Neuromuscular Disorders 33 (2023) 884–896

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Workshop report

261st ENMC International Workshop: Management of safety issues arising following AAV gene therapy. 17th-19th June 2022, Hoofddorp, The Netherlands

Laurent Servais[a,b,*], Rebecca Horton[a], Dimah Saade[c], Carsten Bonnemann[d], Francesco Muntoni[e,f], 261st ENMC workshop study group[1]

[a] MDUK Oxford Neuromuscular Centre & NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK
[b] Division of Child Neurology, Centre de Référence des Maladies Neuromusculaires, Department of Pediatrics, University Hospital Liège and University of Liège, Avenue de l'Hôpital 1 4000 Liege, Belgium
[c] Department of Neurology, University of Iowa Carver College of Medicine, Iowa City, IA, USA
[d] National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA
[e] UCL Great Ormond Street Institute of Child Health, The Dubowitz Neuromuscular Centre, London, UK
[f] National Institute for Health Research, Great Ormond Street Institute of Child Health Biomedical Research Centre, University College London, London, UK

ARTICLE INFO

Article history:
Received 15 August 2023
Revised 17 September 2023
Accepted 24 September 2023

Keywords:
Gene therapy
Duchenne
Spinal miuscular atrophy
X linked myotubular myopathy
Safety
Serious adverse event
Immunity

ABSTRACT

Adeno-associated virus (AAV) gene therapies are demonstrating much promise in the area of neuromuscular disorders. There are now therapies in clinical trials or real-world use for several disorders including spinal muscular atrophy and Duchenne muscular dystrophy. However, there have been several concerning reports of serious adverse events, including deaths. Reporting and monitoring of these is not consistent between trials. Therefore, a group of clinicians, investigators, industry and patient representatives met the weekend of 17th–19th June 2022 to discuss safety issues arising from the use of these therapies. The group shared information on safety events across a spectrum of AAV gene therapy products, both in clinical trials and commercial use. Patterns of serious adverse events were identified and the group discussed methods of identification and management of these as well as new ways of improving information sharing across industry in order to improve the safety of these promising treatments.

1. Introduction

A group of clinicians, investigators, industry and patient representatives met the weekend of 17-19th June 2022 to discuss safety issues arising from the use of adeno-associated virus (AAV) gene therapies in neuromuscular disorders. The meeting included 10 investigators involved in both pre-clinical and clinical science, a representative from the European medicines agency (EMA), Muscular Dystrophy Association (US), 3 parents and patient representatives and 9 industry representatives from Pfizer, Aavantibio, Sarepta, Solid, Novartis, Roche, Audentes/ Astellas and Genethon.

AAV gene transfer therapies show promise across a wide spectrum of rare genetic disorders, many of which are neuromuscular. They thus provide a potential treatment option for a large number of diseases in which there is still a significant unmet therapeutic need. However, in clinical trials and in clinical use several serious adverse reactions, including death, have emerged following these therapies [1]. Notably, there are a constellation of adverse reactions which appear related to the use of AAV vectors as a vehicle, as opposed to being related to production of a specific transgene or in the context of a specific disease. However there also exist adverse reactions triggered by the production of the target transgene. Finally, adverse events have emerged in specific disease contexts which are related to a previously unrecognised vulnerability of that group. A recurrent theme amongst these serious adverse events is the lack of predictability from the preclinical toxicology studies performed in animal models and therefore complexities arise in modelling methods of prevention, follow up and management.

* Corresponding author at: John Radcliffe Hospital Level 2, Childrens Hospital Headley Way Headington, Oxford, OX3 9DU UK.
E-mail address: laurent.servais@paediatrics.ox.ac.uk (L. Servais).
[1] 261st ENMC workshop study group authors list is available at the end of this article.

https://doi.org/10.1016/j.nmd.2023.09.008
0960-8966

The aims of the workshop were to review the range of adverse events in AAV mediated gene therapies for neuromuscular diseases with focus on hepatotoxicity, thrombotic microangiopathy (TMA), thrombocytopenia, cardiac toxicity (direct toxicity vs anti-transgene immunity) and anti-transgene immune reactions. The workshop also intended to facilitate discussion on potential mechanisms of these adverse events using both pre-clinical and clinical data and initiate a consensus process for screening, monitoring, prevention and intervention when these adverse events arise. This workshop was intended as an opportunity for collaboration and data sharing from all relevant stakeholders to help de-risk AAV mediated gene therapy and improve the recognition, prevention and treatment of these adverse events, resulting in proposal of new models for collaboration and data sharing.

1.1. Pre-clinical models and translatability to humans

Many of the adverse reactions observed in human gene therapies are likely to be immune mediated. Immune responses to AAV gene therapies in humans can be directed against the viral capsid or the transgene product and are a product of innate, humoral and cell mediated immunity. Prior exposure to wild type AAV has an impact on adaptive responses in a capsid specific manner, while the packaged DNA content can influence innate responses. Potential antitransgene responses are governed in large part by the recipient genotype regarding the specific defective gene for which the therapy was initiated, while other individual recipient genetic and environmental factors likely play a role in the susceptibility to rAAV triggered events. In general, immune reactions can govern both adverse events as well as durability of gene transfer. These immune responses are not or only incompletely recapitulated in traditional preclinical animal models including mice, large animals, or non-human primates. Because of this, the emergence of some of the serious adverse events was not predicted in pre-clinical studies including regulatory toxicology, and therefore extra caution is warranted when translating safety data into the clinical setting. Careful consideration is needed on how to monitor and modulate these different immune responses.

Cellular immunity against the AAV capsid was first recognised with use of AAV2-factor IX at a dose of 2e12vg/kg for haemophilia B [2]. An asymptomatic transient transaminitis was observed 4 weeks after vector infusion, which correlated with AAV2 capsid responsive IFN-y secreting T cells on ELISpot testing in peripheral blood leading to subsequent loss of transgene expression in the target organ liver. Presumably, loss of transgene expression was due to cytotoxic T-cell mediated destruction of transduced hepatocytes and this occurred predominantly in individuals with pre-existing detectable AAV2 neutralising antibodies. This had not been anticipated based on Non-Human Primate (NHP) [3] studies where the response was not typically recapitulated, however in one instance it was reported [4].

Accurate characterisation of the different aspects of the immune response against the AAV capsid is often difficult in clinical trials as the required measurements are not always systematically obtained. Some of the measurements also have limitations. Whilst detection of circulating IFN- γ secreting T cells specific to AAV capsid epitopes (via interferon-γ enzyme-linked immunosorbent spot (ELISpot) assay) is commonly used as an assay for T cell mediated immune response to the viral capsid, it does not necessarily indicate activation of a cytotoxic T cell response that would also eliminate transduced cells. This was shown in clinical trials for haemophilia where the participant with the highest IFN-y response had no evidence of hepatocellular injury [5]; similarly in an alpha-1 antitrypsin (AAT) deficiency trial where participants retained sustained expression of M-ATT despite IFN- γ positive ELISpot assays suggesting an effector T cell response developing within 14 days post vector delivery [6]. Findings from these two clinical trials highlight the multifunctional aspect of T cell response to AAV; indeed, identification of capsid responsive T cells does not predict a cytotoxic response. A broader repertoire of tests for antigen specific classes of T cells, including T-regs for tolerance induction, is also needed.

Phenotyping of peripheral capsid IFN- γ secreting T cells in healthy individuals reveals a wide panel of profiles that may be AAV serotype dependent and includes terminally differentiated effector memory T cells [7] (TEMRA cells) a particular memory T cell subpopulation, not present in mice, that has been implicated in solid organ rejection and graft vs host disease and could therefore constitute a barrier to viral vector immune tolerance [8].

To address the above challenges, Oumeya Adjali presented ongoing studies which attempt to model human T cell mediated immune responses. These include a humanized mouse model injected with AAV8, and ongoing studies of peripheral and in situ T cell immune responses in a rat model injected with AAV9, and NHP immune data following IM AAV1 administration.

HLA-A2 transgenic immunodeficient mice (NSG-HLA-A2/HHD mutant mice), humanized with PBMCs from anti-AAV8 ELISpot positive HLA-A2 donors and injected with IL-15 to promote survival and activation of memory T cells (TEMRA cells), received rAAV8 (IV, 1e14 vg/kg) to assess immune response (ELISpot, immune infiltrates and flow cytometry) and liver toxicity. Preliminary findings in the group receiving PBMCs and showing IFN-γ response to AAV8 include weight loss, increased engraftment of hCD45 + cells in the spleen, and increased mononuclear cell infiltrate in the liver. Ongoing studies show continued transgene expression at 1 month and a predominance of CD8 T cell infiltrates in the liver. Investigations are now focused on determining whether these T cell infiltrates are specific and reactive to the capsid and whether this can model human hepatotoxicity (Gernoux et al., unpublished data).

Rats are not commonly used in pre-clinical studies for gene therapy but may be a relevant animal model to assess AAV capsid immunogenicity as it is a relevant model for allogenic immune organ rejection and is a better model than mice for complement activation. Peripheral and in situ adaptive immune responses to AAV and transgene product were assessed after injection of wild type seronegative, Sprague Dawley rats with AAV9-GFP vector (Masri et al., unpublished data). The AAV9 serotype was studied in more detail at the time of emergent safety concerns with AAV9 in several human clinical trials and an IV dose of 1e14vg/kg was selected to approximate doses used in human clinical trials. Analysis of peripheral (IFN-γ ELISpot assay) and in situ adaptive immune response (HPS staining and liver infiltrate cell characterization) showed no T cell response against GFP (transgene) but identified a dose dependent anti AAV9 T cell capsid response with a threshold around 1e14 vg/kg. Liver infiltrates were observed with identification of CD3 T cells which are currently undergoing further characterization. Loss of GFP transgene expression was observed in 3 out of 8 animals (analysis up to 180 days post injection), but there were no specific biomarkers or clinical features of these animals which made them distinguishable from those which did not lose transgene expression. Future studies will utilize the AAV8 vector to compare anti-capsid T cell responses between AAV9 and AAV8 serotypes.

One advantage of the NHP animal model is the ability to compare and correlate peripheral vs in situ immune responses using liver and muscle biopsies. A recent study of IM injection of AAV1-rtTA/Epo (Journou et al., under revision) showed persistent inducible transgene expression despite anti-capsid and anti-rtTA T cell responses and muscle cell infiltrates at the site of AAV delivery. This indicates that detection of peripheral IFN- γ T cell response

and cellular infiltrates are not necessarily reliable markers of a cytotoxic T cell response. Characterization of the muscle infiltrates identified CD8 T cells (likely silenced or inefficient since they did not eliminate transduced cells) as well as CD4/CD3 foxp3 cells consistent with a regulatory T cell (Treg) phenotype, which is known to mediate immune tolerance. This finding is consistent with the previously reported persistent transgene expression after IM rAAV1 gene transfer in NHP that is mediated by Tregs and exhausted T cells [9]. This finding also highlights the fine, and not yet fully understood, balance between immunorejection and immune tolerance which may be influenced by the route of administration, dose, serotype, target tissue and state of the target tissue (ie diseased vs healthy tissue).

Complement responses have not been sufficiently characterised in animal models. Indications of the thrombocytopenia and thrombotic microangiopathy seen in humans have only been reported in NHP in one instance (Hordeux et al, unpublished data) and this was in the context of associated liver toxicity.

Sam Hopkins (Sr VP Therapeutics, Sector Lead-Liver, AskBio), presented pre-clinical findings from the Pompe disease mouse model 6^{neo} and the clinical translation to the human phase 1 clinical trial of systemic liver directed AAV2/8-LSPhGAA gene therapy (NCT03533673) in adults with late onset Pompe disease on stable Enzyme Replacement Therapy (ERT). An undisclosed, but relatively low dose (below the published No Observed Adverse Effect Level of 1.6e13 vg/kg) was selected with an aim of achieving ~3–5 % liver transduction with the aim to induce immune tolerance and limit total study agent dose in order to reduce adverse events, while still allowing for discontinuation of ERT at 24 weeks post gene transfer. Multiple mechanisms of toxicity need to be considered, including the high total vg doses as well as the potential for toxicity from transgene overexpression. Concomitant prednisone immune suppression (60 mg/day x4 weeks followed by slow taper out to ~15 weeks) was used in the human clinical trial. As with other preclinical gene therapy studies no transaminitis or T cell response was observed in response to gene transfer in the mouse model. An additional limitation of the Pompe mouse model is the extremely high (i.e 100 %) liver transduction and supra-physiological expression of GAA with AAV based gene transfer,at doses as low as 2e11 vg/kg, which hindered dose translation to the human clinical trial where a much lower transduction rate is desired.

Three individuals had been dosed at the time of the workshop and this was associated with mild elevation in ALT/AST/GGT in one individual. AAV ELISpot assays in that study participant showed slow increase with ELISpot activity roughly coinciding with the wean from prednisone and with a slow rise in ALT which continued up to a year post gene transfer. This was paralleled by a transient decrease in serum GAA activity, which later seemed to stabilize (after week 38). All three participants had increased GAA activity in muscle and serum at week 52 compared to baseline demonstrating continued bioactivity after withdrawal of ERT.

Ana Buj-Bello (France) discussed immune response and cardiac toxicity following systemic AAV8 mediated gene transfer of a human *MTM1* transgene in preclinical mouse and dog models [10] of X-Linked myotubular myopathy (XLMTM) [11]. Immune responses were not assessed in the preclinical knock out (KO) mouse model but histology showed dose dependent asymptomatic focal inflammatory infiltrates (CD11b macrophages and CD3 positive T cells) and areas of fibrosis in the heart. This was observed in the *mtm1* KO context and not when wild type (wt) mice were treated at the same doses. De-targeting expression of myotubularin in the hearts of KO animals using a miRNA target sequence in an AAV construct eliminated lesions (unpublished and abstracts ASGCT, ESGCT congresses 2013).

This suggests that the pathogenesis of this could be related to myotubularin overexpression in the hearts of mtm1 KO mice. Transcriptomic analysis showed no significant difference between wt and mtm1 KO mice. The exact mechanism remains under investigation and could be due to immune response against the transgene, ER stress due to unfolded protein response or a species-specific effect. Mice might be more susceptible to this as a similar complication was not observed in the treated *mtm1* dog model. Myocarditis was also observed in 2 boys in the clinical setting and this is discussed in more detail in the anti-transgene immunity section. In the XLMTM dog model (carrying a missense mutation and expected to have some residual myotubularin expression) treated with AAV8 mediated gene therapy at 10 weeks of life, there was no detectable antibody or T cell response to myotubularin, no cardiac infiltrates and normal cardiac function as measured by echocardiography[10,12]. In addition, no liver toxicity was observed in treated dogs, another example of poor translation of animal models to humans with respect to adverse events, in view of the severe events leading to multiple deaths in the clinical trial. Treated XLMTM dogs have now survived several years post gene transfer with no evidence of clinical diminution of efficacy.

Key points:

Animal models do not necessarily recapitulate human disease and immune responses and can therefore not fully predict adverse events.

Animal models which more accurately predict adverse events in humans would be desirable to fully investigate the risks, along with methods for mitigation of adverse events

Detection of circulating IFN-γ capsid T cells is not always a problem (may indicate tolerance or CD8 exhaustion). Peripheral responses does not necessarily mean immune toxicity the target tissues

There is a need for multiparametric monitoring of immune responses with more than one biomarker to fully understand the impact of specific responses.

Immunogenicity assessment is a case-by-case issue that dependents on an interplay between several factors: AAV serotype, route, dose, organ state, manufacturing methods

1.2. Regulatory and ethical challenges

Patient and family acceptance of risk in the context of AAV gene therapy was also discussed. Elisabeth Vroom (The Netherlands) reported the results from several surveys conducted in parents of children with DMD and adult DMD patients. These surveys converge to demonstrate a significant level of risk acceptance, with participants deeming up to a 1:10 risk of death acceptable. It was discussed that the surveys suggest participants are more accepting of risk than their treating clinicians. Frank Van Leperen (The Netherlands) shared his personal opinion as an adult with DMD. He explained the differential acceptance of risk dependant on current function, for example that he was more accepting of risk whilst there was still the option of preserving his upper limb function. It was discussed that it can be difficult to glean appreciation of risk from surveys as responders may not fully represent the complexity and different functional stages of individuals affected by DMD.

Marta Kollb-Sielecka from the European Medicines Agency highlighted differences between traditional medicines and Advanced Therapeutic Medicinal Products (ATMPs.) As ATMPs are usually administered in highly specialised centres, the terms of licence include detailed instructions for use and training materials. The administration process also plays a

big role in defining safety profile. Patients' preconditioning, immunosuppression, surgery and anaesthesia bring additional risks which have to be characterised and managed appropriately. ATMP legislation mandates establishing extensive post-approval follow-up programme. Durability of response and long-term safety can be uncertain at the time of initial authorisation. Therefore, early planning of registries or other post-approval studies to bridge the gap on long term efficacy and safety is essential. EMA has set up several initiatives to support the sponsors with this task. EMA encourages the sponsors to use EMA Early Access Programmes such as PRIME which can provide valuable advice during the development stage. In particular, the PRIME pathway can be used for medicines targeting unmet medical need and can be accessed on the basis of promising non-clinical data.

A key regulatory, ethical, and scientific challenge raised during discussions was the need for autopsy data in patients who die after gene therapy product infusion. Several instances were noted where it was not possible to perform an autopsy, either due to lack of pathologist availability, lack of a standard operating procedure, or lack of prior consideration and preparation by families and clinicians. Consent for autopsy may be improved by anticipatory counselling in a similar manner to organ donation, prior to entry into clinical trials. Patients and families involved in natural history studies could also be offered the opportunity to donate their bodies at the time of death as indeed any member of the public can do. Some clinical trials have started to mention that an autopsy will be requested in the unlikely event of death during the trial as part of the consenting process. This can help to prepare clinicians and families in advance of a patient death. A standard operating procedure for undertaking autopsy in participants of gene therapy trials would be useful in the out of hours setting, allowing pathologists who are not experienced in the use of gene therapies are able to take and store the appropriate samples. A network of pathologists specialised in the assessment and storage of samples from autopsy of patients post gene therapy would be valuable both for clinical trials and routine practice.

1.3. Toxicity in humans

1.3.1. AAV and hepatic toxicity

Liver toxicity has been recognised with AAV gene therapy products since the early studies in gene replacement for haemophilia [5]. Elevations in liver enzymes typically occurred within days of dosing and correlated with positive ELISpot Assays against the AAV9 capsid and destruction of transduced hepatocytes. The main initial concerns with this observation were with reduced durability in liver targeted therapies. Abnormalities in liver function tests and even liver failure have been observed in patients receiving different AAV transgenes for various neuromuscular conditions raising a more general concern for liver toxicity, with likely different underlying pathological mechanisms. In the specific context of XLMTM, fatal cholestatic liver failure following gene therapy has unveiled a previously underrecognized cholestatic liver disease in children affected by this condition.

1.3.1.1. Clinical course of the AAV toxicity. There have been three seemingly distinct patterns of liver toxicity apparently conserved across different AAV gene therapy programs, hence not restricted to a specific disease, transgene or vector serotype.

The first is a transient rise in liver enzymes which typically occurs around 2-8 weeks and is responsive to steroids. This has been seen in spinal muscular atrophy (SMA) with Onasemnogene abeparvovec [13,14] (SMN, Zolgensma, Novartis, AAV9, 1.1×10e14 vg/kg), and in DMD with rAAVrh74.mhck7.micro-dystrophin (Micro-dystrophin, Sarepta, AAVrh74, 1.33 × 10e14 vg/kg)[15], PF-06939926 (Minidystrophin, Pfizer, AAV9, 2 × 10e14 vg/kg) and SGT-001 (microdystrophin, SOLID, 2 × 10e14 vg/kg, AAV9). It was also noted that Sarepta has ongoing trials for seven AAVrh74 gene therapy products across a spectrum of disorders and that liver enzyme elevations have been noted in all of them. All of these products are administered with concomitant prednisone/prednisolone at 1 and 2 mg/kg/day for at least 4–8 weeks afollowed by a slow wean (boys with DMD receive chronic corticosteroids and following the period of higher doses revert to their baseline steroid dose).

Teji Singh (Sarepta) and Kathryn Wagner (Roche) reported the preliminary findings of the clinical trials of SRP9001, an AAVRh74 delivered microdystrophin construct. About 100 DMD boys had received the product at the time of the conference and all boys received 1 mg/kg prednisone in addition to their baseline background daily steroids pre-infusion and continued daily steroids post infusion. In the initial part of the study, steroids were tapered already at 4 weeks and this was followed by a transient rise in GGT in several patients. GGT is a necessary biomarker in addition to AST and ALT as these are already significant elevated in patients with DMD. Following a protocol change to taper steroids at 8 weeks post-infusion, this particular adverse event was significantly reduced. In the cohorts of patients in the Sarepta study, one patient has had a rise in liver enzymes which persisted at 4 months. This child also had a rise in bilirubin and a biopsy demonstrated liver inflammation without necrosis.

Dan Levy (Pfizer) reported on 40 boys having received PF-06939926, an investigational AAV9 miniaturised dystrophin product. GGT and GLDH were observed to rise modestly and transiently between days 14 and 21.

In another DMD trial, sponsored by Solid, 9 DMD boys have received SGT-001 and one patient had a steroid responsive immune hepatitis at week 4, and some others with mild increases in AST/ALT in the first two weeks of dosing.

Daniel Grant (Novartis Gene Therapies) reported on Onasemnogene abeparvovec commercial use. Approximately 2,300 patients have received onasemnogene abeparvovec either in clinical trials or through commercial use. The discussion identified two seemingly distinct patterns of transaminitis. The first usually occurs within two weeks of dosing or at the time corticosteroids (used for prophylaxis) are tapered. Although liver failure has been a significant adverse event with postmarketing use, it is important to note that what is more common is asymptomatic elevation of transaminases which resolves with use of corticosteroids. Synthetic liver failure appears to be a progression of this associated with steroid taper and can occur even after protracted (more than 4 week) courses of prophylactic corticosteroids and even where there has been no transaminitis in the initial period during steroid treatment. This has in some cases progressed to acute liver failure and there have been two fatalities reported following therapy in the commercial setting [16,17]. This underlines the need for compliance to the steroid regimen, caution during steroid tapering, consistent monitoring and vigilance with initial signs of liver damage. Workshop attendees agreed that transaminases should be monitored weekly with the patient having regular visits to the hospital for at least the duration of prednisolone prophylaxis. Some patients may need corticosteroid prophylaxis for as long as six months after gene therapy infusion.

A second, more persistent transaminitis less responsive to steroids has been observed with Onasemnogene abeparvovec (SMN, Novartis, AAV9, 1.1 × 10e14 vg/kg) and rAAVrh74.mhck7.micro-dystrophin (Microdystrophin, Sarepta, AAVrh74 2 × 10e14 vg/kg).

Daniel Grant (Novartis) explained that this later rise in transaminases has also occurred in several patients receiving onasemnogene abeparvovec and usually occurs after 6 to 8 weeks of treatment. In most cases, this has been prior to the withdrawal

of corticosteroid prophylaxis and increasing the dose did not, in many cases, have a clear effect on the transaminitis. This has been consistent across sites as reported by some of the clinical investigators at the meeting, i.e. Laurent Servais (Belgium), Francesco Muntoni (UK) and Barry Byrne (University of Florida). A catalogue of steroid sparing immunosuppressants has been used to treat the transaminitis, but not necessarily has led to resolution of this adverse event. The significance of this later transaminitis is unknown and while it has not been associated so far with liver failure, it does nevertheless bring the concern that long term cytolysis might eventually lead to clinically significant liver pathology. Patient related factors increasing risk of prolonged transaminitis appear to be older and heavier patients. For example, Diana Bharucha-Gobel (USA) reported three patients treated with onasemnogene abeparvovec with this late, non-steroid responsive transaminitis, who were notable amongst the rest of the cohort treated at her hospital for their age, close to the limit of exclusion. Indeed, two of these three patients were 23 months old, which was the upper age limit of the population treated. The third patient was six months old, but exceptional as he had a baseline anti AAV9 antibody titre of 1:25 which was the upper limit of treatment eligibility. In these patients the transaminitis persisted at least six months after the AAV vector infusion.

Barry Byrne also reported that the initial, transient peak in transaminases was observed in NHPs studies for onasemnogene abeparvovec, but the later peak was not. Corticosteroids were not used as prophylaxis or treatment in NHPs, but B cells were modulated with rituximab. It was suggested that this later transaminitis could therefore be an antibody mediated response.

1.3.1.2. Mechanisms of liver injury. The overarching mechanism for the transaminitis occurring after gene therapy is likely to be immune mediated damage to the hepatocytes. There are a number of different factors which contribute to the liver injury. Transaminitis occurring within days of infusion could be attributed to direct toxicity of the AAV capsid as it is delivered to the liver. This occurs in the first few days after infusion. Possible causes of direct capsid toxicity involve Endoplasmic Reticular (ER) stress due to the high protein load or activation of the innate immune system by non-vector genome nucleic acids which, owing to the production processes, are transported along with the AAV capsid. This non-human DNA is likely to be highly immunogenic, aggravating the innate immune system. There is not yet evidence whether product purification reduces immunotoxicity, however recent unpublished data demonstrate that administration of empty AAV capsids to healthy individuals leads to an immune response and subsequent hepatic transaminitis [18]. This suggests that removal of empty capsids, but also of contaminating DNA as part of product purification could reduce the recipient's immune response and therefore hepatic transaminitis. Whole genome sequencing of the capsids has recently been used to characterise the contaminating DNA.

The observation that higher absolute doses of AAV gene therapy products (ie in heavier patients) are associated with increased probability of liver injury supports the hypothesis that total capsid load influences risk.

The best-established mechanism for early, steroid responsive transaminitis is a cytotoxic T cell response against the AAV capsid. Direct toxicity against the transgene is also possible, but theoretically this would depend on the promotor, leading to increased expression in the liver in case ubiquitous promotors are used. There does not appear to be more liver inflammation in products with expression in the liver (onasemnogene abeparvovec has a ubiquitous promotor) than those without (XLMTM and various DMD AAV GT carrying muscle specific promotors). This T cell mediated response was first measured in the haemophilia gene therapy trials [19], where a clear T cell mediated response was measured correlating with the rise in liver enzymes and destruction of transduced hepatocytes.

Humoral immunity is also implicated in AAV gene therapy associated liver toxicity. Participants with pre-existing anti-AAV2 antibodies were more likely to experience transaminitis and lack of transgene expression with the use of an AAV2 gene therapy product for haemophilia, and due to widespread seropositivity in the general population for anti-AAV2 antibodies this capsid is now not used for products requiring systemic administration. The majority of gene therapy products exclude patients with measurable antibodies against the viral capsid used for the administration. However, a lack of measurable antibodies does not entirely rule out previous exposure and it is possible that the humoral immune system might still be implicated in the absence of seropositivity. It was discussed that B cell modulation may be useful to prevent an antibody response and associated hepatoxicity and rituximab has been used in some trials.

Specific conditions or genetic predisposition may increase the risk of gene therapy related liver damage. This could be due to liver manifestations of the index disease, an unrelated co-existing liver disease or an additional concomitant insult such as an infection. Previous exposure to AAV capsids may also modulate the individual response and this was suggested as a reason for the poor translatability in adverse events between animal models and clinical trials. The role of concomitant infections was discussed in relation to two cases and is an important consideration as use of concomitant immune suppression increases the likelihood. A child receiving fordadistrogene movaparvovec (Pfizer, AAV9) with 14 days of prednisolone (2 mg/kg) prophylaxis for DMD had a protracted rise in GGT and GLDH which occurred at 30 days and persisted for 90 days post dosing. He was found to have EBV reactivation which was thought to contribute to the liver damage. Another child with DMD who received rAAVrh74.mhck7.micro-dystrophin (SRP9001, AAVrh74) also had a protracted and more severe rise in liver enzymes and was found to have parvovirus hepatitis. There may also be underlying genetic susceptibilities to immune reactions against AAV gene therapy products and this was cited as an area requiring further investigation.

Recommendations for liver monitoring

Increased monitoring of liver enzymes after gene therapy administration. minimum of weekly until corticosteroid prophylaxis is completed.

Availability of common protocols between trial sponsors with information on methods and timing of screening

Wider screening for pre-existing liver disease and viral hepatitis

Next generation sequencing of contaminating genetic material and precise evaluation of the empty/full capsid ratio

In depth natural history studies of rare diseases targeted for gene therapy development in order to assess for underlying liver pathology

Clearer standards for manufacturing and quality control of AAVs

Collaboration between manufacturers to standardise labelling of gene therapy products. For example, rAAVrh74.mhck7.micro-dystrophin (Sarepta, AAVrh74, 1.33x10e14 vg/kg) has 45% empty capsids in its product, meaning that the dose of capsid here is significantly higher than the stated dose. Onasemnogene abeparvovec (Novartis, AAV9, 1.1x10e14 vg/kg), however has 15% empty capsids. It is recommended that consistency in measurement is achieved, possibly by labelling with the protein content of products so that they can be more clearly compared.

1.3.2. Adverse events and pre-existing liver conditions

The liver toxicity seen in boys with XLMTM treated with AT132 appears distinct from the transaminitis seen with other AAV products. It appears that a previously under-recognised disease-specific predisposition to cholestatic liver disease was exacerbated with exposure to an AAV8 viral vector. This highlights the need for careful consideration being given to the full manifestations of the disease to be treated, informed by natural history studies and a deep understanding of often complex phenotypes, especially in the context of rare disorders.

1.3.2.3. Clinical course. Wes Miller (Astellas Gene Therapies), reported the findings of the ASPIRO study which evaluated safety and efficacy of an AAV8 vector delivering a full length human MTM1 gene under the control of the muscle specific desmin promotor (AT132) to treat XLMTM. In ASPIRO to date, 24 boys with ventilator-dependent disease have received AT132. Seven have received 1.3e14 vg/kg, and 17 have received 3.5e14 vg/kg. Four boys, one who received 1.3e14 vg/kg and three who received 3.5e14 vg/kg, have died following treatment as a result of complications related to cholestatic liver failure. The final causes of death in three cases was sepsis, and upper GI bleed in one case [20].

No liver safety signals had been observed in pre-clinical murine or canine models [21] or in natural history [22]. The patients were between 2 and a half and 6 years of age at dosing and had baseline levels of myotubularin protein of between 1.2 and 10.8 %. Importantly, despite a severe phenotype these baseline levels of protein antigen indicate that the patients were cross-reactive immune material (CRIM) positive and suggest that they were not immunonaiive for epitopes on myotubularin.

All 4 boys developed cholestatic liver failure, with bilirubin rises prior to rises in transaminases in most cases, sometimes by months. Histopathology demonstrated intrahepatic cholestasis within hepatocytes and canicular cells, with hepatocellular ballooning demonstrating hepatocellular stress. A ductal reaction was present around the bile draining ducts and the patients progressed from fibrosis to frank cirrhosis and liver failure, with three dying of sepsis and one following a variceal bleed. Death occurred between 20 and 40 weeks after dosing. Multiple immunomodulation regimes were trialled, none of which resolved the cholestatic liver failure. In particular corticosteroids did not appear to have any effect on the disease course, with the patient who had the fastest taper from methylprednisolone having the slowest progression to liver failure. Tapering the steroids had to be considered as high and prolonged corticosteroid dosing may exacerbate sepsis in these participants.

The mechanism of the liver damage in XLMTM is likely to be different from immune mediated AAV associated transaminitis. Retrospectively, natural study data shows that many boys with XLMTM have a pre-existing degree of cholestasis and it is likely that this is then exacerbated by the viral load to the liver with the administration of AT132. While these children fulfilled all inclusion criteria, some had laboratory or historical evidence of sub-clinical cholestasis pre-dosing. No particular immune signals were noted, and immune modulation did not alter the course of disease progression as mentioned above. In the final patient, complement (C3 and C4) levels were measured and were within normal limits at week 1 day 7, 14 and week 5. Week 5 soluble C5b9 normal. Cytokine panel day 7 and 14 no significant elevations above baseline.

The boys who had liver biopsies or autopsy material had very high levels of vector copies by diploid genome observed. 80–400 vector copies were seen by diploid genome at autopsy or at living biopsy and this is likely to be an underestimate as there will likely be dilution due to the chronic regenerative cycle of liver disease following the initial insult. For reference, NHPs receiving much higher doses (8e14 vg/kg) had relatively lower amounts of vector copies per genome in the liver of approximately 200 at earlier sampling (week 12) and without the dilution effect from severe liver inflammation. It is possible that there is increased liver targeting in humans compared to the preclinical model studied before.

Further investigations in the livers of these boys revealed an immunohistochemical deficiency of bile salt export pump protein (BSEP) at the canicular membrane. The pathology studies in these boys' livers appeared similar to the pattern seen in children with a congenital absence of BSEP in whom there is a primary deficiency of this protein (a form of Progressive Familial Intrahepatic Cholestasis – PFIC). Bile salt levels in serum have subsequently been measured retrospectively, revealing elevations in serum bile acids in these boys prior to dosing. This was present without the clinical appearance of jaundice and might therefore provide an important indicator of intrahepatic bile salt transportation pathology and an increased vulnerability to AT132 mediated liver failure. It was discussed whether the changes in BSEP expression were a consequence of myotubularin deficiency, however transcription and protein data in liver tissue are not conclusive at this time. It was important to note that data from the livers at autopsy of 6 untreated boys with XLMTM in a natural history study is available. One of these had peliosis, and one showed intrahepatic cholestasis at autopsy. None of these boys' livers had abnormal expression of BSEP. It is possible that the combination of myotubularin deficiency, pre-existing sub-clinical cholestasis and liver directed injury of AAV gene therapy all coalesce to induce the liver failure. It was hypothesised that therapeutic myotubularin expression in the liver, perhaps with the use of a different promotor, may mitigate risk of this adverse event, as the desmin promoter used in AT132 does not allow for liver expression.

Following the initial observation, it is becoming more clear that the propensity for cholestatic liver disease is an intrinsic feature of XLMTM [23–25]. Of the 20 surviving patients in the ASPIRO study, almost all have some degree of liver related abnormality although there have been no other instances of progressive disease with synthetic failure. One has had a biopsy due to a cholestatic episode, this patient had significant cholestasis prior to dosing. Some cases of pure transaminitis which resolved without an increase in steroids have also been seen- this is possibly due to the mechanisms of general liver inflammation with AAV gene therapies discussed earlier.

Astellas is exploring a statistical model to predict risk of liver injury with AT132, as not all boys with pre-existing liver problems went on to develop liver failure after receiving the AAV gene therapy product. Initially, the liver failure was thought to be related to absolute dose, as the oldest and heaviest boys in the highest dose cohort were the first three who died. However, the 4th patient who died most recently was in the lower dose cohort and received one of the lowest capsid burdens overall, indicating that individual susceptibility plays an important role in addition to total dose. 104 independent patient-related predictors for rise in post dose bilirubin were found. This was done on a continuous scale. No clear single predictors related to the product (dose or % full capsids) were identified. The models with the best fit were increased age, pre-dose bilirubin, pre dose AST and pre dose ALT.

1.4. 22 AAV induced liver injury: Discussion

The group discussed that careful natural history studies and deep phenotyping are required prior to intervention with AAV

gene replacement therapies especially in ultrarare conditions. Careful attention must be paid to liver disease- while there were some references to possible liver disease in patients with XLMTM, this was focused on hepatic peliosis, and the inherent risk of haemorrhage after liver biopsy; but the frequency and the implications of the cholestatic condition had not been previously appreciated as this typically did not lead to clinically significant morbidity or mortality. It is now clear that it is this background susceptibility which in some, but not all cases, leads to fatal complications after AAV gene therapy. It remains difficult to predict which patients are at increased risk and this will be important for future use of AT132 which is currently on FDA clinical hold, or any other AAV product for this condition. It was also discussed that in time there is a need to mitigate this risk and treat the liver susceptibility- as opposed to excluding boys deemed to be at an increased risk as to do this would deny a large group of the XLMTM group an effective treatment. A pre-clinical model of XLMTM liver pathology is needed to evaluate the mechanism and potential treatments more easily. The fact that it is not seen in pre-clinical (mouse and dog) models of XLMTM suggests species specific differences. Promotor choice may be needed to target the liver disease- it is possible that even transient liver expression may modify the disease course enough to halt the progression of liver failure.

1.4.1. AAV mediated haematologic adverse events

1.4.1.4. TMA. Thrombotic Microangiopathy (TMA) has become a recognised adverse event observed across different gene therapies for neuromuscular conditions. It has been observed with use of onasemnogene abeparvovec for SMA [26] and in two miniaturised dystrophin products used for Duchenne Muscular Dystrophy. Dan Levy (Pfizer) reported the cases which occurred within the Pfizer (AAV9) minidystrophin trial, Dan Grant (Novartis) spoke about post marketing data for onasemnogene abeparvovec (AAV9) and Roxanna Dreghici (Solid bio) reported on the SOLID (AAV9) trial. Teji Singh (Sarepta) reported that no decreases in C3,C4 or Ch50 were observed in the phase one Sarepta (AAVrh74), and Serge Braun that no complement activation was observed in the single patient injected in the Genethon (AAV8) trial. Sam Hopkins reported that no complement activation (C2,7,14), thrombocytopenia or renal signs were observed in the ASKbio (AAV8) trial for late onset Pompe disease. TMA was not observed in the (AAV8) trial for XLMTM with Aspiro.

TMA usually becomes apparent between days 5 and 11 following gene transfer, with peak incidence at days 6 to 9. It is initially characterised by anaemia and thrombocytopenia. The thrombocytopenia occurs early and is thought it be either due to direct AAV mediated platelet damage, damage to the endothelial lining resulting in platelet clumping, or both. Clinical manifestations include: nausea and vomiting, poor feeding and irritability and decreased urine output. The anaemia is often accompanied by raised LDH, decreased haptoglobin and haemoconcentration. Fragmented red cells (schistocytes) may be observed on a peripheral blood film, reflecting intravascular haemolysis. In severe cases that could lead to death, damage of the renal microvasculature occurs leading to renal failure requiring dialysis [27]. Predictors of susceptibility and possible therapeutic interventions need further research. Further research is also needed to determine whether this is a risk common to all AAV mediated gene therapies or whether it is preferentially (or even exclusively) observed for some vectors.

It is now widely accepted to be complement mediated with predictable decreases in C3 and C4, followed by a measurable increase in the membrane attack complex, C5b-9, which fit timing wise with the biochemical changes. Platelet levels fall early, usually in the first 1-2 days and ultimately with a nadir between days 7-9. Some groups have detected IgM within the plasma at 48 hours post exposure, leading to activation of the classical complement pathway beginning with C1q. Clinically, oedema and haemoconcentration may be observed. Haemolytic anaemia with low haptoglobin and schistocytes on the blood film can develop. A wide range of clinical severity has been observed, ranging from a self-limiting biochemical abnormality not needing intervention to renal failure needing acute dialysis and sometimes resulting in death. There appears to be a direct effect of the AAV9 capsid on C3 in plasma after vector exposure, leading to an amplification loop and production of the membrane attack complex. This possibly also occurs with AAV- antibody complexes. Participants with DMD are possibly even more susceptible to this due to underlying inflammatory environment which characterises the condition, although a recent report has also found evidence of a subclinical vasculopathy in SMA, which could also contribute to this adverse event [28]. A complement reaction against the transgene (as opposed to the vector capsid) is significantly less likely as a cause due to the timing of this adverse event as transgene expression does not occur until several weeks following gene therapy administration. Interplay with the humoral immune response is possible; screening widely for AAV seropositivity at baseline could aid in identification of at-risk groups, but as previously discussed lack of AAV-antibodies does not entirely correlate with immune naivety. The relative frequency of this occurrence is lower in onasemnogene abeparvovec (9 out of approx. 2,300 exposed patients) than with the miniaturised dystrophin trials. It is hypothesised that this is because older and heavier patients are recruited in the DMD trials with higher viral load. The issue of a weight related toxicity might also be applicable to SMA; indeed the safety profile of OA appears to be much more favourable in infants identified by newborn screening and injected soon after birth (Servais et al. Submitted). Admittedly the denominator of the current newborn screening is very limited compared to the real world data on onasemnogene abeparvovec; other factors such as the limited immune exposure and the higher immune tolerance in very young babies could also play a role.

Genetic alterations in complement pathways have been suggested in one participant with severe TMA following onasemnogene abeparvovec [26], indicating that the identification of individual susceptibilities will be important. More data and comparison with patients who did not develop TMA is needed, but this further substantiates the claim of the role played by complement activation and could be another method of identification of at risk groups and a target for prophylactic treatment.

Treatment has been predominantly supportive, but intensive treatments such as acute dialysis have been needed in some cases. Eculizumab, a C5 inhibitor, has been tried with variable and unverified success. There has been no discernible difference in either the time course or outcome of TMA when using eculizumab and its use in patients who received Onasemnogene abeparvovec has not demonstrated any clear benefit. It has been used in both the SOLID and Pfizer miniaturised dystrophin trials and adopted as prophylaxis in the former. Pneumococcal and meningococcal vaccines are recommended prior to eculizumab treatment and this is another consideration if its use is implemented.

Key points: Thrombotic microangiopathy

Has been observed across multiple AAV products, most commonly those using AAV9 vectors
Usually occurs between days 5 and 11 following gene transfer
Early features include a fall in platelets relative to baseline
Clinical features are vague and may include nausea and vomiting, irritability and poor feeding
Biochemical features include: that of intravascular haemolysis: anaemia, decreased serum haptoglobulins, increased LDH, schistocytes on peripheral blood film
Renal dysfunction
Thrombocytopenia
Elevated d-dimer
Elevated ferritin
Decreased C3&C4
The likely mechanism is complement mediated against the vector capsid
It is possible that AAV9 has a direct effect on C3
Monitoring of platelets is important in the first days-weeks following gene transfer, with further tests for TMA (d-dimer,blood film,LDH, haptoglobulins) if platelet counts decrease
Anaemia and renal dysfunction should also be monitored for regularly
The mainstay of treatment is supportive (and may include dialysis)
Eculizumab has been used in some instances but it is not yet clear whether this is effective

1.4.1.5. Other. A transient decrease in platelets without evidence of TMA has been reported across trials. This is proposed as a likely clumping effect due to acute inflammation. Transient decreases of lymphocytes (XLMTM) have also been noted but these are difficult to interpret in the context of corticosteroid administration. These have been self-limiting and recovered without treatment. Haemophagocytic lymphocytosis (HLH) has been reported once in a three year old child following use of Onasemnogene abeparvovec for SMA [29]. This child had been diagnosed with SMA at three months of age and treated with Nusinersin until three years of age. Fever, rash, hepatosplenomegaly accompanied by the laboratory findings of pancytopenia, liver dysfunction, hypertriglyceridemia, hypofibrinogenemia and elevated ferritin occurred at 36 hours post therapy. This resolved with methylprednisolone therapy. Similar findings have been observed with administration of another AAV9 gene therapy product at a similar dose at the University of Florida [30]. These resolved with treatment with the IL-1 receptor antagonist anakira. HLH is a rare immunological condition which results in an uncontrolled inflammatory response. There are various secondary causes, including viral infection. This represents another presentation of AAV gene therapy's effect on the innate immune system.

1.4.2. Directly AAV mediated cardiomyopathy (non-transgene mediated)

Acute cardiomyopathy has been observed in one patient in the Pfizer trial for DMD. This 16-year-old-boy had a nonsense mutation in exon 47. His baseline ejection fraction was reported as 53 % by the central readings of cardiac MRI, but possibly lower when read at the study site, but still within the eligibility range. He was the first, and only, patient to have been treated with sirolimus as additional prophylaxis against thrombotic microangiopathy.

He was being treated as an outpatient following gene transfer and had been clinically stable, aside from nausea and vomiting which is common after gene transfer. The first indication of cardiac damage was a rise in troponin I from a baseline of $<$ 0.03 ng/ml to 0.45 on day 2. Due to centralised reporting of results, this result was not available to the clinical team until day 5 when the patient came back for another visit. At this point, the patient's troponin I was 2, but this again was not available at the time of the visit, he was clinically stable and went home. He returned the next day in clinic, six days after receiving the gene therapy product, with complaints of nausea, anxiety and difficulty sleeping. Examination initially demonstrated normotension but was significant for a prolonged peripheral capillary refill time and tachycardia. He was urgently seen by a cardiologist, where an ECHO demonstrated markedly reduced ventricular contractility and increased left ventricular thickness due to myocardial oedema. Over the course of the day, the patient had episodes of tachycardia and hypotension. Volume depletion, as evidenced by high blood urea and haemoconcentration was possibly contributing to the patient's condition. He was moved to the intensive care unit where he was given milrinone and furosemide. After a discussion with his family, care was re-orientated towards comfort and he died of cardiogenic shock on that evening. An autopsy was declined.

Whilst the cause of death is still unknown, a likely possibility is an innate immune system reaction against the capsid. An adverse reaction with some clinical similarities has subsequently been seen in an adult patient with DMD treated with AAV9 delivered CRISPR [31]. The timing post dosing makes an anti-transgene reaction extremely unlikely. A localised complementopathy such as the above described TMA was raised as another possibility. The timing, haemoconcentration and myocardial oedema support this theory although, unlike the systemic TMA seen he had no alterations in his renal function or levels of C3 and C4. This patient had a lower reduction in their platelet count than is typical for children receiving this therapy, but interpretation may be difficult due to haemoconcentration. A T Cell mediated response is unlikely due to timing, as typically it will take 10 days for T Cells to be activated. The sponsors initially also considered parvovirus b19 reactivation as a possible contributing factor, but high levels of parvovirus are often seen in the tissue of patients with chronic heart failure and this is therefore more likely an incidental finding. The rest of the screen for infectious agents was negative. Less severe cardiac events have been observed with use of other gene therapy products, they have been reported in the real-world use of onasemnogene abeparvovec in SMA, in one patient in the Sarepta trial and two in the Solid trial. Participants all had a rise in troponin I in the first few days after gene therapy administration, peaking at day eight and considerably improved by day 14. In the Sarepta trial the reason for admission and measurement of troponin I was nausea and vomiting.

Since this incident the Pfizer trial now mandates a seven-day period of hospitalisation with daily measurements of troponin I and at least two weeks of close outpatient monitoring with participants residing in close proximity to the treatment centre.

1.4.3. Anti-transgene immunity

Presumed immune reactions against the transgene may occur when there is no residual protein expression (from bi-allelic or hemizygous non-sense variants in recessive or X-linked disorders respectively) or when the residual protein product is aberrant and missing crucial epitopes from the normal gene product. This constellation is referred to as Cross-Reactive Immune Material (CRIM) negative. Overarching topics or questions related to anti-transgene immunity can be grouped in the following categories: (1) Genotype based participant selection/ inclusion; (2) Immune monitoring; (3) Risk mitigation and immune strategies for potential or observed anti-transgene responses; (4) Impacts of anti-transgene responses on efficacy.

There have been five events observed across three different sponsors for gene replacement for DMD consisting of muscle

weakness (in proximal and distal musculature), bulbar weakness, acute respiratory distress, and myocarditis. These events occurred in trials utilizing the following capsids: AAVrh74, AAV8, or AAV9, at doses ranging from 1e13 vg/kg to 2e14 vg/kg in participants ranging from 7 to 9 years old. It is important to note that doses are not directly comparable due to differences in processing and measurement. Onset of symptoms ranged from Day 24 to Day 42 post gene transfer, which corresponds to the time taken for microdystrophin expression and did not coincide with the expected timing of complement activation or part of the innate immune response. Three of these patients had cardiac involvement evidenced by reduced ejection fractions, MRI changes or troponin rises. All eventually recovered but required intensive care treatment including ventilatory support and were given multiple treatments including plasmapheresis, tacrolimus and steroids. None of these patients became haemodynamically unstable.

These events are thought to be related to an anti-transgene response because of the nature, timing and characteristics of the participants. The participants all had deletions which overlapped with the N terminal region of the transgene construct (Bonnemann et al. In press). However, it is important to note that a patient with a smaller deletions in this region did not experience this SAE, perhaps because of a greater chance of development of tolerance to adjacent sequences.

In two participants who experienced a presumed transgene mediated reaction, T cell reactivity against transgene epitopes deleted in the patient were detected by ELISPot. Where they were not, this was postulated as either due to lack of sensitivity in ELISpot testing or the effects of participants using heavy immunosuppression at the time of sampling (Genethon). Antibodies against the micronized dystrophin transgene were also detected (Sarepta and Pfizer) in these participants when this had not been seen in participants without anti transgene adverse event.

Muscle biopsy was not available for the participant in the Pfizer trial. In the Genethon trial, this demonstrated infiltrates of macrophages and T cells. In the Sarepta trial, muscle biopsy at about 2½ weeks after the onset of weakness (6 weeks post gene transfer) showed some T cell infiltrates. There was discussion between participants regarding whether these histological findings could be defined as myositis; however, there was no uniform MHC Class I staining as is commonly seen in autoimmune myositis. There was a positive and markedly elevated ELISpot response (mapped to exons 8 and 9 to the N terminal peptide pool) which persisted for several weeks and also a positive antibody response to microdystrophin. Interestingly this region involves Hinge 1, a region not conserved in utrophin, while most of the remaining microdystrophin domains are also conserved in utrophin. This conservation, presumably, reduced the possibility of a complete new epitope exposure after AAV gene therapy.

Other participants may develop anti-transgene antibodies or ELISPot reactivity (sometimes even at baseline) and then not go on to have a serious adverse reaction [32]. Other factors associated with anti-transgene responses need to be more fully elucidated in order to better predict and mitigate risk. Possible causes could be related to differences in HLA between participants.

Following review of these events, sponsors provided mitigation strategies that are being implemented. First, currently, sponsors are excluding the following potential at-risk genotypes: (1) Sarepta – mutations involving exons 1–17; (2) Genethon – mutations involving exons 1–17 or exons 29–30; (3) Solid – mutations involving exons 8–11 or exons 42–45 deletions; 4) Pfizer mutations involving exons 9–13 or exon 29–30. Sponsors are increasing monitoring for T-cell mediated responses. There is a need to evaluate further strategies for T cell directed immune modulation and tolerance. Jeff Chamberlain (USA) proposed the concept of de-immunization by epitope redesign using ROSETTA software, which predicts immunogenicity of certain epitopes, allowing them to be removed from the transgene construct. Chamberlain commented on the observed immune reactions against the hinge 1 region of dystrophin in Duchenne muscular dystrophy. It is theorised that this hinge 1 epitope could be recognised as a non-self antigen in DMD boys with a deletion removing this region also as hinge 1 is not expressed in utrophin, therefore not allowing for induction of immune tolerance. Through this approach, the group has successfully redesigned constructs that are still efficacious in mice. They have not yet been tested for immunogenicity in humans. This strategy was discussed as a potential strategy of interest. In the future, once safety can be better established with the above mitigation strategies, the goal will be to identify mechanisms to allow dosing of participants with all genotypes. One suggestion for DMD was to start with those participants who have smaller deletions around what appears to be the critical region in the dystrophin gene, as they may be at lesser risk of an anti-transgene reaction. Mass spectrometry measurement of dystrophin peptides at baseline may aid in assessing immune-naivety more accurately than relying on genotype, although this may need to be caveated with the fact that some proteins may prime the immune system to react rather than acting in the induction of tolerance. Anti-transgene responses clearly pose a safety concern in these clinical trials, and need to be mitigated; however, it is still not entirely clear whether these responses are linked to a decrease or loss of efficacy. Long term follow up will be needed to better answer this question.

It should be noted that certain classes of DMD associated variants are unlikely to be associated with anti-transgene responses following dystrophin re-expression. Specifically, patients with single-exon duplications are likely to have expressed full-length dystrophin in revertant fibres due to low-level splicing events. As a result, U7snRNA-directed exon skipping of single exon duplications restoring a normal reading frame (NCT04240314) is less likely to induce anti-dystrophin immune responses.

Some predicted CRIM-negative participants were included in the XLMTM intravenous gene transfer study with Astellas (rAAV8-Des-hMTM1). Astellas reported that ELISpot has been problematic in the ASPIRO clinical trial due to difficulty of the test. Predicted CRIM negative participants in the MTM1 trial did have an observed higher anti-MTM1 antibody response. There were two predicted CRIM negative participants who developed myocarditis (presenting with tachycardia and troponin I elevations), however, there were also some predicted CRIM negative participants who had developed an anti-transgene antibody response and associated myopathy who did not develop myocarditis.

The trial for Giant Axonal Neuropathy (GAN) at NIH (NCT NCT02362438) (AAV9/JeT-GAN) has included predicted CRIM negative participants. Fourteen patients have been exposed to the gene therapy product delivered intrathecally at 4 dose levels. Four patients had genotypes which predicted CRIM negativity. They were treated with an immune modulation pathway similar to that used in solid organ transplantation with tacrolimus and rapamycin as well as intravenous methylprednisolone on the day of gene transfer followed by daily oral prednisolone. No anti transgene responses have been observed by ELISpot analysis in the GAN clinical trial (at this time an anti-gigaxonin antibody is not available).

1.5. Screening and monitoring, prevention and treatment

On site processing of samples is essential in the first week after gene therapy administration as changes can happen quickly and be apparent on samples prior to clinically. Delays in receipt of blood test results have been implicated in serious adverse events

previously. Point of care tests may be useful to expedite this. Use of a PICC or midline was suggested to assist with more regular blood drawing in children.

The group discussed that it would be useful for essential tests to be standardised between gene therapy trials, as well as harmonisation of the dose measuring methods. Better documentation and data sharing of all previous adverse events is warranted so that risk stratification of adverse events and prophylactic treatment can be implemented.

For monitoring of liver disease, liver function tests including transaminases, GGT and bilirubin should be performed at baseline, day 5, day 7 and day 9 and then at least weekly until corticosteroid tapering has completed. The blood results should be reviewed by the team responsible for administration of the gene therapy products up to six months following gene transfer. It is important to include at least two timepoints in the first week for comparison. Serum bile acids may be important in monitoring for cholestasis and specific attention should be paid to pre-existing liver involvement either as a separate co-occurring morbidity, or as an integral part of the condition, such as in XLMTM. Liver biopsies may be necessary to decide the optimal management especially of chronic liver involvement not responsive to steroid therapy.

To monitor for thrombotic microangiopathy a full blood count, urea and electrolytes, serum serum haptoglobulins, d-dimer and ferritin should be performed at days 5,7 and 9 or whenever a patient has evocative symptoms. The appearance of schistocytes on a peripheral blood film is sensitive early sign. A relative drop in platelet count, even if this does not go below normal limits, could trigger further monitoring or use of further prophylactic methods such as eculizumab or i.v. methlyprednisone. Measurements of C3/4, and the complement membrane attack complex (C5b-9) is helpful however results may not be available in real time at most clinical centres to guide clinical decision making. D-dimer elevation is a sensitive early marker.

Cardiac monitoring should consist of an echocardiogram and possibly a cardiac MRI at baseline in DMD. Daily troponin I measurements in the first week following gene therapy is helpful, however turnaround time for result reporting is variable. Abnormal results should trigger electrocardiogram (ECG), echocardiogram and possibly cardiac MRI along with consultation with a paediatric cardiologist.

Immunological monitoring is needed at baseline and at timepoints throughout the study (in clinical trials). At present, it is not possible to determine whether a patient is truly immune naïve to AAV with anti-AAV antibody screening as seronegativity does not mean an immune naïve status. Multiparametric serial assessments of antibodies including IgM and IgG may be useful as kinetics of AAV immunoglobulin response may determine whether there was prior exposure. However, this is unlikely to be feasible in the real world setting for approved therapies. Assays should be standardised between trials and there should be clarity if the test performed refers to binding or neutralising antibodies. Measures of T Cell activity are needed, ideally an assay which is more practical than IFN-γ ELISpot. Assays for memory B cells may be useful in determining previous antigen exposure, but sampling difficulties may make this impractical. Multi-parametric measurement is required, especially to also capture the initial innate immune response with involvement of the complement cascade. D-Dimers are readily available and as a point of care test and could potentially be employed as a surrogate for complement C5b-9 which are more difficult to obtain. Development of a point of care complement test may be useful for future detection and monitoring of immune mediated adverse events.

In some trials (e.g. Pfizer miniaturised dystrophin) hospitalisation is planned for at least one week following gene transfer. This is for acute events such as risk of cardiac failure which may occur in the first 7 days, be initially subtle and be amenable to prompt treatment.

Autopsy should be considered in the unfortunate event of participants dying either on trial or natural history studies or receiving therapies in the real world. This should be discussed at the time of informed consent, and a standard procedure put in place for processing of samples.

Key Points: Identifying Adverse Events

Key biochemical parameters such as full blood count, liver function tests, urea and electrolytes need close monitoring in the days-weeks following gene transfer (Fig. 1)

To monitor for thrombotic microangiopathy a full blood count, urea and electrolytes, serum serum haptoglobulins, d-dimer and ferritin should be performed at days 5,7 and 9 or whenever a patient has evocative symptoms.

Liver enzymes need monitoring during the time of steroid taper and after until stabilised. Steroid taper should be slowed if transaminitis appears

Cardiac monitoring should include baseline echocardiography, and use of some AAV products may warrant daily troponin I testing in the week following administration

It is important that results are available at the site of the patient and that centralised reporting of results is not solely relied upon

Lab results, especially time critical such as troponin, should be reviewed on the same day as they are taken

Point of care blood results might be useful to make monitoring more efficient

Use of a PICC or midline might aid frequent blood drawing in children

1.6. Additional considerations

Live vaccinations should not be given at the time or in the weeks preceding gene therapy treatments. It is recommended that vaccinations are given prior to commencement of gene therapy allowing a sufficient interval of time to avoid close concomitance between the vaccination (which triggers a strong immune response) and the AAV gene therapy. An interval of 3-4 weeks between a live vaccination and the AAV gene therapy; or 2 weeks in case of an attenuated vaccine is often advised. In case eculizumab might be considered, pre-vaccination with pneumococcal and meningococcal vaccination is recommended, and the timeline for these vaccinations therefore needs to be considered. This is of course not applicable for patients identified by newborn screening, in whom however the risk of TMA appears lower. if eculizumab is indicated in an unvaccinated patient post dosing immunization and a course of prophylactic antibiotic are recommended (per the FDA label).

Immunosuppression protocols vary widely between trials and harmonisation and data sharing is likely to be useful for future developments. Corticosteroid prophylaxis is used in most trials of systemic gene therapy. Sirolimus is an emerging T cell active agent with the potential benefit that it does not act against T-Reg cells hence promoting the development of tolerance, and it is unclear whether should be offered prospectively to all participants, or only to those deemed to be at higher risk. There has been some effort to use heavy immunosuppression in order to enable re-dosing, and in order to mitigate anti-transgene reactions.

Whole genome sequencing of participants may help identify individual susceptibility factors potentially predisposing to adverse events. Prospective collection of WGS data with adequate patient consent should be considered in patients treated by AAV therapies.

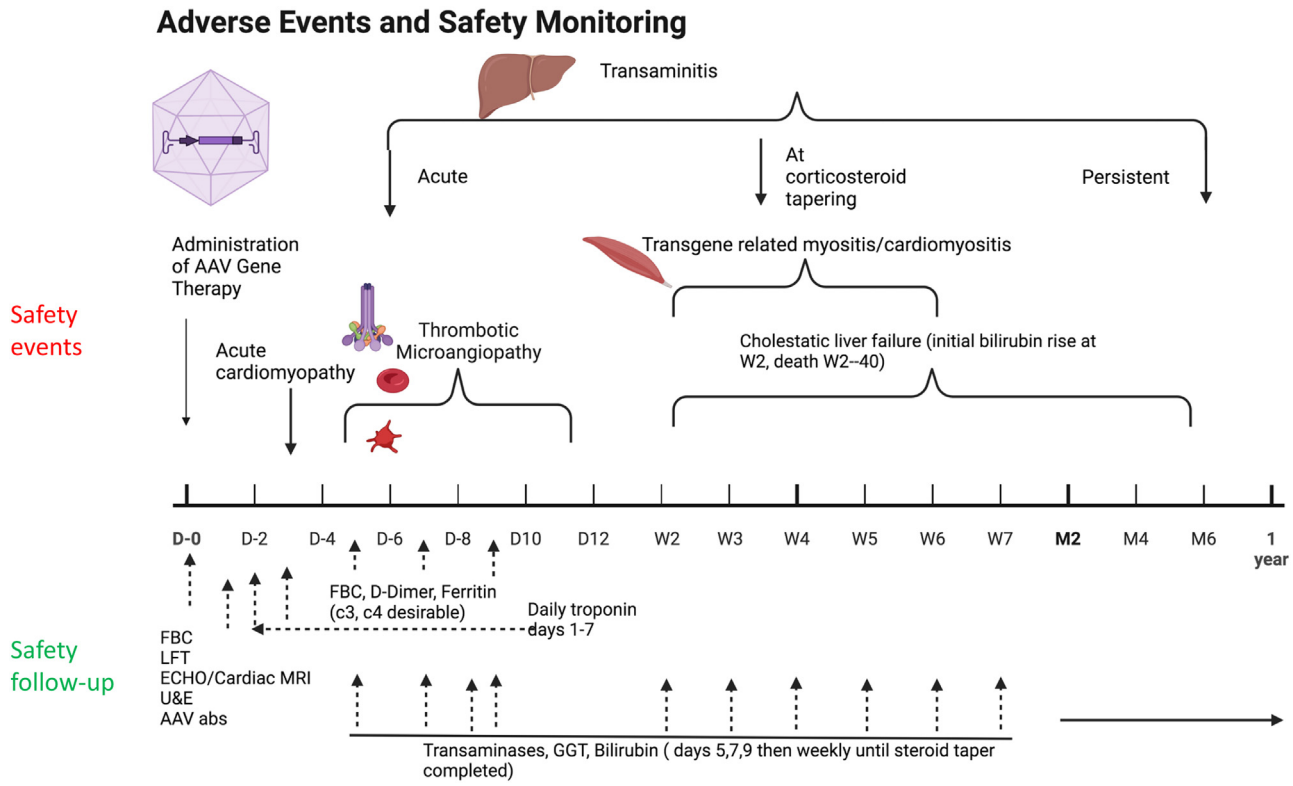

Fig. 1. Typical adverse events timeline following AAV infusion and standard follow up.

1.7. Future research in the field and pre-competitive collaboration

The collaborative data sharing consortium put together between Pfizer, Sarepta, Solid, Roche and Généthon to jointly analyse the host genetics and transgene epitope content of microdystrophin trials clearly demonstrates the power and value of prepublication data sharing across disparate studies. The model should be refined and extended to address additional challenges facing the development of safe and effective GT. Although broad collection of data and specimens across multiple domains for extended periods of time would be desirable, business, financial and ethical/privacy considerations make this a challenging goal to accomplish.

One possibility would be to focus on one or two idiosyncratic classes of SAE that appear to be occasional complications across multiple different trials and related to a class or platform effect toxicity. An important consideration will be identification of a minimal set of Common Data Elements and tests to be reported across trials, with consistent longitudinal measurements relevant to the SAE class under consideration (i.e., haematological tests for TMA, serum bile acids, LTFs for hepatotoxicity, etc.). Working groups across trials could work on these.

Angela Lek (MDA) discussed the importance of pre-competitive data sharing and collaboration between trial sponsors. Incentives for sharing of adverse events include early identification of drug class effects, potential stratification issues across trials and provide better guidance for patient selection to avoid SAEs. Collaborative data sharing models were discussed in the context of pooling of safety data. A federated data sharing platform was raised as a means of avoiding sole ownership or a single custodian of the data. A set of findings across studies, as well as adverse event reporting could be harmonised, and be used to flag safety signals. Coding of data and quality of data input would be important considerations for the quality of this data.

2. Conclusions

Better collaboration and improved data sharing can mitigate the consequences of emerging serious adverse events or aid their more rapid detection and treatment. This is critical in the rare disease field where small numbers of participants will be treated per site. As the majority of safety issues surrounding AAV gene therapies appear to be immune mediated, a greater appreciation of the human immune response after AAV is needed. A recurring theme was the poor predictive value of animal models, due to differences in immune architecture, immune exposure and disease natural history. The urgent need for careful natural history studies of rare diseases to determine any underlying susceptibility for organ damage (the pertinent example being predisposition to cholestasis in XLMTM) was addressed. Careful natural history studies could be used to guide promotor choice at the outset of new therapies in order to maximise both safety and efficacy. Harmonisation of product release criteria reporting and labelling, immune protocols and monitoring will lead to improved ability of detection and treatment of serious adverse reactions, and pave the way to safer use of gene therapies.

Author agreement

All authors and contributors have approved the final version.
All authors match with ICMJE criteria.

Declaration of Competing Interest

FM has received honoraria for scientific advisory boards from Novartis Gene Therapies Inc., Biogen, Novartis, PTC, Roche, Pfizer, Dyne Therapeutics and Sarepta. LS has received consulting fees from Sarepta, Pfizer, Roche, Affina, Renegex Bio, Audentes, Evox Therapeutics and Novartis. He has received honoraria from Sarepta, Roche, Audentes and Novartis. He is the secretary of the World

Muscle Society. DS, RHH and CGB have no conflicts of interest to declare.

Acknowledgments

The workshops and next generation programme are made possible thanks to the financial support of the European Neuromuscular Centre (ENMC) and its Full Partners: Association Française contre les Myopathies (France), Deutsche Gesellschaft für Muskelkranke (Germany), Muscular Dystrophy Campaign (UK), Muskelsvindfonden (Denmark), Prinses Beatrix Spierfonds (The Netherlands), Schweizerische Stiftung für die Erforschung der Muskelkrankheiten (Switzerland), Spierziekten Nederland (The Netherlands), Telethon Foundation (Italy). In addition, we would like to thank the Associated Partners: Finnish Neuromuscular Association (Finland), Österreichische Muskelforschung (Austria), SMA Europe and World Duchenne Organisation, and the members of the ENMC Company Forum: Amicus Therapeutics, Astellas, Biogen, Ionis Pharmaceuticals, Lupin Neuroscience, Novartis, PerkinElmer, Roche, Sanofi, and Sarepta. A special thanks to MDA USA for their support to cover travel costs from USA participants. The figure was generated using biorender.

261st ENMC workshop study group

Dr Oumeya Adjali (France), Dr Alan Beggs (USA); Dr Diana Bharucha (USA); Dr Carsten Bönnemann (USA); Dr Serge Braun (France); Dr Barry Byrne (USA); Dr Manuela Corti (USA); Dr Ana Buj-Bello (France); Dr Jeff Chamberlain (USA); Dr Ana Ferreiro (France); Dr Kevin Flanigan (USA); Mrs Olga Germanenko (Russia); Dr Nathalie Goemans (Belgium); Dr Daniel Grant (USA); Dr Sam Hopkins (USA); Dr Rebecca Horton (UK); Dr Marta Kollb-Sielecka (The Netherlands); Dr Caroline Le Guiner (France); Dr. Dan Levy (USA); Dr Angela Lek (USA); Dr Weston Miller (USA); Dr Carl Morris/ Dr Roxana Dreghici (USA); Dr Francesco Muntoni (UK); Dr Dimah Saade (US); Dr Laurent Servais (UK); Dr Teji Singh (USA); Drs Elisabeth Vroom (The Netherlands); Dr Kathryn Wagner (Switzerland). Mr Frank Van Ieperen (The Netherlands)

References

[1] Horton RH, Saade D, Markati T, Harriss E, Bonnemann C, Muntoni F, et al. A systematic review of adeno-associated virus gene therapies in neurology: the need for consistent safety monitoring of a promising treatment. J Neurol Neurosurg Psychiatry 2022;93(12):1276–88 [published Online First: 2022/10/04]. doi:10.1136/jnnp-2022-329431.

[2] Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med 2006;12(3):342–7 [published Online First: 2006/02/14]. doi:10.1038/nm1358.

[3] Nathwani AC, Gray JT, McIntosh J, Ng CY, Zhou J, Spence Y, et al. Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. Blood 2007;109(4):1414–21 [published Online First: 2006/11/09]. doi:10.1182/blood-2006-03-010181.

[4] Hinderer C, Katz N, Buza EL, Dyer C, Goode T, Bell P, et al. Severe toxicity in nonhuman primates and piglets following high-dose intravenous administration of an adeno-associated virus vector expressing human SMN. Hum Gene Ther 2018;29(3):285–98 [published Online First: 2018/01/31]. doi:10.1089/hum.2018.015.

[5] Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med 2011;365(25):2357–65 [published Online First: 2011/12/14]. doi:10.1056/NEJMoa1108046.

[6] Brantly ML, Chulay JD, Wang L, Mueller C, Humphries M, Spencer LT, et al. Sustained transgene expression despite T lymphocyte responses in a clinical trial of rAAV1-AAT gene therapy. Proc Natl Acad Sci USA 2009;106(38):16363–8 [published Online First: 2009/08/27]. doi:10.1073/pnas.0904514106.

[7] Vandamme C, Xicluna R, Hesnard L, Devaux M, Jaulin N, Guilbaud M, et al. Tetramer-based enrichment of preexisting anti-AAV8 CD8(+) T cells in human donors allows the detection of a T(EMRA) subpopulation. Front Immunol 2019;10:3110 [published Online First: 2020/02/11]. doi:10.3389/fimmu.2019.03110.

[8] Benichou G, Gonzalez B, Marino J, Ayasoufi K, Valujskikh A. Role of memory t cells in allograft rejection and tolerance. Front Immunol 2017;8:170 [published Online First: 2017/03/16]. doi:10.3389/fimmu.2017.00170.

[9] Gernoux G, Gruntman AM, Blackwood M, Zieger M, Flotte TR, Mueller C. Muscle-directed delivery of an AAV1 vector leads to capsid-specific T cell exhaustion in nonhuman primates and humans. Mol Ther 2020;28(3):747–57 [published Online First: 2020/01/27]. doi:10.1016/j.ymthe.2020.01.004.

[10] Mack DL, Poulard K, Goddard MA, Latournerie V, Snyder JM, Grange RW, et al. Systemic AAV8-mediated gene therapy drives whole-body correction of myotubular myopathy in dogs. Mol Ther 2017;25(4):839–54 [published Online First: 2017/02/27]. doi:10.1016/j.ymthe.2017.02.004.

[11] Childers MK, Joubert R, Poulard K, Moal C, Grange RW, Doering JA, et al. Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. Sci Transl Med 2014;6(220):220ra10 [published Online First: 2014/01/24]. doi:10.1126/scitranslmed.3007523.

[12] Elverman M, Goddard MA, Mack D, et al. Long-term effects of systemic gene therapy in a canine model of myotubular myopathy. Muscle Nerve 2017;56(5):943–53 [published Online First: 2017/04/04]. doi:10.1002/mus.25658.

[13] Day JW, Finkel RS, Chiriboga CA, Snyder JM, Lawlor MW, Meng H, et al. Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of SMN2 (STR1VE): an open-label, single-arm, multicentre, phase 3 trial. Lancet Neurol 2021;20(4):284–93 [published Online First: 2021/03/21]. doi:10.1016/S1474-4422(21)00001-6.

[14] Chand D, Mohr F, McMillan H, Tukov FF, Montgomery K, Kleyn A, et al. Hepatotoxicity following administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal muscular atrophy. J Hepatol 2021;74(3):560–6 [published Online First: 2020/11/14]. doi:10.1016/j.jhep.2020.11.001.

[15] Mendell JR, Sahenk Z, Lehman K, Nease C, Lowes LP, Miller NF, et al. Assessment of systemic delivery of rAAVrh74.MHCK7.micro-dystrophin in children with duchenne muscular dystrophy: a nonrandomized controlled trial. JAMA Neurol 2020;77(9):1122–31 [published Online First: 2020/06/17]. doi:10.1001/jamaneurol.2020.1484.

[16] Feldman AG, Parsons JA, Dutmer CM, Veerapandiyan A, Hafberg E, Maloney, et al. Subacute liver failure following gene replacement therapy for spinal muscular atrophy type 1. J Pediatr 2020;225:252–8 e1[published Online First: 2020/05/31]. doi:10.1016/j.jpeds.2020.05.044.

[17] Novartis reports Zolgensma caused two deaths from liver failure 2022 [Available from: https://www.reuters.com/business/healthcare-pharmaceuticals/novartis-reports-zolgensma-caused-two-deaths-liver-failure-2022-08-11/.

[18] Gojanovich GA. Using a systems biology approach to unravel the immunogenicity of AAV8 empty capsids in healthy volunteers. ESGCT Congress; 2023.

[19] Nathwani AC. Gene therapy for hemophilia. Hematol Am Soc Hematol Educ Prog 2019;2019(1):1–8 [published Online First: 2019/12/07]. doi:10.1182/hematology.2019000007.

[20] Gangfuss A, Schmitt D, Roos A, Braun F, Annoussamy M, Servais L, et al. Diagnosing X-linked myotubular myopathy - a German 20-year follow up experience. J Neuromuscul Dis 2021;8(1):79–90 [published Online First: 2020/11/10]. doi:10.3233/JND-200539.

[21] Lawlor MW, Dowling JJ. X-linked myotubular myopathy. Neuromuscul Disord 2021;31(10):1004–12 [published Online First: 2021/11/06]. doi:10.1016/j.nmd.2021.08.003.

[22] Amburgey K, Tsuchiya E, de Chastonay S, Glueck M, Alverez R, Nguyen CT, et al. A natural history study of X-linked myotubular myopathy. Neurology 2017;89(13):1355–64 [published Online First: 2017/08/27]. doi:10.1212/WNL.0000000000004415.

[23] Molera C, Sarishvili T, Nascimento A, Rtskhiladze I, Muñoz Bartolo G, Fernández Cebrián S, et al. Intrahepatic cholestasis is a clinically significant feature associated with natural history of X-linked myotubular myopathy (XLMTM): a case series and biopsy report. J Neuromuscul Dis 2022;9(1):73–82 [published Online First: 2021/08/10]. doi:10.3233/JND-210712.

[24] Neese JM, Yum S, Matesanz S, Raffini LJ, Whitworth HB, Loomes KM, et al. Intracranial hemorrhage secondary to vitamin K deficiency in X-linked myotubular myopathy. Neuromuscul Disord 2021;31(7):651–5 [published Online First: 2021/06/15]. doi:10.1016/j.nmd.2021.04.009.

[25] D'Amico A, Longo A, Fattori F, Tosi M, Bosco L, Chiarini Testa MB, et al. Hepatobiliary disease in XLMTM: a common comorbidity with potential impact on treatment strategies. Orphanet J Rare Dis 2021;16(1):425 [published Online First: 2021/10/14]. doi:10.1186/s13023-021-02055-1.

[26] Chand DH, Zaidman C, Arya K, Millner R, Farrar MA, Mackie FE, et al. Thrombotic microangiopathy following onasemnogene abeparvovec for spinal muscular atrophy: a case series. J Pediatr 2021;231:265–8 [published Online First: 2020/12/02]. doi:10.1016/j.jpeds.2020.11.054.

[27] Guillou J, de Pellegars A, Porcheret F, et al. Fatal thrombotic microangiopathy case following adeno-associated viral SMN gene therapy. Blood Adv 2022;6(14):4266–70 [published Online First: 2022/05/19]. doi:10.1182/bloodadvances.2021006419.

[28] Zhou H, Hong Y, Scoto M, Thomson A, Pead E, MacGillivray T, et al. Microvasculopathy in spinal muscular atrophy is driven by a reversible autonomous endothelial cell defect. J Clin Invest 2022;132(21) [published Online First: 2022/09/14]. doi:10.1172/JCI153430.

[29] Galletta FC, Marseglia U, Cacciola L, Gallizzi A, Cuzzocrea R, Messina S, et al. Hemophagocytic lymphohistiocytosis following gene replacement therapy

in a child with type 1 spinal muscular atrophy. J Clin Pharm Ther 2022;47(9):1478–81 [published Online First: 2022/08/05]. doi:10.1111/jcpt.13733.

[30] Byrne BJ, Elder M, Leon-Astudillo C, Cort M. Secondary hemophagocytic lymphohistiocytosis following Zolgensma therapy: An evolving story on the innate response to systemic gene therapy. Mol Ther 2022;30(12):3503–4 [published Online First: 2022/11/28]. doi:10.1016/j.ymthe.2022.11.007.

[31] Lek A, Wong B, Keeler A, Blackwood M, Ma K, Huang S, et al. Death after High-Dose rAAV9 Gene Therapy in a Patient with Duchenne's Muscular Dystrophy. New Engl J Med 2023;389(13):1203–10. doi:10.1056/NEJMoa2307798.

[32] Anthony K, Ala P, Catapano F, Meng J, Domingos J, Perry M, et al. T cell responses to dystrophin in a natural history study of Duchenne muscular dystrophy. Hum Gene Ther 2023 [published Online First: 2022/12/02]. doi:10.1089/hum.2022.166.