports the shared decision-making process for clinicians and individuals considering gene therapy. These key steps start after vector infusion, including receptor engagement, cellular uptake, endosomal escape, nuclear entry, and transgene expression. All of these stages could influence transduction efficiency, which could result in differences in treatment outcomes between individuals. The natural history of how AAV persists in cells could provide insights into long term efficacy and safety. The immune system may also pose an obstacle to successful outcomes. Studies have shown that pre-existing immunity to natural AAV is common and may exclude some individuals from receiving gene therapy. Additionally, asymptomatic liver enzyme elevation which can result in loss of expression is an important area in which more research is required. Ongoing advances in vector engineering and a better understanding of host-pathogen interactions are helping to address these challenges. This review provides a primer on the hepatic lifecycle of AAV and an introduction to the cellular and immune processes involved in AAV transduction and persistence in the liver.

Keywords: Adeno-Associated Virus; Gene Therapy; Hemophilia; Liver

Introduction

Gene therapy is a novel advanced therapy that aims to treat or prevent disease by introducing, removing, or editing genetic material within a patient's cells. By addressing the root cause of genetic disorders at the molecular level, gene therapy offers the potential for long-term or even curative treatments. Adeno-associated virus (AAV) vectors currently lead the way for approved clinical applications [1]. AAV is a small, replication-deficient virus that has been engineered into an effective gene delivery system due to a favorable safety profile and ability to transduce both dividing and non-dividing cells. Recombinant AAV vectors (rAAV) are made in the laboratory via co-transfection of producer cell lines, with separate plasmids encoding the therapeutic gene, capsid proteins and an adenoviral helper. Following production, rAAV is purified using methods such as ultracentrifugation or column chromatography and formulated for clinical use [2, 3].

Among the various applications of this technology, liver directed AAV gene therapy has emerged as a leading strategy for treating monogenic disorders such as hemophilia [4, 5]. The liver is particularly well-suited for this approach due to its intrinsic capacity to produce therapeutic proteins and a relatively immune-tolerant environment. Moreover, hepatocytes are the natural site of Factor IX (FIX) synthesis, aligning with the physiological context of its expression. Initial proof-of-concept came from Herzog et al. (1997), who demonstrated FIX expression in mice following intramuscular AAV delivery [6]. Building on this, subsequent studies - originally targeting alpha-1-antitrypsin deficiency - highlighted the liver as a promising site for effective gene transfer [7]. These findings were extended to the hemophilia B (HB) dog model, where gene transfer via liver directed AAV gene therapy, using mesenteric vein delivery, resulted in phenotypic correction and therapeutic levels of FIX expression [8, 9]. Following this, Nathwani et al. demonstrated successful delivery of a self-complementary liver-specific AAV-FIX vector in mice and non-human primates [10]. These seminal preclinical studies laid the groundwork for the transition to human trials. An important early trial from Manno et al supported the safety of liver-directed AAV gene therapy in humans using centrally delivered AAV-FIX vector into the hepatic artery. Although patients did not retain FIX expression long term, this trial highlighted an early transient asymptomatic elevation of liver enzymes with accompanying decline in transgenic

expression [11]. These studies culminated in the landmark University College London (UCL) / St Jude's study, which demonstrated sustained long-term FIX expression after peripheral vein infusion of an AAV-FIX vector containing a liver-specific promoter in ten men with severe HB [12]. The discovery of a gain-of-function F9 mutation (FIX Padua) in a young man diagnosed with a proximal deep vein thrombosis in Northern Italy, marked a further milestone for hemophilia gene therapy [13]. FIX Padua is a naturally occurring point mutation (R338L) in the protease domain of FIX which interacts with the A2 domain of Factor VIII. This variant confers an 8-fold increase in coagulant activity compared to wild-type FIX. This gain-of-function variant has since been incorporated into clinical AAV-FIX vectors to enhance their therapeutic potency. The pivotal phase 3 HOPE-B study, using an AAV5 vector containing the FIX Padua transgene (etranacogene dezaparvovec), demonstrated sustained therapeutic expression of FIX in patients with hemophilia B, reduced bleeding rates, ability to stop prophylaxis and an improvement in quality of life [14].

Successful gene delivery for hemophilia A (HA) has proved more complex, mainly due to *F8* being a much larger gene, which exceeds the AAV packaging capacity. A key breakthrough involved the use of B-domain deleted FVIII constructs to fit into AAV's limited packaging capacity, similar to those used in recombinant FVIII concentrates [15]. Early preclinical work demonstrated that hepatic delivery of AAV vectors encoding B-domain deleted human factor VIII (FVIII) could result in sustained therapeutic expression in murine and canine models [16, 17]. These advances translated into successful studies showing long-term expression and safety in dogs [18, 19] and non-human primates [20]. The first successful human clinical trials that followed was the landmark phase 1/2 study by Rangarajan et al. [21], which used an AAV5 vector (valoctocogene roxaparvovec) to deliver B-domain deleted FVIII to the liver, resulting in therapeutically-meaningful FVIII expression and reduction in bleeding episodes. Following on from this important study, this treatment was expanded into a Phase III trial in 134 men with severe HA, which showed superiority of AAV gene therapy over the current standard of care [22]. Regulatory approvals in the United States (FDA) and Europe (EMA) of valoctocogene roxaparvovec for HA, and etranacogene dezaparvovec for HB marked a significant milestone for in the field [23, 24].

Notably, programmes differ in how they treat pre-existing anti-AAV antibodies: some approvals and trial protocols require absence of neutralizing antibodies to the relevant serotype, whereas others (including the pivotal programme for etranacogene dezaparvovec) permitted patients with low–to–moderate anti-AAV titres; this heterogeneity in eligibility criteria is discussed in more detail below.

However, despite these advances, AAV's interaction with cells in the liver remains a complex and dynamic process that extends beyond initial gene delivery. Once administered systemically, the vector must navigate host immune defenses, bind to target cells (i.e., hepatocytes) receptors, enter the cell, escape endosomes to avoid lysosomal degradation, and successfully convert into a transcriptionally active form. Each step in this process can influence the efficacy, variability and safety of therapy. In this review we will explore the hepatic journey of AAV in gene therapy, focusing on its cellular entry, nuclear processing, immune interactions, and long-term implications. We will discuss what is currently known, identify knowledge gaps, and highlight future directions for improving the safety and efficacy of AAV-based therapies for treatment of hemophilia.

Structure and Biology of AAV

AAV is a small, non-enveloped virus within the Parvoviridae family, possessing a single-stranded DNA genome. This genome consists of two essential coding regions, *Rep* and *Cap*, flanked by inverted terminal repeats (ITRs) (Figure 1). The rep sequence encodes four *Rep* proteins, which play key roles in AAV replication and genome packaging [25]. Natural (wild-type) AAV persists in cells predominantly in episomal structures, which are circular DNA molecules that remain extra-chromosomal. This means that although AAV persists in the nucleus, the viral genome does not combine or integrate at significant frequency with the host cell's genome [26]. However, natural AAV does possess the mechanism to integrate at specific sites using a process dependent on the *Rep* proteins, even if at low frequencies [27, 28]. Wild-type AAV is replication-deficient and requires co-infection with another helper virus - typically adenovirus or herpes simplex virus to provide essential functions required for productive replication [25]. The *Cap* region encodes three viral capsid proteins VP1, VP2, and VP3, which assemble

in an approximate 1:1:10 ratio to form the protective protein shell (capsid) of the virus. This capsid protects the viral genome from degradation and determines AAV's ability to engage with and be taken up by different cell types. This process, called tropism, refers to the selective affinity of a virus to particular cells or tissues. This is governed by interactions between the viral capsid and host surface molecules that mediate attachment and internalization. Different AAV serotypes have evolved unique receptor interactions and entry pathways, affecting their efficiency in transduction and gene delivery [29, 30]. In gene therapy applications, rAAVs retain only the outer capsid and the viral genetic components (*Rep* and *Cap*) are replaced with a therapeutic transgene and promoter/enhancer elements (Figure 1). The ITRs at both ends of the genome are retained as these required for replication, packaging and episomal persistence [31, 32].

While all AAV serotypes share a general capsid architecture, differences in the amino acid composition and surface-exposed loops result in diverse biological properties. These include variations in receptor binding, cell and tissue tropism, transduction efficiency, and susceptibility to neutralizing antibodies. For instance, AAV2 binds to heparan sulphate proteoglycans and shows high transduction efficiency in liver and muscle tissues, whereas AAV9 interacts with terminal galactose residues and demonstrates broad tropism, including efficient central nervous system and cardiac transduction [33, 34]. (Figure 1).

Hepatic Lifecycle of AAV: From Infusion to Protein Expression

Cellular Uptake

After intravenous administration, rAAV vectors rapidly disseminate and are taken up into target tissues. Liver-tropic capsid serotypes show particularly high efficiency in transducing hepatocytes. This specificity is largely determined by interactions with cellular receptors and co-receptors. While the AAV receptor (AAVR) - a conserved transmembrane protein - plays a central role in facilitating endosomal trafficking and nuclear delivery across multiple serotypes [12], it is not the sole determinant of transduction. Initial binding is influenced by surface glycans and proteins, including heparan sulphate (AAV2), galactose (AAV9), and sialic acids (AAV1, AAV5), as well as by integrins and

other cell-specific co-receptors (Figure 2) [30, 35, 36]. The combination of these interactions dictates both tissue specificity and transduction efficiency for each serotype. Following receptor engagement, AAV particles are internalized, primarily via clathrin-mediated endocytosis, in which clathrin-coated vesicles transport the virus into the cell. Once inside the cell, particles are trafficked through the endosomal system. While clathrin-mediated endocytosis is the predominant pathway, other mechanisms - such as caveolin-dependent endocytosis and micropinocytosis - may also contribute to cellular uptake [37].

Road to the nucleus

Once internalized, AAV particles are trafficked through the endosomal system and trans-Golgi network (TGN) (Figure 2). This process is facilitated by the microtubule network and involves interactions with host cell proteins including syntaxin 5, which is critical for retrograde transport to the TGN [30]. Successful escape from endosomes and/or the TGN is an essential step in the AAV life cycle, as failure to exit from these compartments would result in lysosomal degradation. This process is initiated by acidification of AAV within the endosome, which triggers conformational changes in the capsid. This leads to externalization of a VP1 unique (VP1u) region, which contains an enzymatic phospholipase A2 (PLA2) domain. This domain is normally hidden and protected from immune recognition and proteolytic cleavage. When the PLA2 domain becomes exposed at low pH, this facilitates penetration of the endosomal membrane and release of AAV into the cytoplasm [38, 39]. Recently, another host membrane protein, G protein-coupled receptor 108 (GPR108) has been identified as another key factor required for efficient cytoplasmic escape for most AAV serotypes [40]. AAV particles then accumulate in the perinuclear region and are actively transported into the nucleus through the nuclear pore complex. This receptor mediated process is mediated by nuclear localization signals present on the capsid [37].

Inside the nucleus

Once inside the nucleus, the viral capsid disassembles, releasing the single-stranded DNA (ssDNA) genome (Figure 2). Since host cells use double-stranded DNA (dsDNA) for transcription, this single-stranded AAV genome must use host-mediated second-strand synthesis to form a transcriptionally active dsDNA template. This process occurs either through host polymerases using the ITRs as primers or by forming complementary base pairs in self-complementary AAV (scAAV) vectors, which bypass the need for second-strand synthesis. The resulting double stranded AAV genomes then predominantly form circular episomal structures. Episomal persistence is thought to contribute to its favorable safety profile in gene therapy, although episomes may be lost due to cell division and are thought to lead to progressive expression loss over time [41, 42]. Rare integration events, where fragments of the rAAV genome become inserted into host chromosomes have also been reported [43]. While episomal forms are considered the primary template for transgene expression, recent data from non-human primates suggest that integrated genomes may also contribute to sustained expression in some contexts [44]. Although the clinical relevance of integration remains uncertain, current evidence does not suggest a genotoxic risk in patients treated with rAAV gene therapy [45].

The Immune Response to AAV

The interplay between AAV vectors and the host immune system is a critical determinant of gene therapy efficacy and safety (Figure 3). Both innate and adaptive immune responses can act as barriers to successful treatment by reducing vector transduction, impairing transgene expression and contributing to inflammatory or cytotoxic events following administration.

Innate immune response

The innate immune system serves as the body's first line of defense against foreign pathogens, including viruses. It recognizes conserved molecular patterns, known as pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs). Upon administration, AAV vectors are sensed by the innate immune system through several PRRs, most notably Toll-like receptor 9 (TLR9), which detects unmethylated CpG motifs in the AAV genome, and the cGAS-STING pathway,

which responds to cytosolic DNA [46, 47]. This detection leads to the production of type I interferons and pro-inflammatory cytokines, potentially reducing transduction efficiency and contributing to acute liver inflammation or transaminitis, particularly at higher vector doses [48].

Beyond these canonical sensors, complement activation also plays a role in shaping the innate immune response to AAV [49]. In rare cases, particularly in neurological indications, AAV-mediated complement activation has been associated with thrombotic microangiopathies (TMAs) [50-53]. A recent study has highlighted evidence of sinusoidal endothelial injury, resembling those seen in transplant patients or post drug injury, in livers from NHP exposed to high dose systemic rAAV [54]. Although the clinical significance of complement activation is still under investigation and evidence of AAV-associated TMAs has not been seen in hemophilia clinical or pre-clinical studies, this is an area in which further research is required to improve understanding of systemic toxicity profiles of high-dose AAV therapies.

Adaptive immune response: humoral immunity

One of the first major barriers posed by the adaptive immune response is the presence of pre-existing neutralizing antibodies (nAbs), resulting from natural exposure to AAV. The presence of nAbs can block AAV uptake into cells, reducing or nullifying transduction efficiency. Antibody prevalence varies widely depending on the AAV serotype, geographical region, age and previous viral exposure. For example, anti-AAV2 nAbs are seen in 30-80% of the population, while serotypes like AAV5 or AAVrh10 tend to have lower seroprevalence [55, 56]. Importantly, these antibodies can exhibit cross-reactivity, meaning that antibodies generated against one AAV serotype may partially neutralize another, thereby limiting the effectiveness of switching serotypes [57]. Once a patient receives rAAV gene therapy, the immune system recognizes the viral capsid as foreign and mounts a robust humoral immune response, leading to the generation of high-titer nAbs. This response begins when antigen-presenting cells, such as dendritic cells or Kupffer cells, internalize the AAV and process its capsid proteins into peptides. These capsid-derived peptides are presented on MHC class II molecules to $CD4^+$ helper T cells, which in turn activate B cells. These B cells differentiate into plasma cells that secrete high-affinity antibodies

targeting the AAV capsid. These newly formed antibodies persist long-term and effectively neutralize any subsequent AAV administration of the same or even closely related serotypes [58]. Patients who have previously received AAV gene therapy would not currently be able to have a second rAAV treatment in the presence of circulating the nAbs. In the pivotal UCL / St Jude's trial for HB, patients developed durable anti-AAV8 antibodies shortly after vector infusion, with titers remaining elevated for at least 10 years [12].

Strategies to overcome this limitation, such as plasmapheresis, immune suppression, or capsid engineering to evade pre-existing immunity, are being explored but remain experimental at this stage [59-63]. Although pre-existing nAbs are an exclusion criteria for most clinical trials, this was not the case in the HOPE-B trial with etraconagene dezaparvovec [14], after no difference in AAV transduction efficiency was seen in humans and NHP [64, 65]. Post hoc analyses from the HOPE-B trial suggested that patients with pre-existing nAbs titers below certain thresholds could still achieve therapeutic FIX expression, while higher titers were associated with treatment failure. Based on these findings, UK guidance currently recommends excluding patients with titers ≥1:678 (7-point assay) or ≥1:898 (9-point assay), as advised by the manufacturer [66].

Adaptive immune response: cellular immune response

In addition to humoral immunity, cellular immune responses can also limit AAV efficacy. After vector administration, antigen-presenting cells also process and present peptides via MHC class I, leading to activation of $CD8^+$ cytotoxic T lymphocytes [67]. Evidence from early clinical trials suggested that T cell–mediated responses were responsible for declining transgene levels, based on observations in patients receiving AAV2-mediated gene therapy for HB [11]. In this study, individuals who initially achieved therapeutic Factor IX expression experienced a subsequent decline that coincided with elevated liver transaminases and the emergence of AAV capsid-specific $CD8^+$ T cells in peripheral blood. These findings implicated an adaptive cellular immune response in the clearance of transduced hepatocytes and prompted the use of immunosuppressive regimens in later trials. CpG content within

the AAV genome has been also shown to prime the adaptive immune system, including cytotoxic T-cell responses. This mechanism, initially demonstrated in preclinical models and supported by findings in clinical settings, has been associated with increased transgene-specific T-cell responses and vector clearance[68-70]. In addition to this, a distinct population of plasmacytoid dendritic-like cells has also been shown to sense CpG motifs within AAV genomes, leading to innate immune activation and a subsequent negative impact on transgene expression [71]. Interestingly, there is growing evidence that regulatory T cells (Tregs) play a role in modulating the immune response to AAV. Tregs can suppress both cellular and humoral immune responses, potentially contributing to long-term transgene expression [72, 73].

Long-Term Implications and Knowledge Gaps

Despite the growing number of successful AAV-based gene therapy trials, important questions remain regarding the durability, safety, and immunogenicity of treatment over the long term.

Hepatotoxicity

One of the commonest adverse events seen in clinical studies of AAV gene therapy has been an asymptomatic rise in liver enzymes, particularly alanine aminotransferase (ALT) [4]. These elevations are typically transient and occur within weeks to months after vector infusion, hence the need for more intense monitoring during the first months post-treatment. They have been reported across multiple trials involving both hemophilia A and B gene therapies, with incidences ranging from 20% to over 80% depending on vector dose, serotype, and transgene [14, 22]. Notably, differences in the incidence and severity of ALT elevations have been reported between HA and HB gene therapy trials. The reasons for the more frequent and pronounced ALT elevations observed in HA trials remain incompletely understood. This may be related to several factors, including the larger size and complexity of the FVIII transgene, higher vector doses required for therapeutic expression, and the fact that FVIII is not normally synthesized in hepatocytes, potentially eliciting greater immune surveillance. ‘Ectopic’ FVIII production in hepatocytes - particularly at supraphysiologic levels - has

been associated with unfolded protein response activation and endoplasmic reticulum stress in preclinical models. Recent reviews have provided comprehensive insights into this issue [74]. While most post-gene therapy ALT elevations in hemophilia have been asymptomatic and self-limiting, it is important to recognize that such elevations can exist on a broader pathological spectrum. In other AAV-based gene therapy indications, more severe hepatotoxicity, including cases of acute liver failure and death [75] .It is hypothesized that some ALT elevations may result from capsid-specific CD8+ T cell responses directed against transduced hepatocytes, leading to subclinical cytotoxicity [67, 76]. Importantly, most ALT elevations respond well to short-term corticosteroid therapy, and do not appear to have long-term clinical consequences, though continued monitoring remains essential. Although the use of corticosteroids has been effective in managing transaminitis in the most part, the impact and duration of immune suppression remains a significant concern, as well other risks such as cardiovascular, metabolic, endocrine, musculoskeletal and neuropsychiatric adverse effects [77]. The Exigency study has highlighted the significant negative impact of immunosuppression on quality of life in patients with hemophilia post gene therapy [78].

There are questions whether AAV has any long-term chronic liver sequelae, which warrants further investigation. So far, long-term follow-up in preclinical [19] and clinical studies [12, 22] have shown no evidence of chronic liver disease in patients post gene therapy. A major limitation in elucidating these mechanisms is the lack of faithful recapitulation in preclinical animal models. While mice, dogs and non-human primates have been used extensively to study AAV gene transfer, they rarely exhibit the same pattern of liver enzyme elevations seen in human studies [79]. This suggests that human-specific factors - such as individual variability in immune response, pre-existing liver conditions, or subtle differences in vector-host interactions - may contribute to hepatotoxicity.

It is therefore essential to screen patients for underlying liver conditions - particularly those that may not only impact gene transfer efficacy but also increase the risk of hepatocellular injury following AAV gene therapy. Conditions such as non-alcoholic steatohepatitis (NASH), chronic viral hepatitis and non-

alcoholic fatty liver disease (NAFLD) warrant particular attention. Comprehensive assessment of liver health should be conducted prior to and following gene therapy, using serum liver enzymes, viral serology,, ultrasound and vibration-controlled transient elastography (FibroScan), working with Hepatologist within the multidisciplinary team[80] [81].

Variability and Loss of expression

One important issue is the variability in transgene expression levels seen between individuals treated with the same dose of gene therapy, which has been observed across all clinical studies. For instance, in the pivotal trial of valoctocogene roxaparvovec [22], three participants who all received the same 6×10^{13} vg/kg dose exhibited markedly different FVIII activity levels at one year post-treatment - ranging from approximately 7 IU/dL to over 60 IU/dL. This variability likely reflects the complex intracellular journey of AAV, interactions with the immune system and variability in liver physiology [82]. Transgene expression has generally been more stable in HB than in HA [12, 22, 82]. In HA, multiple studies have demonstrated a gradual decline in FVIII expression during the first-year post-gene therapy [83].

Because AAV vectors primarily persist as episomal DNA, any process that increases hepatocyte proliferation - such as liver regeneration, growth in pediatric patients, or underlying liver disease is thought to lead to loss of transgene copies and gradual decline in expression [84]. Partial hepatectomy models have shown that liver regeneration leads to significant loss of episomal AAV genomes, resulting in reduced transgene expression [85]. However, this is in contrast to evidence showing no significant reduction in transgene expression in neonatal dog models of HA and HB, despite liver growth [86, 87].

Integration and genotoxicity risk

Although AAV vectors predominantly persist as episomes, both pre-clinical and clinical studies have shown that a small proportion of vectors integrate into a recipient's genome [45]. Although integration could mediate stable transduction, there are concerns that this could result in insertional mutagenesis. Insertional mutagenesis is the process via which integration leads to disruption in gene regulation or

proto-oncogene activation. This could act as one stage in tumor formation, which although not reported for AAV, has been seen for integrating viral vectors such as gamma-retroviruses and lentiviruses [88]. Notably, the frequency of AAV genome integration is several orders of magnitude lower than these integrating vectors [45]. Evidence from wild-type AAV studies are conflicting: while Nault et al. reported the presence of integrated wild-type AAV sequences in hepatocellular carcinoma (HCC) genomes [89], other studies have presented differing findings. For instance, a study analyzing liver cancer cohorts from Thailand and Mongolia (N=317) found only one HCC patient with a potentially oncogenic AAV integration, suggesting a minimal risk of AAV-induced hepatocarcinogenesis in these populations [90]. Importantly, findings from these wild-type AAV studies are not directly translatable to rAAV, as these would be devoid of the *Rep* sequence. Some murine models have demonstrated a higher propensity for rAAV integration leading to tumorigenesis [91], which has not been observed in large animal models or human clinical trials [92]. The recurrent integration site identified in mice, known as *Rian* - which encodes a microRNA and was associated with tumorigenesis in the study by Donsante et al. [91] - does not have a homologous locus in humans. Moreover, a recent longitudinal study in mice exposed to AAV5-FVIII gene therapy reported no evidence of tumorigenesis or fibrosis resulting from vector integration [93]. So far, seven published cases of cancer have been reported in patients treated with rAAV gene therapy [12, 45]. Detailed analysis in these cases demonstrated no evidence of rAAV integration in these tumors that could have contributed to these tumors. Nevertheless, long-term follow-up of gene therapy recipients remains essential to fully understand the risks associated with AAV persistence and rare integration events.

Unmet needs and limitations

Despite recent advances, significant unmet needs remain in AAV gene therapy. A major limitation, particularly for HA patients, is the exclusion of individuals with pre-existing nAbs against the vector, which are present in a substantial proportion of the population. Patients who develop alloantibodies (inhibitors) against FVIII or FIX also represent an important unmet clinical need. Additionally, current

therapies are not approved for use in children due to concerns about durability of expression in the setting of liver growth and hepatocyte turnover. Accessibility is also limited by high treatment costs and restricted regulatory approval, with AAV-based gene therapies currently licensed in only a small number of countries, further constraining global availability and equity of care.

Conclusion and Future Directions

Liver-directed AAV gene therapy has advanced treatment for monogenic diseases, but key aspects of the AAV lifecycle in human hepatocytes remain unclear. To advance the field further, deeper mechanistic insight into how AAV behaves within the human liver, particularly within hepatocytes and the interaction with the immune system. Greater access to post-treatment liver biopsy data will be essential to bridge the gap between preclinical models and clinical outcomes to guide the development of next generation vectors. In the evolving landscape of gene therapy, clear communication, transparency about uncertainties, and meaningful shared decision-making are critical for individuals considering these advanced therapies.

Author contributions

GS wrote the first draft, reviewed and edited the manuscript. PB edited and reviewed the manuscript.

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Figures

Figure 1. (A) *Natural adeno-associated virus (AAV) structure.* Wild-type AAV's genome contains *Rep* and *Cap* sequences, followed by a terminal sequence of adenosine elements (polyA), The genome is flanked by inverted terminal repeats (ITRs), which are hairpin-like structure, essential for AAV's stability and structural integrity. ***(B)*** *Recombinant AAV (rAAV) schematic.* The *Rep* and *Cap* sequences are removed to make space for a promoter and enhancer, followed by the transgene of interest in the form of a complementary DNA sequence (cDNA). The polyA in rAAV is a synthetic smaller version of the natural polyA.

Figure 2. *Journey of AAV through the cell.* After systemic administration, AAVs bind to glycan- and protein-based receptors on hepatocytes, with co-receptors facilitating entry. The virus is internalized via endocytosis and must escape endosomes to avoid degradation. It then enters the nucleus through the nuclear pore complex (NPC), where the capsid disassembles and the single-stranded genome undergoes second-strand synthesis. The resulting double-stranded DNA persists mainly as episomes, enabling transcription, translation, and secretion of the transgenic protein.

Figure 1