A systematic review of adeno-associated virus gene therapies in neurology: the need for consistent safety monitoring of a promising treatment

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Introduction

Gene therapies have been spotlighted in the rare disease field due to hopes that they can provide substantial benefit to a variety of life-limiting conditions. Many of these are neurological disorders for which no disease-modifying options exist. Gene therapies act by replacing, editing or silencing known disease-causing genetic mutations in a target tissue.

Adeno-associated virus (AAV)-mediated gene therapies have grown exponentially since their clinical trial debut in 2003,[1] and are currently one of the leading platforms for gene therapy. Recombinant AAVs are unable to replicate in humans and have superseded previously used adenoviral platforms due to their potential for long-lasting gene expression, coupled with reduced immunogenicity.[2] The AAV attaches into the target cell and is taken in by receptor-mediated endocytosis. After endosomal transport and escape, the virus is transferred into the nucleus, where uncoating takes place allowing for second strand synthesis of the transgene, unless it is self complementary.[3] Integration into the host genome is considered a rare phenomenon as AAVs mostly remain as extrachromosomal episomes.[2] AAV capsids determine their serotype and tissue-specific tropism enables a degree of specific transduction. AAV9, for example, allows for central nervous system (CNS) transduction. Specific tropism can be selected for by the creation of novel engineered AAV capsids.

Three AAV-based gene therapy products have obtained commercial licences, although one has subsequently been removed from the market due to commercial failure.[4–6] Voretigene neparvovec-rzyl is used in inherited retinal disease and administered by local injection in the retina. Onasemnogene abeparvocec-xioi is the only product currently available for systemic administration, approved by the Food and Drug Administration (FDA) in 2019 for the treatment of spinal muscular atrophy (SMA). To date, approximately 2300 patients have received this treatment. The number of products being manufactured and reaching clinical trial is increasing at speed and, while the majority are early phase, initial reported efficacy rates are in some cases high. It is important to note that, while these initial reports are generating much excitement in the field, efficacy is in most cases based on surrogate end points, such as successful gene transduction. Clinically significant improvements on a scoring system are rarer, and there is no available evidence from randomised control trials supporting efficacy as yet, although several such studies are currently underway, as for Duchenne muscular dystrophy (DMD).

Alongside the excitement felt for the potential of new therapeutic options, there is a growing need for caution. Several serious treatment-related adverse events have been reported, including nine deaths.[7–11] The predictors of severe adverse events, along with the potential methods for mitigation, are yet to be fully elucidated. Possibilities span product attributes, such as capsid serotype (AAV9) and dose (higher dose range), as well as patient characteristics such as stage of disease, weight and pre-existing immunity. Potential patient genetic susceptibility has not been assessed in any of these studies but is likely to be important. These data remain largely unpublished, accessible only from press releases and conference abstracts. Many of the adverse reactions

observed in humans have not been observed in preclinical studies.7 Equally, reactions seen in animals do not necessarily translate into humans12 . This casts a doubt on the predictability of safety from animal models and raises the need for better modelling and increased caution when translating results from preclinical to clinical studies for AAV gene therapies.

There is, therefore, an urgent need to carefully consider factors associated with adverse events of AAV gene therapies in humans. Effective management of serious adverse reactions is critical in order for the promise of gene therapy to be fully realised. There is growing evidence that such reactions are likely to be class effects of types of gene therapy products and their specific interaction with the host rather than attributable solely to a specific manufactured product. Variables include serotypes, transgenes and a constellation of immunological factors including specific problems associated with patients who have bi-allelic null mutations leading to a loss of cross-reactive material (CRIM negativity) and resultant lack of immune tolerance. To aid with improving safety we have therefore systematically reviewed the literature in order to help compile information thus far.

Methods

We sought to identify safety and efficacy results of human clinical studies testing AAV-based gene therapy products for neurological diseases, defined here as studies which have a clinical neurological outcome measure. AAV gene therapy products with either systemic (intravenous) or CNS-directed (intrathecal, intracranial) administration were included. Sponsors' press releases and conference abstracts were included if they were published after 2020 or no peer-reviewed information about that trial existed. There was no limit on language, sample size or phase of the trial.

Search strategy and data extraction

The search strategy was designed by RHH, DS, TM (investigators) and EH (research librarian). The full search strategy is available in the appendix. EH and RHH searched Ovid Medline, Ovid Embase and Cochrane CENTRAL. Titles and abstracts were independently screened for inclusion by RHH and DS. RHH and TM searched for relevant studies on ClinicalTrials.gov. Google searches and searches of recent conferences were also used. A matching process was then employed to group publications, editorials, conference abstracts and press releases with their ClinicalTrials.gov reference. Each identified study was checked for updates manually on 08 March 2022.

Appraisal

Out of the records, 32 were peer-reviewed journal articles. The other information was obtained from conference abstracts and press releases. Evidence hierarchy is noted in the tables of efficacy and is used to interpret results according to trial design. All reports of adverse events were weighted equally. The American Academy of Neurology risk of bias tool13 was used by RHH and TM, the full risk of bias assessment can be found in the supplementary informationonline supplemental file 1.

Results

Existing trials and products

We identified 61 clinical trials for 52 gene therapy products, covering a broad spectrum of disorders with neurological manifestations including neurodegenerative, neuromuscular and metabolic disease. Table 1 summarises AAV gene therapy clinical trials and reported efficacy outcomes.

Technical details

Systemic administration was through a peripheral limb vein. Direct CNS administration was intrathecal or intracranial, with some studies administering the product using image-guided neurosurgery. Nine different AAV serotypes were used, the most common being AAV9. Notably, AAV2 serotypes were more commonly used in earlier years with AAV9 and AAVrh serotypes used in later years. Promotors were used to govern transgene expression in the target tissue.

Efficacy

Efficacy data are presented for each product in table 1. 24 products report at least preliminary evidence of efficacy, although this is only reported in peer-reviewed publications in 12 instances. Six products have progressed to phase III trials. Onasemnogene abeparvocec-xioi has been approved and is in clinical use.

Signals of efficacy used in trials include biochemical markers (NCT03952637, GM1 gangliosidosis, AAV9-GLB1), neuroimaging,[14] clinical scoring systems,[15] milestone assessments[16] and measures of transgene expression[17] (see table 1). Most trials do not use a direct comparator but rather reference either natural history studies[14 18] or the patient's own baseline.[15] Only one study reported efficacy in comparison with a concomitant control group (NCT03199469, AAV8, XMTM, AT132) and the only randomised placebo-controlled trial reported no efficacy (NCT00876863, CERE-110, Alzheimer's disease). Work is being done on validating outcome measures to aid precision[19] and phase III randomised controlled trials are emerging.[20]

Safety

Serious adverse reactions have been reported with uses of 14 gene therapy products as presented in table 2. In the case of Onasemnogene abeparvocec-xioi for SMA, serious adverse reactions have occurred with commercial use as opposed to in clinical trials. [10 11 21 22] Key serious adverse events conserved between trials of systemically delivered gene therapies are liver toxicity[15 16 21–23] and thrombotic microangiopathy (TMA).[23–26] Neurosurgical administration of gene therapy products (see tables 1 and 2) such as conducted in Alzheimer's, Canavan and Parkinson's diseases carries its own risks.[14 27 28] Due to vast heterogeneity in the reporting of safety and monitoring between trials, a full assessment of risk is difficult to produce.

A review of treatment-related deaths

Nine children have died following gene replacement therapy due to either the product (n=8)[7–11] or its administration (n=1)[9](see online supplemental table 4). Four of these nine children were in the ASPIRO Trial for X linked myotubular myopathy (XMTM) (NCT03199469). Three received intravenous remsarigene bilparvovec (AAV8, desmin promotor) at the highest dose (3.5×10^{14} vg/kg).[7] These three boys developed progressive cholestatic liver failure, with a rise in bilirubin out of proportion to the transaminitis. The investigators have not reported a definitive cause for this liver failure but reported that all three boys had some pre-existing hepatic pathology and developed progressive liver failure postdosing.[29] The children died between 20 weeks and 40 weeks after receiving the gene therapy product, but the rise in bilirubin began within the first 2 weeks after initial dosing.

Initially, the investigators reported that these children differed from the average child in this study, in that they were among the oldest and heaviest children involved and, therefore, received among the highest total doses. This is the highest reported systemic dose for any AAV gene therapy (see table 1A). The trial was suspended. In December 2020, the FDA released the clinical hold with the proviso that only the lower dose (1×10^{14} vg/kg) was used and that the age of participants was limited to those under 5 years. Unexpectedly, the first and only participant who was dosed after the lifting

of this trial has subsequently died.30 The cause of death remains to be fully investigated. Prior to his death, it is known that he had a rise in serum transaminases and evidence of cholestatic liver disease, which caused Astellas to place a hold on further dosing. It is now becoming apparent that a propensity to cholestatic liver disease is an integral component of XMTM29, which may be exacerbated by the high viral load delivered to the liver during gene therapy. Therefore, these adverse events are best viewed as disease-specific susceptibility exacerbated by specific gene therapy toxicity, the details of which remain to be fully elucidated.

One child died of a suspected treatment-related cause after receiving onasemnogene abeparvocec-xioi under its commercial license for SMA. The child died following TMA and underwent exchange transfusion and dialysis before unfortunately dying 6 weeks after administration of the product. Recently, two deaths from acute liver failure occuring 5-6 weeks after onasemnogene aveparvovec-xioi were reported in Russia and Kazhakstan, but very few details have been publically disclosed so far.10 There have been two other fatalities in children receiving onasemnogene abeparvocec-xioi, and these children showed evidence of thrombocytopenia and haemolysis, but the cause of death was not cited by the authors as directly related to the product.31

One child died following intracranial administration of an AAV2 vector for CNL2 Batten disease, following status epilepticus.9 It is thought that this is most likely due to neurosurgery rather than the gene therapy product itself, but the exact cause remains unclear. Postadministration electroencephalogram monitoring has been included for subsequent trial participants. No other incidences of neurosurgical-related deaths have been reported in AAV gene therapy trials.

A further child has died 7 days following infusion of PF-06939925 for DMD (NCT03362502) of cardiomyopathy.32

Complement activation syndromes

TMA has been observed with three gene therapy products, SGT-001 (NCT03368742),23 PF-06939926 (NCT03362502)26 for DMD and onasemnogene abeparvocec-xioi for SMA.24 All of these gene therapy products use an AAV9 vector. There is evidence of anticapsid antibodies as a possible contributor, and this has led to the use of rituximab as a preventative measure in some trials (see table 3A). These syndromes have affected a total of nine children, one of whom has died.11 TMA is characterised by arteriole and capillary endothelial pathology and microvascular thrombosis. It is usually associated with activation of the alternate complement pathway and presents clinically with thrombocytopenia, then haemolytic anaemia and acute kidney injury.33 Isolated thrombocytopenia, which is one of the earliest biological signs of TMA, has been reported as a treatment-related adverse event following onasemnogene abeparvocec-xioi for SMA, SGT001 for DMD and PF-06939926 for DMD. It is not clear whether this is due to complement activation.

Other features of TMA include proteinuria, hypertension, haematuria and vomiting.34 Onset was within 6–11 days of administration. These symptoms were seen across the three drug products and the children were treated with a combination of glucocorticoids, plasma exchange, dialysis and complement monoclonal antibody therapy. The monoclonal antibody eculizumab acts against C5 in the complement pathway and was used in four patients following SGT001 (n=2), PF06939925 (n=1) and onasemnogene abeparvocec-xioi (n=1) with inconclusive results. A case series of three patients developing TMA after OA detailed the complement panel studied (see table 3A).24 Complement activation was evidenced by low C3 and C4 and an increase in soluble C5b-9 complex. An increase in this complex has also been observed with use of SGT-001 for DMD and this trial now uses eculizumab prophylactically (see table 3A). Notably, all of these complications in OA have occurred in commercial use, and preliminary signs were not monitored in clinical trials. Updated safety

information from the EMA recommends stringent monitoring of platelet counts in the week following administration,35 and that the child should be screened for haemolysis after relative drops in platelet counts, even if this does not fall below recognised normal limits. Other disease markers include troponin rise, uraemia, raised D-dimers, anaemia and schistocytes on the blood film. Whether this syndrome is more widespread and whether there is the potential for early detection has not yet been fully investigated.

Measurement and reporting of complement activation is inconsistently performed between trials, if done at all, as it is measured in response to suspected complement activation and not routinely. Thrombocytopenia relative to the patient's baseline appears to be an early sign,35 but this is not reported consistently, unless platelets drop <150,000/mL. In a cohort of 76 patients treated for SMA by onasemnogene abeparvocec-xioi, no TMA was reported although 78% of patients developed a degree of thrombocytopenia.36 Only one case series reports the measures of the different complement fractions and products.24

Hepatotoxicity

Elevation of liver enzymes has been consistently associated with administration of AAV gene replacement products, in some incidences this has been severe.10 In some cases, liver enzyme elevation appears to be associated with cytotoxic T cell responses against the AAV capsid.37 It has been successfully treated with prednisolone and it is not usually associated with progressive liver failure.22 It appears distinct from the cholestatic liver failure observed in the ASPIRO Trial (NCT03199469) for XMTM, which was outlined earlier as likely interplay with disease-specific specificity. Hepatic transaminitis classed as severe was reported in participants in trials and real-world use of onasemnogene abeparvocec-xioi and in DMD, following SGT00123 and rAAVrh74.MHCK7.*microdystrophin* (NCT03375164).15 These participants were successfully treated with glucocorticoids. Clinical trial and postmarketing safety data for onasemnogene abeparvocec-xioi have demonstrated 375 hepatic events, 14 of which were associated with clinical signs and symptoms and 4 of which progressed to acute liver failure.21 In the case of the four patients who went on to develop acute liver injury with the use of OA, hospital admission and intravenous methylprednisolone was required. All of these patients recovered, although in some cases they required a protracted course of corticosteroids, which in itself adds to the side effect and monitoring burden.38 Subsequently, two cases of fatal liver failure were reported10. This pattern of significant numbers of recipients developing steroid-responsive transaminitis with few becoming symptomatic and fewer progressing to acute liver failure is typical of other systemically administered AAV gene therapies. A recent observational study of children receiving onasemnogene abeparvocec-xioi in the postmarketing era demonstrated that liver enzyme elevation was significantly increased with weight and age at treatment, consistent with the previous results.21

Cardiac toxicity and transgene-specific immunotoxicity

Cardiac toxicity has recently been reported with three separate *microdystrophin* products. Five patients treated developed myopathy; three of these developed myocarditis.39 This is hypothesised to be related to patients with CRIM negativity (mutation exons 9–13; mutation exons 29 and 30), where a reaction is developed against the transgene due to lack of immune tolerance in patients whose immune systems are naive to this gene product.40

Patients with mutations leading to CRIM negativity were excluded from the trial, but another adolescent patient died of acute cardiomyopathy 7 days after the injection.41 Given the delay between the infusion and the cardiomyopathy, an immune reaction against the transgene is highly unlikely and alternative mechanisms such as viral myocarditis in the context of immunosuppression

have been proposed. Raised troponin I has been reported with use of onasemnogene abeparvocec-xioi, but this has not been associated with clinical signs except in one child who had a raised troponin I at the time of death—the level being taken after 2 hours of cardiopulmonary resuscitation. These troponin elevations have not been reported as serious treatment-related adverse events. One child in a postmarketing observational study[21] developed a haemodynamically significant pericardial effusion 9 months following gene replacement therapy; it is unclear whether this was associated with the treatment.

Other

One incidence of vomiting requiring hospital admission was reported as a severe adverse event with the PF-06939926 *microdystrophin* product (NCT03362502).[26] One patient receiving onasemnogene abeparvocec-xioi developed hydrocephalus post-treatment; this was reported as possibly related and was managed with a ventriculo-peritoneal shunt.[21] Two patients receiving rAAVrh74.MHCK7.micro-dystrophin (NCT03375164) experienced severe rhabdomyolysis.[20 42] Twelve of 14 patients treated for AADC deficiency developed dyskinesias, which were treated with risperidone.[43] Meningoradiculitis has been reported following intrathecal gene therapy in one instance.[44]

Systemically administered therapies and anticapsid immunity

Cut-off values of neutralising serum antibodies directed against the capsid are used as inclusion criteria in the majority of systemic clinical trials as presented in table 3A,B. Where it has been reported, systemically administered therapies in seronegative hosts induce a robust anticapsid humoral immune response, which persists for at least a year[45] and likely beyond, severely complicating opportunities for redosing. A possible preventative but still exploratory strategy exception is with the use of prophylactic non-glucocorticoid immune suppression which could provide opportunities for re-dosing, but this is still largely exploratory. An ongoing single-patient trial is using a combination of rituximab and sirolimus with the aim of enabling re-dosing.[46] The authors report that no immune response against the AAV capsid has been seen with this immunotherapy.

Interferon-γ ELISpot is used to measure anticapsid T cell responses which have been detected after systemic administration in most cases. Reporting of immune-related tissue damage is predominantly manifesting as transaminitis, and tapered courses of prednisolone are implemented to alleviate this.[15 16] Alternatively, doses can be titrated in response to anticapsid T cell activity.

Direct CNS delivered therapies

Direct CNS administration of AAV gene therapy products can induce a peripheral humoral immune response as presented in table 3B. Where T cell responses are seen, these are mostly transient. There are no documented incidences of complement activation. Prednisolone is used as prophylaxis in some instances (table 3B). There has been one instance of meningoradiculitis associated with peripheral T cell and B cell responses in a patient with amyotrophic lateral sclerosis after intrathecal gene therapy. There was also evidence of dorsal root ganglion inflammation, presenting as pain syndrome. This was not repeated in the second patient receiving this treatment, who had immunosuppression with sirolimus and rituximab during gene therapy and also did not demonstrate the same T cell and B cell response against the capsid.[44]

Discussion

AAV-mediated gene therapies have reached clinical trials for a wide range of neurological conditions, including both central nervous system and muscle tissue. There have been many positive efficacy reports, causing excitement in the rare disease field. However, targeting the CNS and muscle brings specific safety issues. The poor specificity of rAAV for muscle compared with the liver, coupled with the difficulty of directly accessing the CNS have led to doses as high as 3.5×10^{14} vg/kg (NCT031994691) being used, over 100 times higher than those for haemophilia.[47] This leads to a dramatically different safety profile. Consistent reporting and collaboration are urgently needed to mitigate the risk of these promising therapies.

While efficacy has been clearly reported with use of onasemnogene abeparvocec-xioi for SMA,[16 36 37 48–50] the picture is less certain for other products. A large proportion of results have not yet been reported in peer-reviewed journals (table 1). Randomised controlled studies are still missing, and only one completed study (for XMTM) was conducted with a concomitant control group.[19] Efficacy is therefore claimed predominantly in comparison with natural history cohorts,[16] or the patient's own pretreatment condition.[14] Additionally, most trials are reliant on surrogate measures of efficacy (eg, transgene expression) without knowledge on whether these will translate into a clinically significant benefit for patients. In disorders with dramatic, universal and rapid evolution, such as SMA type 1, this is a valid strategy. However, complexity arises in disorders with a slower, more variable and heterogenous course such as DMD, or when comparing multiple therapeutic options. It is important to consider the impact that premature claims of efficacy have in the rare disease community. Managing expectations of families is an increasingly important skill for clinicians as the constellation of treatments for rare disease expands.

Information on persistence of effect or long-term safety remains speculative. The only treatment with robust evidence of efficacy is onasemnogene abeparvovec, which is used for SMA. As yet, no long-term safety concerns have been noted either in the case of SMA, or with direct neurosurgical administration of products for Parkinson's and Canavan diseases which were carried out in the early 2000s.[51–53] To date, no washout effects or loss of efficacy have been observed for the treatment of SMA, with durability of 5 years post-treatment recently being reported.[54] However, when a muscle is targeted, such as in DMD, washout is anticipated as the number of muscle cells increases with growth, and muscle tissue undergoes physiological remodelling. This is particularly important given that options for re-dosing are limited, although use of sirolimus and rituximab prior to gene transfer is being trialled to mitigate the effects of immune reactions on second dosing.[46 55]

Randomised controlled trials are challenging in rare diseases. Nevertheless, it is only with robust evidence of efficacy that the risk-to-benefit ratio of novel therapies with potentially serious adverse events can be fully evaluated. Mitigation of adverse events with a better identification of patients at risk of severe reactions, immunosuppressive regimens, specific follow-up and more specific engineering of capsids will be paramount not only to improve safety, but also to allow the delivery with an optimal dose. It will also become important to benchmark the efficacy of AAV-mediated gene therapy against other gene-modifying treatments, such as antisense oligonucleotides. Only then can an informed decision about risk-to-benefit and longevity of outcome and cost be made.

Recently, several treatment-related adverse events have been reported, although they also remain largely unpublished. AAV-mediated gene therapy has classically been considered safe.[2] However, CNS and muscle-targeted AAV gene therapies have a drastically different risk profile to the liver-directed therapies, such as for haemophilia. This is most likely due to the large difference in dose. We have identified multiple serious adverse reactions, including nine deaths. Eight of these are almost certainly related to the study product.[7 8 10 11 30 32] One death was most likely related to neurosurgical intervention.[9]

The serious adverse events from systemically administered gene therapies outlined below appear to be more specific to situations where extremely large doses are required. High doses are required (online supplemental table 4) when the product must target an abundant tissue, such as muscle, or achieve adequate CNS penetration via peripheral administration. These specific serious adverse events have not been seen with trials of products for haemophilia,56 as mentioned above. This is also the case where the drug target is non-CNS, such as in Pompe disease where the liver is targeted to produce GAA to improve neurological function. Much lower doses are used (see table 1B) and the same pattern of serious adverse events is not seen—although an increase of liver enzymes is frequently observed. This pattern is also observed within a product, with incidences of severe adverse reactions occurring in the heaviest participants in the highest dose cohort who therefore received the greatest total dose of the product. This has been observed in the XMTM ASPIRO Trial7 real-world use of onasemnogene abeparvocec-xioi for SMA21 and in trials of *microdystrophin* products for DMD.57 Nevertheless, severe adverse reactions have also been observed in lower-dose cohorts30 and with younger children.21

As well as the consideration of dose, certain genetic susceptibilities may constitute an additional risk factor for serious treatment-related adverse events. An immune reaction against the transgene itself is a major concern in patients with biallelic null mutations which could not only put them at risk but also jeopardise transgene durability. These patients, who are termed CRIM-negative, do not produce any of the protein endogenously and are at risk of recognising the transgene as foreign and generating an immune response against it. They need special consideration to suppress an antitransgene immune attack and instead induce immune tolerance. This has been seen in all three trials of *microdystrophin* products for DMD where patients with certain null mutations (mutations exon 42 and 42, mutations exon 1–17) developed myositis and in some cases myocarditis as the presenting features of an immune-mediated attack on the transgene. Trials of these products now exclude patients with this genetic background, and this is also the tactic used in trials for MPSIIIB which also excludes participants with null mutations. Interestingly, production of antitransgene antibodies has not been observed in the subjects enrolled in haemophilia trials, despite the fact that some of them were carriers of null mutations in the *FIX* gene.58 59 Having said this, participants with a history of antibody formation to therapeutic products were excluded from the trial, meaning that this data may well underestimate the potential risk. The possibility of peripheral immune cell activation is not completely removed by direct CNS administration of the gene therapy product, and therefore immunosuppression is likely still required, especially if these patients are included.60 61

There are two emerging safety syndromes which appear to be conserved between AAV gene therapy products. These are liver toxicity and TMA, both being a consequence of immune reactions against the AAV capsid. The TMA picture seen in AAV9 capsids used for SMA and DMD is possibly linked to T cell and complement activation. Complement panels are an emerging addition to postgene therapy monitoring regimes. Multiple trials taper the course of prednisolone according to measures of T cell activity such as ELISpot. The utility of this remains unclear. Three trials have included the C5 inhibitor eculizumab, two on an as-needed basis and one prophylactically. Robust evidence of efficacy is still awaited. Anticapsid T cell responses have been documented to correlate with tissue damage in some instances. It remains unclear whether the liver failure in XMTM was immune-mediated, due to the lack of inflammatory liver parenchymal infiltrates and the pre-existing subclinical cholestasis in this condition.

We have identified four main ways in which these reactions have been targeted: exclusion of specific patients' genotypes to avoid immune reactions against the transgene, adaptation of immune suppression protocols, reduction of the number of immunogenic capsids and supportive measures when early biological signs appear. Reduction of immunogenic capsids includes improved product purification; specifically, moderating the full-to-empty capsid ratio,57 as well as engineering of novel

capsids which evoke a lesser immune response.62 The mainstay of prevention of these adverse reactions has been the introduction of immune suppression and screening of participants for pre-existing immunity. There is good evidence that several of the adverse drug reactions experienced are due to activation of an anticapsid immune response and resultant tissue damage. This was initially observed in haemophilia, where administration of an AAV2 gene therapy product gave rise to a humoral anticapsid immune response.59 AAV serotypes with less pre-existing immunity in the population (eg, AAV8, AVV9) were then selected for future development.47 It has since become standard practice to measure levels of pre-existing antibodies directed against the AAV capsid and most protocols have cut-off values above which the product is not given.

Difficulty arises in a lack of parity in measurement of immune and tissue parameters after administration of gene therapy. A consensus on monitoring, both in clinical trials and with real-world use, is urgently needed so that factors associated with adverse events can be more clearly identified. Indeed, real-world data from use of onasemnogene abeparvocec-xioi have highlighted early biological signs, including a decrease in platelets relative to the patient's baseline, which is often not reported in clinical trials as it does not dip below a laboratory threshold.24

Identification of early signs such as this, and increased parity in measurement should give a way to improved management and recognition of serious adverse reactions. Transparent and complete reporting, likely moving towards information sharing on a meta-database, on all biological findings during clinical trials is also needed to better understand early safety signals. Consistency in monitoring and reporting of early safety signals is urgently needed in order to fully understand and tackle serious adverse events.

A recent initiative has led four sponsors to share emerging serious adverse events related to autoimmunity against the transgene in a group of boys with DMD. They were carrying a range of deletions removing an epitope present in the AAV transgene. This initiative led to rapidly shared information regarding the at-risk genotypes, and to modification of the inclusion criteria of the ongoing clinical trials to reduce risk for participants. This initiative of collaboration between the different sponsors should be taken as a positive example in the field of AAV gene therapy.40

Conclusions

AAV-mediated gene therapies hold out much hope for otherwise untreatable and devastating neurological conditions. However, outside of SMA-1, there is a lack of robust evidence supporting use of these therapies. There is, however, clear evidence of mostly immunologically mediated toxicities that are not without significant risk, including deaths. Such toxicities will continue to emerge during larger, later-phase trials and in postmarketing experience, when larger numbers of patients will be dosed. A clear understanding of these risks and their mechanisms will be the basis for effective risk mitigation to ensure that the promise of gene therapies continues to be realised. For this to succeed, a transparent collaboration between all stakeholders is urgently needed.

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