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INVITED REVIEW

Hepatology

Adeno-associated viral vector gene therapy: Challenges for the paediatric hepatologist

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Abstract

Hepatoxicity associated with recombinant adeno-associated virus gene therapy is being increasingly encountered by hepatologists in tertiary and quaternary referral units due to the recent increase of these therapies for neuromuscular and haematological disorders. The challenges in managing the condition stem from a lack of good-quality evidence on the appropriate protocols for immunosuppressants due to lack of representative animal models. There is a need for protocols for diagnosing and treating hepatotoxicity and this possible with further research to understand the problem and its management. The review also highlights the importance of a multidisciplinary team in managing hepatotoxicity and recommends further research to better identify at-risk individuals, define the extent of the problem and assess the long-term effects of liver injury and immunosuppressants.

KEYWORDS

hepatotoxicity, Mycophenolate mofetil, sirolimus, steroids, tacrolimus

1 | INTRODUCTION

An increasing number of gene therapy (GT), including those approved for clinical use and undergoing human trials, use recombinant adeno-associated virus (rAAV) as their viral vector. Hepatotoxicity being a common adverse event, has led to increasing involvement of hepatologists.[1–3] Although most instances of hepatotoxicity are mild, acute liver failure and death have been documented. Even when mild, the hepatoxicity can result in decreased efficacy of GT when the liver is the target organ of GT. Immunosuppression to treat GT-related hepatotoxicity may lead to associated adverse events. The burden of immunosuppression can be significant and prolonged, especially with persisting hepatitis.[2,4–7] The protocols for immunosuppression to treat hepatotoxicity have varied between trials. We summarise the hepatotoxicity reported with rAAV-vector-based gene therapies, challenges in management and the use of various immunosuppressive agents and protocols to treat hepatotoxicity.

2 | rAAV GT

GT products with rAAV vector in children that have received approval or conditional approval for clinical use from the US Food and Drugs Administration or the European Medicines Agency include Zolgensma® (onasemnogene abeparvovec) for spinal muscular atrophy (SMA) in infants and Elevidys® (delandistrogene moxeparvovec-rokl) for Duchenne muscular dystrophy. Other GT approved in the past and also in adults include, Glybera[â] (Alipogene tiparvovec) for lipoprotein lipase deficiency, Hemgenix[â] (etranacogene dezaparvovec) for severe and moderately severe haemophilia B in adults who do not have factor IX

Abbreviations: CK, creatinine kinase; CpG, cytosine–guanine dinucleotide; GGT, gamma glutamyl transpeptidase; GT, gene therapy; MMF, Mycophenolate mofetil; Nab, neutralising antibodies; PIS, prophylactic immunosuppression; rAAV, recombinant adeno-associated virus; RIS, reactive immunosuppression; SMA, spinal muscular atrophy; TLR-2, Toll-like receptor-2; TLR-9, Toll-like receptor-9; ULN, upper limit of normal; XLMTM, X-linked myotubular myopathy.

inhibitors and Roctavian[â] (valoctocogene roxaparvovec) for severe haemophilia A. Other emerging rAAV-GT include those for glycogen storage disorder—type 1a, Wilson disease and Ornithine transcarbomylase deficiency.

The factors contributing to the preference for AAV include the broad tropism of the virus, presumed lack of pathogenicity, nonintegration of the viral genome into the host genome, presumed low immunogenicity relative to other vectors and the ease of production.[8] The rAAV genome is composed of two inverted terminal repeats flanking a promoter, transgene and a poly-A tail. The resultant rAAV can infect and transduce the transgene but cannot replicate or produce capsids. rAAV binds to cell surface receptors enters the cell by endocytosis, enters the nucleus and releases the genome. Before transcription and translation of the transgene is possible, the single-stranded DNA needs to be converted to double-stranded DNA. This conversion may not be necessary if the rAAV is packaged with self-complementary DNA. rAAV that is released from the endosome is ubiquitinated before being degraded by proteosomes (Figure 1).

What is Known

- Paediatric hepatologists are increasingly encountering hepatotoxicity related to recombinant adeno-associated virus gene therapy.
- The hepatoxicity may result in life-threatening complications, loss of transgene activity and adverse events related to immunosuppression use.

What is New

- There is a need for research to better understand the problem and its management to guide immunosuppression protocols for treating hepatotoxicity. Long-term outcomes of hepatotoxicity and immunosuppression also need to be studied.
- Early and reliable identification of individuals at risk for hepatotoxicity could decrease morbidity and mortality.

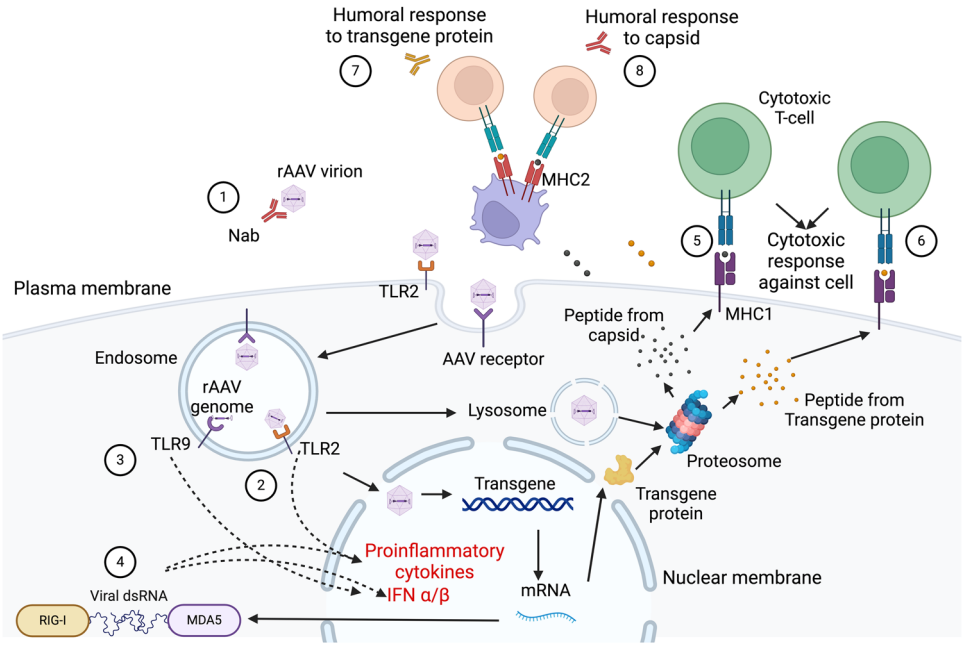

FIGURE 1 Immunological response to aAAV: (1) Pre-existing Nab to capsid antigen bind to rAAV and inhibit cellular entry, trafficking and uncoating of rAAV; (2) binding of rAAV by TLR-2 activates innate immunity; (3) binding of unmethylated CpG of rAAV genome by TLR-9 activates innate immunity; (4) double-stranded RNA derived from RNA generated from inverted terminal repeat sequences activate innate immune response by binding to MDA-5; (5) capsid peptide from proteosome presented to CTLs with MHC-1; (6) transgene protein derived peptide from proteosome presented to CTL with MHC-1; (7) transgene peptide presented on MHC-2 to elicit a humoral response to the transgene protein; (8) capsid peptide presented on MHC-2 to elicit a humoral response to the capsid protein. Adapted from Jagadisan and Dhawan.[9] *Created with BioRender.com*. CpG, cytosine–guanine dinucleotide; CTL, cytotoxic T cell; dsRNA, double-stranded RNA; MDA-5, melanoma differentiation-associated protein; MHC-1, major histocompatibility complex-1; mRNA, messenger RNA; NAb, neutralising antibodies; IFN, interferon; rAAV, recombinant adeno-associated virus; TLR-2, Toll-like receptor-2; TLR-9, Toll-like receptor-9.

2.1 | Immunological mechanism of hepatotoxicity

The immunogenicity of rAAV affects the transduction and is responsible for observed adverse events of the GT (Figure 1).[2,10,11] Transduction is impaired by pre-existing neutralising antibodies (Nab) against capsid proteins. Nab to wild type AAV is quite prevalent in the population early in life with cross-reactivity across serotypes, even though the seropositivity is less in infants compared to older children.[12] Studies in capsid engineering and evolutionary biology of AAV have yielded viral vectors such as AAVLK03 and AAV-Anc80L65 with lesser affinity to prevalent Nab and better transduction.[8,13,14] Capsid-derived peptides released by the proteasome are presented with major histocompatibility complex class II receptors leading to CD4+ T cells and B-cell mediated adaptive immune response and generation of Nab that subsequent administrations rAAV GT.[2] A similar process may result in the production of antibodies to the transgene protein.[15] This process does not contribute to hepatotoxicity.

Hepatoxicity is mediated by innate immunity and T-cell-mediated immune response. The mechanism of immunological reaction against rAAV is briefly summarised in Figure 1.[9] In humans, the innate immune response is a modest response that accounts for the early hepatotoxicity a week after GT administration.[16–18] This is mediated by Toll-like receptor-2 in Kupffer cells, binding of cytosine–guanine dinucleotide of rAAV genome to Toll-like receptor-9 and binding of double-stranded RNA (dsRNA) derived from messenger RNA to cytoplasmic dsRNA sensor. With the use of prophylactic immunosuppressants, the reported hepatotoxicity is a peak in transaminases without reports of serious adverse events.[2] The release of pro-inflammatory cytokines from this process through nuclear factor kappa-light-chain-enhancer of activated B cells and type-1 interferons also drives the subsequent adaptive immune response by causing maturation of antigen-presenting cells and activation of neutrophils, macrophages and natural killer cells.[2,16,19–21]

The onset of the T-cell response, mediated by CD8+ T cells is often a month after rAAV GT destroying transduced hepatocytes.[17,22,23] Although rAAV cannot produce new capsids, the T-cell response to the administered capsid proteins continues for months.[24] rAAV capsid has been detected for long periods in tissues depending on the route of administration—6 years after subretinal injections and 12 months follow-up after intramuscular injection.[25,26] The process terminates with infiltration of CD4+ CD25+ FoxP3+ T-regulatory cells and exhaustion of CD8+ cells.[26]

Variations in the immune response may result from polymorphism in the IL6R gene.[27] Recent literature demonstrated an association of Indeterminate Paediatric Acute Liver Failure outbreak in the United Kingdom with adeno-associated virus-2 infection in the presence of other viruses. This raised the possibility of immunological injury against the virus leading to liver injury with a high association with *HLA DRB1*04:01*.[28] It is not known if this would also apply to rAAV GT-induced hepatotoxicity.

2.2 | Pattern of hepatotoxicity

The pattern of liver injury seen in rAAV-GT for SMA is a hepatocellular liver injury. Children with X-linked myotubular myopathy (XLMTM) who have an underlying cholestatic liver disease have an exacerbation of cholestasis (Table 1).[2,3,5–7,9,10,27,29–46] The early peak in transaminases in the first week is partly dampened by the high doses of prophylactic immunosuppression (PIS) used. Deaths have not been reported in this phase in humans. The second peak of transaminase elevation is noted with the tapering of steroids. This flare has resulted in acute liver failure and death, especially if the steroid tapering is too rapid. It often responds to an increase in immunosuppression. The transaminases normalise eventually over a variable period.[2,6,7,10,30,32,33] The hepatotoxicity results in reduced transgene expression if the liver is the target of transduction.[5]

Given this hepatoxicity, patients receiving GT are screened for pre-existing liver injury to exclude them from trials to avoid severe liver injury that may be compounded by the immune-mediated injury related to the rAAV. These parameters for exclusion are likely to be different for different disorders and are ill-defined and at best empirical currently.[2,47] Disorders treated with rAAV may have pre-existing liver abnormalities that exacerbate the immunological injury due to GT. Thus, the hepatotoxicity may be a double-hit on the liver leading to the catastrophic results noted in patients with SMA and XLMTM. Patients receiving GT are screened for significant liver injury to preclude them from trials and clinical use. Irrespective of such associations, all such patients will need screening for other liver co-morbidities (Table 2).[9]

2.3 | Mitigation of hepatotoxicity by patient selection

Mitigation of the risk of hepatotoxicity should focus on:

(i) Using lower vector doses to achieve gene transduction whenever possible.

The hepatotoxicity is closely linked to the vector dose administered. Vector doses necessary to achieve transduction of transgene into the cell can

TABLE 1 Baseline liver dysfunction and hepatotoxicity in patients receiving GT.

Disease/disease group and details of GT	Liver injury/dysfunction in the underlying disease	Liver injury documented with GT
SMA—AVXS-101—nonintegrating, fully functional copy of SMN1 into motor neuron cells using self-complementary AAV and AAV9.[2]	• Mild elevation in liver transaminases in 61% of patients in clinical trials.[2] • Release of enzymes from muscles could also cause mild elevation of transaminases • Hepatic steatosis—possible due to abnormal beta-oxidation of fatty acid (e.g., dicarboxylic aciduria).[29–31] • Reye-syndrome (acute liver failure) requiring liver transplantation after prolonged intraoperative propofol and sevoflurane exposure, postoperative acetaminophen, stress-induced lipolysis and reduced perioperative glucose intake.[32] • Susceptible to paracetamol toxicity with therapeutic doses drug.[33]	• 90% treated with AVXS-101—ALT and/or AST elevation during therapy irrespective of the baseline elevation. 90% elevations <3× ULN, 5% elevations >20× ULN.[2] • Dual peak in elevation in liver enzymes—First peak a week after drug infusion, second peak a month after infusion when steroids tapered commenced. • Acute liver failure in two children 3–8 weeks after infusion, with rapid steroids tapering. Both responded to high-dose steroids.[6] • Two deaths reported from Russia and Kazakhstan during steroid taper.[34]
XLMTM—AT132 was a rAAV-8 vector expressing *MTM1* gene.	• Raised transaminases, alkaline phosphate and GGT, hepatomegaly, cholestatic jaundice, pruritis, cholelithiasis, acute cholecystitis, hepatic steatosis and peliosis hepatis • Amburgey et al. (33 patients studied over 1 year)—Abnormal serum liver enzymes (22.5%), hepatomegaly (11.8%), jaundice (14.7%), liver haemorrhage (5.9%).[35] Death due to bleeding in hepatic peliosis. • ASPIRO study—T132 in 24 XLMTM patients—Baseline evidence of liver disease in >50%—intermittent direct hyperbilirubinemia, transaminase elevation or cholestasis in the past.[7,10,36,37] • Hypothesis on the mechanism of liver injury—defects in autophagy, impaired lipid layer trafficking.[38]	• ASPIRO study - Death in three children in the high-dose cohort within 20–40 weeks—All older children >5 years of age, received high vector doses (range: $4.8–7.7 \times 10^{15}$ vg), had pre-existing hyperbilirubinemia and on the drug developed direct hyperbilirubinemia leading to a decompensated liver disease. - Autopsy showed intrahepatic and canalicular cholestasis, bile ductular reaction, fibrosis and absence of prominent liver parenchymal inflammatory infiltrates. One child showed lesions consistent with hepatic peliosis. • The first patient administered the lower dose (1.3×10^{14} vg/kg) on trial recommencement died, leading to study termination. • Dose labelled as 'low dose' significantly high by the standards of trials in other diseases.[7,10,36,37]
Haemophilia—Regardless of the natural endogenous source of the proteins (liver sinusoidal endothelial cells and hepatocytes for factor VIII and factor IX respectively), the cellular target for transgene expression in AAV GT of Haemophilia A and B is the hepatocyte for both haemophilia A (factor VIII deficiency) and haemophilia B (factor IX deficiency)[39–41]	• Liver disease may be a result of parenterally transmitted chronic hepatitis C virus infection via contaminated plasma-derived coagulation factors in the past • Human immunodeficiency virus infection in some of these patients can add to the liver injury.[42] • Patients with advanced fibrosis, use of hepatotoxic medications such as antiretroviral therapy and chronic viral hepatitis are contraindications to rAAV-GT. • Fibroscan may be useful in assessment of fibrosis. Patients with mild degree of fibrosis detected on fibroscan may need closer monitoring.[3]	• Early innate immune response against CpG motifs not blunted completely by corticosteroids.[27] Vector codon optimisation with reduced CpG content and high specific activity factor IX Padua variant enables lower bleeding rates with lower vector doses. Immune-mediated transaminase elevation amenable to treatment by steroids.[43,44] • Liver transaminases elevation with higher vector doses. Rise in transaminases associated with drop in factor IX levels due to the destruction of the transduced hepatocytes by cytotoxic T-cells.[5] • Transient courses of rescue corticosteroids normalise the serum transaminase levels[45] • Delayed initiation of an immune response and transaminase elevation after 3–4 months unique to factor VIII trials. The reactive and prophylactic courses of steroids in factor VIII GT are longer compared to factor IX GT trials.

TABLE 1 (Continued)

Disease/disease group and details of GT	Liver injury/dysfunction in the underlying disease	Liver injury documented with GT
		• Long-term decline in factor VIII level possibly related to its synthesis by hepatocytes rather than liver sinusoidal endothelial cells, natural senescence and turnover of hepatocytes.[47]

Note: Adapted from Jagadisan and Dhawan.[9]

Abbreviations: AAN, adeno-associated viral vector; AAV, adeno-associated viral vector; AAV9, AAV serotype 9; AST, aspartate transaminase; CpG, cytosine–guanine dinucleotide; GGT, gamma glutamyl transpeptidase; GT, gene therapy; SMA, spinal muscular atrophy; ULN, upper limit of normal; XLMTM, X-linked myotubular myopathy.

TABLE 2 Baseline assessment of an infant with SMA being considered for AVXS-101.

Work-up at baseline in an infant with SMA being considered for AVXS-101
Liver function test—Serum levels of total bilirubin, conjugated bilirubin, AST, ALT, alkaline phosphatase, GGT, INR, total protein, albumin
Serum creatinine kinase
Hepatobiliary ultrasound
IgM anti-HAV, anti-HEV, anti-HCV antibody, hepatitis B surface antigen, urine CMV PCR, serum CMV PCR, Adenovirus PCR, PCR for HHV-6, parvovirus, HSV PCR, EBV serology and PCR
Serum lipid profile, uric acid
Plasma amino acids, urine organic acids, acylcarnitine profile, alpha-1-antitrypsin levels and phenotype, very long chain fatty acid, plasma lactate, screening for cystic fibrosis
In infants >6 months of age, antinuclear antibody, antiliver kidney microsomal antibody-1, anti-SMA, serum immunoglobulins
Screening for Coeliac disease in older infants
Drug history and family history of liver disease

Note: Adapted from Jagadisan and Dhawan.[9]

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; EBV, ebstein barr virus; GGT, gamma glutamyl transpeptidase; HAV, hepatitis A virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HHV, human herpes virus; IgM, immunoglobulin M; INR, international normalised ratio; PCR, polymerase chain reaction; SMA, smooth muscle antibody.

be minimised by engineering the rAAV vector which including changes to the capsid. Decreasing the load of CpG motifs decreases the innate immune response. With the use of high-activity transgenes, lower vector doses can be used. The level of pre-existing antibodies that are a contra-indication for GT vary between trials and more recent haemophilia trials using high-activity genes have been able to achieve good transgene activity in spite of relatively higher Nab levels.[48] Avoiding empty capsids (capsids with no genetic material inside) by fine tuning the manufacturing process ensures that the capsid dose administered is less. Careful patient selection also helps. Administering GT to patients with low Nab titre avoids the need to administer high vector doses.

(ii) Screening and excluding children with significant pre-existing liver abnormalities. The degree of hepatic dysfunction that would be an effective exclusion criterion for GT to mitigate hepatotoxicity is less well-defined and variable between studies. The parameter used could be the serum transaminase levels, the serum bilirubin level or the degree of fibrosis. The parameter could vary between diseases and the vector dose used.[2,32,47] The fine balance between safety and benefiting more patients with GT could be difficult to achieve. Until a better safety profile is demonstrated, children with any degree of cholestasis are best excluded from the use of GT.

(iii) Avoiding hepatotoxic medications is an essential part of risk mitigation.

(iv) Avoidance of rapid steroid tapering could prevent severe flares of liver injury that could lead to liver dysfunction.

The utility of measuring any pre-existing cellular immunity to AAV is of doubtful value in predicting hepatotoxicity. As discussed above, the timeline of onset of hepatoxicity is often indicative of an early innate immune response followed by a new onset T-cell response rather than a memory response.[2,44]

2.4 | Clinical use of immunosuppression protocols used in hepatotoxicity

Preclinical and clinical use of immunosuppressant protocols in rAAV GT has been for the following purposes:

(i) depleting circulating pre-existing Nabs to improve transduction and expand the recipient cohort that can receive the GT.[49–53]

(ii) PIS that dampens innate immune response to decrease adverse events during the early post-administration phase and also suppresses the later onset adaptive immune response if the immunosuppressants are continued longer. PIS may be started with the dual aim of preventing

development of Nab and preventing hepatotoxicity but the former objective is often not achieved.

(iii) reactive immunosuppression (RIS) to suppress adaptive immune response that has led to adverse events and/or inhibition of transgene expression.

Innate and cellular adaptive immune response predominantly mediate hepatotoxicity. Hence, drugs such as Rituximab used to deplete Nab and improve transduction have no effect on hepatotoxicity. The following drugs have been used as PIS and RIS for hepatotoxicity in rAAV GT. The goal of PIS should be to preclude any hepatotoxicity with the least drug-induced adverse events. RIS regimens should avoid long courses of steroids and avoid mortality and morbidity in the setting of serious hepatotoxicity. These goals are yet to be achieved and may require second-line immunosuppressants to achieve the goal.[2,3,6,45]

2.5 | Corticosteroids

Corticosteroids suppress both the innate and adaptive immune system. They are often used as PIS in rAAV GT including that in haemophilia, XLMTM and SMA. In XLMTM, AT132 was administered with prophylactic prednisolone at 1 mg/kg/day for 8 weeks after administering the drug and tapered over the subsequent 8 weeks. The steroid use protocol used for steroid use as a prophylactic and reactive therapy for hepatoxicity in AVXS-101 treatment of SMA is described in Table S1.[2,4,5,7,9,10,32,33,43] Chand et al. refer to a revision in their PIS steroid protocol for clinical trials in March 2019 in response to reports of acute liver failure. The revised regimen included initiating prednisolone at 2 mg/kg/day, starting the day before dosing, for 3 days; and then reducing to 1 mg/kg/day for 30 days, with tapering. Further modification of dosing and duration of steroids was left to the clinicians' discretion. Yet, the summary of product characteristics issued with Zolgensma® continues to advise a prednisolone dose of 1 mg/kg/day preinfusion.[2]

The reactive steroid courses in haemophilia GT were in response to transaminase elevation with or without a decline of transgene expression. These were short courses in factor IX GT compared to long courses in factor VIII GT. These were associated with steroid-associated adverse events.[5,45] In hepatoxicity associated with AT132, corticosteroids were used to treat along with ursodeoxycholic acid in patients who worsened and subsequently died. The benefit of corticosteroids was doubtful in these cases, considering that the inflammatory infiltrates were minimal in the liver at autopsy.[7,10,32,33] In SMA patients treated with AVXS-101, the reported mean duration of prednisolone treatment ($n = 100$) was 83 days (range: 33–229 days; SD 60.6 days) without details on the dose burden of prednisolone.[2] Acute liver failure reported 3–8 weeks postinfusion of AVXS-101 was responsive to 20 mg/kg/day of methylprednisolone.[6]

2.6 | Sirolimus

Sirolimus inhibits mammalian target of rapamicin and thereby cytotoxic T-cells, T-helper cells and B-cell response. It also upregulates T-regulatory cells. The clinical use of Sirolimus as a PIS in combination with Rituximab in rAAV GT is based on animal studies aimed at attenuating antibody formation, decreasing pre-existing Nabs and improving transduction.[53] It allows readministration of GT.[51,54] The facilitation of autophagy by Sirolimus also enhances transduction.[55] It may also prevent the development of antibodies to the transgene. Sirolimus may upregulate T-regulatory cells and thereby encourage tolerance to rAAV capsid and the transgene.[56]

Rituximab and Sirolimus (serum trough level of 3–7 ng/mL) were used in a cross-reacting immunologic material-negative child with Pompe disease who initially received enzyme replacement therapy followed by rAAV-GT and was shown to not develop antibodies to rAAV capsid and along with the absence of a T-cell response.[52] In rAAV-GT for Crigler-Najjar syndrome, methylprednisolone, 8 weeks of prednisolone and 12 weeks of Sirolimus were used as PIS.

In the initial patients who received GT for Crigler–Najjar syndrome, hepatoxicity associated with decrease in transgene activity was noted. This required steroids and Sirolimus as RIS. Subsequently, the PIS regimen was revised with Sirolimus being used for a prolonged course of 48 weeks, rather than the 12-week course. Although effective in suppressing the hepatotoxicity, the development of antibodies to AAV could not be prevented.[57]

2.7 | Mycophenolate mofetil (MMF)

MMF suppresses T-cell and B-cell proliferation by inhibiting de novo purine synthesis. Based on the data in mice that MMF reduces vector genome second strand synthesis by depleting intracellular guanosine triphosphate, MMF was felt to be unsuitable as an immunosuppressant for the early phase of transduction using single-stranded rAAV. However, MMF did not impair transduction by a self-complementary rAAV vector, similar to that used in AVXS-101.[58] Studies in nonhuman primates with MMF added 4 weeks after single-stranded rAAV infusion showed that despite the delayed introduction of MMF to allow for second-strand synthesis, transgene expression was inhibited by MMF through an immunological mechanism.[59] In contrast, Jiang et al. did not demonstrate impaired transduction

or factor IX synthesis in rhesus macaques with coadministration of MMF and tacrolimus along with rAAV.[60]

MMF was used as PIS alongside cyclosporine with or without methylprednisolone in Glybera GT in humans but failed to prevent the development of a T cell and B cell response to the capsid or the transgene product.[61]

MMF may be used as a steroid-sparing agent in the reactive treatment of prolonged hepatotoxicity as has been used by our team in the management of persistent hepatoxicity following AVXS-101 use (unpublished literature).

2.8 | Tacrolimus

Tacrolimus is a calcineurin inhibitor. The use of Tacrolimus is accompanied by concerns regarding downregulation of T-regulator cells and a potential loss of tolerance to the transgene product.[56]

Tacrolimus was effective in one patient as a RIS to treat hepatotoxicity in rAAV GT for haemophilia B in the B-AMAZE study in addition to its use as a prophylactic agent in combination with prednisolone.[4] The choice of Tacrolimus over Sirolimus or MMF in the setting of severe hepatotoxicity is prompted by the rapidity of action of Tacrolimus compared to the other two drugs.

2.9 | Challenges in managing hepatotoxicity

The reported prolonged duration of transaminase elevation in rAAV-GT is concerning.[2,3,45] A significant proportion of patients received steroids for >90 days. This could also be partly related to individual variations in the practice of tapering steroids.[2] Its effect on muscle mass and function needs evaluation in SMA. A formal study of steroid adverse events in these patients is lacking.

There are no biomarkers that identify patients who will need prolonged use of immunosuppressants. In those with prolonged transaminase elevation, the time of initiation of second-line immunosuppressants is empirical at the most and in the authors' view, should be around 60 days. It would have to be balanced with the understanding that some patients might normalise their transaminases while drugs such as MMF take time to act from the time of their initiation. Second-line immunosuppressants may not be justified when steroid weaning could be achieved in under 8 weeks and without steroid toxicity.

The threshold of serum transaminase elevation that warrants RIS is likely to be much lower in children where the rAAV-GT is directed towards transgene expression in the liver to avoid loss of efficacy. There is a trend towards maintaining 'normal' serum transaminase levels in these patients.[57] This goal will differ from that in autoimmune hepatitis that hepatologists are used to as it is generally understood that transaminases tend to fluctuate minimally over time even in normal children. There is a risk of overimmunosuppression in these children if we practice to avoid these fluctuations for fear of losing transgene activity. For example, Sirolimus was used for 48 weeks in rAAV-GT for Crigler–Najjar syndrome to avoid hepatotoxicity-induced transgene activity.

Although self-limiting, the effect of prolonged liver inflammation is not known. Performing liver biopsies in patients with prolonged transaminase elevation and neuromuscular weakness is challenging and should be reserved for a select group of patients with acceptable anaesthetic risk.

There is a need for research to better understand the problem and its management to guide immunosuppression protocols for treating hepatotoxicity. Our personal clinical practice for the management of AVXS-101-related hepatotoxicity is described in Figure 2.[62,63] This would generally apply to GT where the liver is not the target for transgene expression and wider fluctuations in serum transaminases may be acceptable. Multidisciplinary team discussions that include a hepatologist should guide management decisions for rAAV GT-related hepatotoxicity.[3,62] The choice of immunosuppression could differ based on the underlying disease, the age group of the patient and the susceptibility to infections.

3 | rAAV AND ONCOGENESIS

Although the article focuses on hepatotoxicity, the concerns regarding possible role of AAV in oncogenesis merit mentioning. The evidence linking wild-type AAV or rAAV to the development of hepatocellular carcinoma (HCC) is controversial. Although rAAV causes episomal integration, there may be a small amount of chromosomal integration, which may be linked to the vector dose.[64] AAV is known to cause HCC in mice.[65,66] The reports of 11 cases of HCC in humans with AAV2 sequence integration, in the absence of risk factors for HCC, raised concerns of oncogenicity due to the virus.[67] Yet, rAAV-GT for haemophilia B had not resulted in HCC after 15 years of follow-up.[68] The only patient in a rAAV-GT clinical trial for factor IX deficiency who developed HCC 1 year after therapy, had co-existent risk factors for HCC in the form of chronic HCB and HBV infections and also nonalcoholic fatty liver disease. rAAV infection targeting oncogenic locus in mice is known to result in HCC in the presence of a proliferative stimulus, either in mice infected as neonates or infection in adult mice with injury due to fatty liver.[66] Hence there is a potential risk

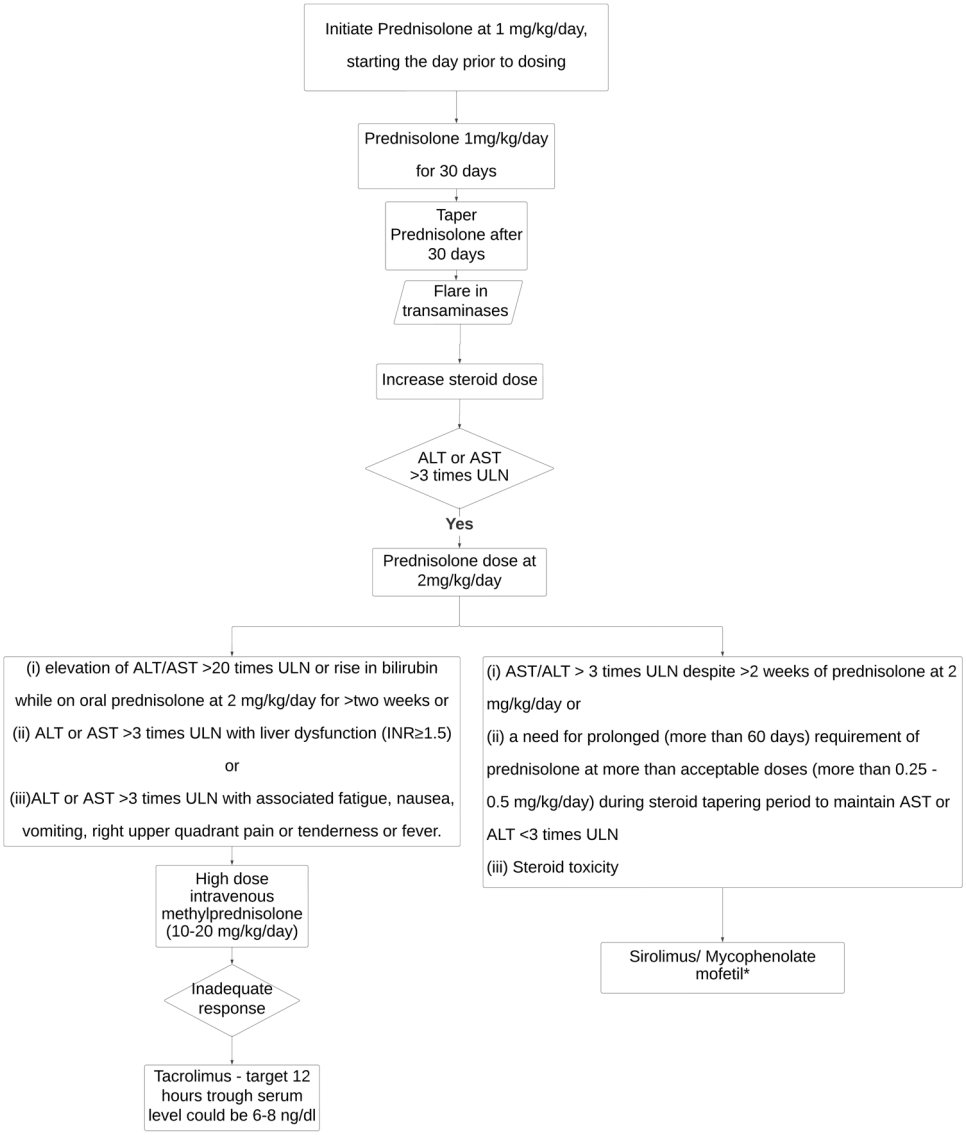

FIGURE 2 Institutional algorithm for second-line reactive immunosuppression in children AVXS-101 related hepatotoxicity. ALT, alanine transaminase; AST, aspartate transaminase; ULN, upper limit of normal.

of recipients of rAAV-GT developing HCC as they grow and develop highly liver diseases such as nonalcoholic fatty liver disease. A proactive strategy to screen for HCC with annual ultrasound and serum alpha-fetoprotein measurement needs further discussion and not currently part of our protocol.

4 | CONCLUSIONS

Hepatotoxicity with rAAV-GT has emerged as an area that will see increasing involvement of paediatric hepatologists. Paediatric hepatologists will need to offer advice with a reasonable understanding of the mechanism of toxicity, the individual needs of liver-targeted or extrahepatic-targeted GT and the options available for immunosuppression. It will involve finding a balance between losing transgene expression, avoiding clinically significant liver injury and the depth and duration of steroid-based or steroid-sparing immunosuppression. Early and reliable identification of individuals at risk for hepatotoxicity could decrease morbidity and mortality. Decisions regarding immunosuppression could benefit from a wider conversation between all professionals involved in care.

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Anil Dhawan is on the Scientific advisory boards of Novartis, BitBio and Aspect Bio. The remaining author declares no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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