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Meeting Report

Meeting Report: 2022 Muscular Dystrophy Association Summit on 'Safety and Challenges in Gene Transfer Therapy'

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Abstract. Muscular Dystrophy Association (MDA) has invested over $125M in the development of gene therapy for neuromuscular disorders (NMD) over the past 20 years. As a lead initiator of progress in this important field of medicine and to help ensure continued progress towards therapies for patients, MDA organized a dedicated summit in January 2022 to address emerging challenges in safely delivering adeno-associated virus (AAV) mediated gene therapies with a focus on their application in NMD. In this meeting, chaired by Carsten Bönnemann (NINDS, NIH) and Barry Byrne (University of Florida), academic and industry experts and stakeholders convened to openly discuss adverse events linked to clinical trials, as well as other challenges emerging in preclinical studies associated with difficulties in the translation of AAV-mediated gene therapies.

BACKGROUND

Gene therapy using various serotypes of adeno associated viral (AAV) vectors for neuromuscular disorders (NMD) has advanced considerably in the last decade, with onasemnogene abeparvovec-xioi (Zolgensma®) having achieved regulatory approval in the United States and Europe [1]. There are currently five different AAV mediated clinical trials in patients for Duchenne muscular dystrophy [2], as well as trials underway for limb girdle muscular dystrophies, a congenital myopathy (MTM1) [3], Pompe disease [4], Danon disease [5], giant axonal neuropathy [6] and ALS [7], with many more in advanced preclinical stages. These trials highlight specific challenges associated with gene therapy for NMDs and related toxicities brought upon by these unique challenges. A thorough understanding of both determinants of efficacy as well as potential drivers of toxicity and risk will help the entire field adjust and advance carefully by proactively addressing potential toxicities to fully realize the translational potential of gene therapy for patients with NMDs.

This meeting focused on identifying and understanding toxicities (rather than on specific applications of AAV), with discussions mainly coalescing around the two main instigators of immunological toxicity of AAV-delivered therapies: *capsid-triggered* and *transgene-triggered* responses. Recognition of and discrimination between these two responses is critical, as they require different approaches to monitoring, prevention and risk-mitigation. Additional discussions relevant to the safety and feasibility of gene therapies for NMD held during the

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Table 1
Listing of meeting presenters and talk titles

Presenter	Organization	Title
Carl Morris, PhD	Solid Biosciences	Potential Solutions of Gene Transfer Therapies
Dan Levy, MD, PhD	Pfizer	AAV Gene Therapy and Complement Activation
Teji Singh, MD	Sarepta Therapeutics	Delandistrogene moxeparvovec (SRP-9001) Micro-dystrophin Gene Therapy Program Experience
Dongsheng Duan, PhD	Department of Molecular Microbiology and Immunology University of Missouri	AAV CRISPR Therapy Induces Cas9-Specific Immune Responses in Dystrophic Dogs
Carsten G. Bönnemann, MD	National Institute of Neurological Disorders and Stroke (NINDS)	Anti-Transgene SAEs in Trials of Gene Therapy for DMD: A Collaborative First Analysis
Lee Sweeney, PhD	UF Myology Institute University of Florida	Potential cardiac toxicity associated with high doses of AAV.microdystrophin gene therapy for DMD
Lindsey A. George, MD	Children's Hospital of Philadelphia University of Pennsylvania School of Medicine	Clinically Observed AAV Toxicities: Hepatic and TMA
Roland W. Herzog, PhD	Indiana University	Immune Response Mechanisms in AAV Gene Transfer to Skeletal Muscle
Francesco Muntoni, MD, FRCPCH	UCL Great Ormond Street Institute of Child Health & Great Ormond Street Hospital London, UK	Skeletal Muscle Organ Toxicity in Clinical Trials
Jeffrey Chamberlain, PhD	University of Washington	Capsid Concentration Influences Systemic AAV Delivery AAV-Durability is Dependent on Expression Cassette Design and Dose
Joe Kornegay, DVM, PhD	Texas A&M University	Canine Microdystrophin Studies: Clinical Translation (or Not)?
Barry Bryne, MD, PhD	Powell Gene Therapy Center University of Florida, College of Medicine	Kinetics of Innate and Adaptive Response to AAV Gene Therapy Findings from NHP Studies and Clinical Translation
J. Fraser Wright, PhD	Stanford University School of Medicine	rAAV Vector Design and Characterization: Defining and Measuring Critical Quality Attributes for Clinical Success
Charles Gersbach, PhD	Duke University Department of Biomedical Engineering	Genome Editing for DMD
Qi Lu, MD, PhD	Atrium Health/Wake Forest University	Modifying Muscle Specific Promoter to Achieve Balanced Transgene Expression for Long-term Efficacy with Low Dose AAV Gene Therapy
Federico Mingozzi, PhD	Spark Therapeutics	Anti- AAV Antibodies and Strategies to Address Them
Kevin Flanigan, MD	Nationwide Children's Hospital The Ohio State University	Considerations in the Treatment of Infants with DMD
Perry Shieh, MD, PhD	University of California	Update on the Efficacy and Safety of AT132 in XLMTH: ASPIRO Study
Emma James, PhD, MFPM	Encoded Therapeutics	Ethical Considerations in Gene Therapy

meeting covered transgene durability, gene-editing approaches, translatability of large animal models and ethical considerations are also summarized in this report. The list of meeting presenters and their talk titles can be found in Table 1.

CAPSID-TRIGGERED RESPONSES

Capsid-triggered safety concerns can be subdivided into two main categories: those that are linked to innate immune responses triggered by AAV particles and their nucleic acids, and those that are linked to adaptive cellular and humoral immune responses triggered by the specific capsid. Innate responses may occur more immediately compared to those requiring an adaptive response, but there likely is cross-talk between the two. Safety events in this category have been reported by all AAV gene therapy trial sponsors and range in severity from moderate effects such as transient vomiting and nausea, to more serious occurrences including various hepatoxicities and thrombocytopenia in isolation or as part of complement triggered thrombotic microangiopathy (TMA) manifesting in renal impairment, anemia, and mutisystemic effects on lungs, heart and muscle. TMA related events currently are amongst the potentially most serious capsid related toxic consequences to address [8].

Due to their earlier onset and commonality across all gene therapy trials, capsid-triggered responses

have been studied in greater detail than transgene-triggered responses. Various efforts by trial sponsors to better understand capsid-triggered safety events include monitoring kinetics of IgG and IgM antibodies, complement activation, platelet levels, liver function tests, and troponin levels post-treatment. The variable adverse responses observed in patients within the same and across different trials continue to be a source of investigation. Adding to the difficulty in comparative studies of adverse events across trials, gene therapy modalities, and diseases, is the fact that trials employ different immunosuppression strategies (steroids, complement inhibitors, sirolimus), further complicating conclusions that can be drawn. Further investigations will also need to focus on the correlation between adverse events to dose levels, serotype, immunosuppression protocols, cross-reactive immunologic material (CRIM) status of patient, disease-specific etiologies, and potential other individual susceptibilities.

Lindsey George (Children's Hospital of Philadelphia) discussed the two main types of capsid-triggered clinical manifestations observed across gene therapy clinical trials – hepatotoxicity and TMA. Hepatotoxicity is independent of target cell type and can be acute (hepatocellular and cholestatic hepatitis), or potentially long-term (fibrosis or genotoxicity due to AAV integration reported in animal models [9, 10]. Hepatoxicities present with elevations in transaminase (ALT) and are prophylactically managed by glucocorticoid administration. Example cases from the Zolgensma trial for SMA were presented to highlight hepatocellular toxicity [11], while example cases from AT132 trial for XLMTM (X-linked Myotubular Myopathy) were presented to highlight cholestatic hepatitis that led to four deaths [12] (Table 2). In her talk, Lindsey George notes that complement-mediated TMA occurred in high-dose ($>10^{14}$ vg/kg) systemic AAV9 gene therapy across four disease cohorts.

Three trial sponsors (Sarepta, Pfizer, Solid Biosciences) of dystrophin gene replacement therapy were represented at the meeting and presented adverse events observed in their respective clinical trials (Table 2). In Sarepta's trial (9001-201) for dystrophin gene replacement therapy (microdystrophin, MHCK7 promoter, AAV-rh74), Teji Singh reported that the majority of adverse events occurred within the first 12 weeks and were classed as mild to moderate, with vomiting being the most common. Elevation in liver enzyme (GGT) was noted in all patients; however, a protocol amendment to increase levels of steroids to 1 mg/kg per day for 2 months resulted in a reduction in the number of affected patients (to 30%). It should be noted that TMA is a laboratory diagnosis and few studies have been done to evaluate terminal complement activation. Teji Singh noted that there was an average reduction of 20% in CH50 (total complement measure) among the study participants. Following Solid Bioscience's dystrophin gene replacement therapy (microdystrophin transgene, CK8e promoter, AAV9), Carl Morris reported that all 9 patients exhibited consistent platelet decline and complement activation. Further *in vitro* experiments were performed to demonstrate the link between seropositive serum samples and capsid-triggered complement activation. In Pfizer's dystrophin gene replacement therapy (minidystrophin transgene, hybrid CK promoter, AAV9), Dan Levy discussed three serious adverse events consistent with TMA/aHUS (atypical Hemolytic Uremic Syndrome), as characterized by complement activation, hemolysis, thrombocytopenia and renal impairment. Frequent platelet, renal and hemolysis laboratory assessments (either daily or every-other-day, over the first 10 days after infusion) now help identify the need for potential intervention. There were persistent high levels of vector in blood until 5–7 days post therapy and type 1 interferon and other cytokine responses were identified despite high-dose glucocorticoid treatment. Additionally, a fatality occurred in the trial of a non-ambulatory sixteen-year-old DMD patient who received 2×10^{14} vg/kg. He died six days post-treatment due to cardiogenic shock -rising troponin levels were noted, without significant thrombocytopenia or systemic reduction of complement. The cause of death currently is believed to be the consequence of an innate immune response in the myocardium causing myocardial edema and heart failure. However, as an autopsy was not obtained, the mechanism will remain under active investigation, especially to identify strategies to mitigate risk.

Following the description of adverse events in specific DMD studies, Barry Byrne described efforts in his laboratory to better characterize post-administration immune responses to capsid. In a study of 20 patients receiving Zolgensma, Barry Byrne has monitored antibody (IgG/IgM) and complement kinetic profile following AAV gene therapy. These subjects were studied in relation to the use of an immune suppression regimen using rituximab and sirolimus. The initial findings confirm that innate immune responses that lead to TMA are entirely due

Table 2
List of AAV gene therapies for neuromuscular disorders and associated adverse events

Disease	AAV Serotype	Promoter	Adverse events	Clinical Trial Status
DMD	AAVrh74	MHCK7	Vomiting, increased transaminases, liver injury, rhabdomyolysis, immune mediated myositis, myocarditis	Sarepta: NCT03769116 (Active) NCT04626674 (Enrolling by invitation)
DMD	AAV9	CK8e	Thrombocytopenia, renal damage, cardiopulmonary insufficiency, myocarditis	Solid Biosciences: NCT03368742 (Active)
DMD	AAV9	MSP	Thrombocytopenia, aHUS/thrombotic microangiopathy, myocarditis	Pfizer: NCT03362502 (Active) NCT04281485 (Recruiting)
SMA	AAV9	CBA	Fever, malaise, vomiting, acute liver failure, thrombocytopenia	Novartis: Zolgensma® (FDA approved)
XLMTM	AAV8	DES	Hepatic toxicity, Hyperbilirubinemia, sepsis	Astellas: NCT03199469 (On clinical hold)

to early IgM and IgG formation. In addition, consideration for the use of Imlifidase (IgG antibody-cleaving enzyme) and plasmapheresis were considered as a means to rescue the onset of high-sustained Ab leading to TMA. Going forward, experts in the field anticipate employing multiple strategies to overcome high antibody titers for vector re-administration in future trials. Animal models have proven to be useful in evaluating the safety of anti-AAV NAb evasion strategies, unlike their utility for anticipating T-cell responses, thus allowing for these strategies to be confidently investigated using preclinical models.

Perry Shieh (University of California Los Angeles) shed more light on the XLMTM gene replacement trial (MTM1 gene, desmin promoter, AAV8) by Astellas [3], where it was noted that three deaths occurred in patients given high dose therapy, while one death occurred in a patient given low dose therapy. Both the high-dose and low-dose related deaths were attributed to cholestatic liver failure in response to capsid-triggered toxicity which exacerbated a pre-existing cholestatic liver disease (Table 2). This propensity is now increasingly recognized as a part of the MTM1 disease phenotype [13, 14], likely directly related to myotubularin deficiency in the liver [15], which is not addressed by the gene therapy due to the muscle/heart restricted desmin promoter.

SOURCES OF CAPSID-TRIGGERED IMMUNE RESPONSES AND MITIGATION STRATEGIES

Several factors are known to play a role in capsid-triggered immune responses following systemic AAV gene therapy [16]. These include the total vector amount required, serotype, potential impurities (e.g. percentages of full vs partially filled and empty capsids, encapsidated host cell or helper component DNA [17], characteristics of the cassette, potential impurities, CpG content of vector genome [18, 19], as well as the presence of pre-existing neutralizing antibodies (NAbs). Roland Herzog (Indiana University) presented evidence of different immune pathways triggered related to vector dose levels [20] and the reduction of CpG vector sequences [18] combined with blockade of specific receptors as a potential mitigation strategy for T-cell immunotoxicity. Fraser Wright (Stanford University) highlighted that capsid-specific immunotoxicities correlated strongly with CpG content of AAV expression cassettes in human clinical trials for hemophilia B [21] and can also arise from product-related impurities such as empty capsids, encapsidated host cell or helper component DNA; thus, highlighting the importance of mitigation through vector design strategies such as codon modification to reduce TLR9 activation potential as well as efficient vector purification and post-manufacturing quality control assays to mitigate these potential sources of immunotoxicity. In addition, Fraser Wright discussed the implications of pre-existing antibody binding to AAV particles beyond neutralization of target-tissue transduction, specifically the unwanted formation of immune complexes that can activate complement. An AAV capsid modification strategy to reduce antibody binding, insertion of albumin-binding domains into the capsid subunits as has been reported for recombinant adenoviruses [18], was proposed as one strategy to reduce immune complex formation and potentially enable vector re-administration.

Systemic administration of AAV is at risk of inactivation by circulating neutralizing NAbs to the AAV capsid. Federico Mingozzi (Spark Therapeutics) proposed strategies aimed at overcoming

pre-existing immunity from exposure to wild-type AAV or even, potentially, re-dosing following AAV gene therapy administration. Large vector doses can theoretically overcome low titers of pre-existing NAbs to achieve successful transduction; however, this increases the risk of inflammatory cytokines and subsequent adverse events. Titers observed post gene therapy are much higher (greater than 100X) [22] than those resulting from natural exposure and may elicit persistent cross-reactive antibodies to other serotypes. These higher post-dosing NAb titers cannot be overcome by higher repeat doses of AAV gene therapies due to safety concerns, thus necessitating different strategies for re-dosing. The strategies discussed to overcome anti-capsid antibodies (which may apply to both re-dosing and overcoming pre-existing immunity) include: i) pharmacological immuno-modulation: Blocking antibody formation, eradicating pre-existing immunity with drugs targeting B and T cells, such as Rituximab and sirolimus, rapamycin nanoparticles (ImmTOR); ii) removal of antibodies: plasmapheresis, enzymatic digestion of IgGs, balloon catheters/perfusion (using isolated perfusion following catheterization to flush out antibodies) [23–25]; iii) vector re-engineering (e.g. evolving capsid from natural serotypes and recognition/neutralization by antibodies).

TRANSGENE-TRIGGERED ADVERSE EVENTS

Transgene-triggered safety concerns typically occur weeks after treatment to allow for expression of the transgene and are linked to immune responses directed at the transgene. In this meeting, evidence for transgene-triggered adverse events were mainly discussed in the context of DMD gene replacement therapy. Emerging evidence point to transgene-triggered responses occurring due to immunoreactive epitopes in the dystrophin transgene with the potential to cause a reaction in certain patients with 'at risk' genotypes, namely deletions that render the patient to be CRIM negative for the transgene epitope. The potential for T-cell immune response to self and non-self dystrophin epitopes was already raised in a study by Mendell et al. [26] which described detection of dystrophin-specific T cells in a subset of DMD patients both pre- and post- gene therapy. However, the serious nature of anti-transgene responses was only revealed in recent collaborative efforts between trial sponsors to understand the similar clinical presentation of myositis and myocarditis observed in several treated patients, summarized by Carsten Bönnemann for a collaborative group that included Francesco Muntoni, Pfizer, Sarepta, Genethon and Solid, and a group of academic experts [27, 28]. Collective observations of transgene-triggered events across these studies confirm an emerging correlation of these adverse events with patient genotypes that have N-terminal deletions of the dystrophin gene in regions that correspond to sequences represented in the micro/mini-dystrophin transgene, resulting in immune-naivete or CRIM negativity for these N-terminal epitopes. These transgene-triggered clinical events are hypothesized to be largely T-cell mediated and directed against the dystrophin transgene.

Francesco Muntoni (University College London) further discussed the importance of considering CRIM status in patients receiving DMD gene replacement therapy and its predictability towards transgene-triggered responses. Factors to consider for anti-dystrophin immunity in DMD patients include residual expression of shorter dystrophin isoforms, revertant fibers and novel-junctional epitopes, deleted epitopes and homology with other proteins, and the inflammatory environment of fibers positive for HLA-I. In essence, which epitopes of the transgene the body has 'seen' or 'not seen' [29, 30]. These considerations can help to predict 'at-risk' genotypes for anti-dystrophin immune responses.

Following the identification of anti-dystrophin antibodies directed at N-terminal regions of microdystrophin, trial sponsors have moved to exclude DMD patients with pathogenic mutations in exons 1–17 from participating in future gene therapy trials until more is understood. It remains to be established whether more patients will be excluded in the future due to the identification of additional immunoreactive epitopes in the dystrophin transgene. As many more DMD patients will undergo microdystrophin gene therapy in the coming years, the nature of transgene-triggered safety events and potential mitigation strategies will require further attention. As a follow-up to this meeting, MDA issued a request for grant applications to further understanding of transgene-triggered adverse events pertaining to DMD gene replacement therapy. Example research areas that may help overcome or mitigate risks associated with transgene-triggered events include: i) identification of 'at risk' DMD mutations for transgene-triggered responses; ii) identification of immunoreactive regions in the dystrophin gene; iii) re-design of the dystrophin transgene for future

gene therapies to exclude immunoreactive epitopes; iv) development of immunosuppression strategies to minimize transgene-triggered responses; v) *in vitro* assays and/or models predictive of transgene-triggered immune responses.

Another possible transgene-specific response relating to DMD gene replacement therapy was discussed by Lee Sweeney (University of Florida) based on preclinical observations of cardiac toxicity in the D2-*mdx* mouse [31], a more severe model than the original BL10-*mdx*, following administration of AAV-microdystrophin gene therapy. Treatment with microdystrophin at clinical trial doses (2×10^{14}vg/kg) resulted in dilated cardiomyopathy and reduced ejection fraction. The two proposed hypotheses for this observation point to the use of promoters that cause over-expression of transgene in the heart. The first hypothesis is that utrophin, which is thought to play a cardio-protective role, is competitively displaced by microdystrophin. The second, is that the heart is generally intolerant of exceptionally high protein expression. These preclinical findings highlight the need for active cardiac monitoring and the use of cardio-protective drugs in DMD patients undergoing gene replacement therapy. Further development of transgene promoters that bias expression in skeletal muscle over heart may also help to address potential cardiac toxicity issues.

PREDICTABILITY OF ADVERSE EVENTS USING LARGE ANIMAL MODELS

Preclinical testing of efficacy and safety associated with AAV gene therapy is routinely performed on rodent models. However, large animal models such as dogs (DMD and XLMTM models) and non-human primates (NHPs; wildtype) have also been employed for preclinical testing. The utility of dog models for testing AAV gene therapy was discussed by Joe Kornegay (Texas A&M University). In many dog studies, systemic delivery of AAV gene replacement therapy has been successful in demonstrating efficacy but has shown minimal adverse events. Systemic delivery of microdystrophin in DMD dogs did not result in any evidence of an inflammatory response (thrombocytopenia, hemolytic anemia or cardiac dysfunction) that have been noted in DMD patients. Similarly, in XLMTM dog models, there is no evidence of liver enzyme elevation or liver dysfunction that led to the death of 4 patients in the Astellas trial (ClinicalTrials.gov Identifier: NCT03199469). In contrast, intramuscular delivery in DMD dogs does elicit both a humoral and a cellular immune response, likely due to higher localized antigen load compared to systemic delivery. Barry Byrne (University of Florida) also led a discussion on the use of NHPs in pre-clinical pharm-tox studies. The question of whether we have sufficiently learned what we need to from healthy NHPs in response to AAV gene therapy was raised, and also the fact that previous NHP studies have not been as predictive of safety and adverse events in human gene therapy trials as anticipated [32]. Barry Byrne also raised the potential issue that dose-to-body weight ratio in primates may not be directly translatable to humans in determining clinical effective doses of gene therapy [33]. Collectively, these findings question the translatability of large animal models for predicting all of the possible immunotoxicity in the context of systemic gene therapy in humans. Healthy large animal models are useful in the context of biodistribution studies, but diseased models are needed to more accurately reflect the potential for adverse events.

TRANSGENE DURABILITY

The important issue of transgene durability in the context of AAV gene replacement therapy in humans remains unknown, however several influential factors have been hypothesized. Jeffrey Chamberlain (University of Washington) presented on two factors related to durability of microdystrophin gene therapy – cassette design and dose. He reported that inclusion or exclusion of specific domains of dystrophin in the transgene cassette influences the persistence in muscle fiber expression, and that vectors resulting in initial high levels of expression are not always reflective of their future durability [34]. He concluded that durability of expression cassettes cannot be easily predicted by their content and can only be established by head-to-head comparisons. Clinical considerations of durability were brought up by Kevin Flanigan (Nationwide Children's Hospital) in the context of transgene loss with continued muscle fiber degeneration and regeneration, and the potential dilution of genomes during muscle growth in treated children. Another factor discussed is the possibility of cytotoxic T-cell targeting of transgene-expressing fibers. Although this has not been observed in preclinical models, preliminary evidence reported in microdystrophin trials point towards reducing levels of transgene expression. Together, the multitude

of factors that can plausibly influence the durability of transgene expression remain speculative and will require longitudinal monitoring of transgene expression levels in treated patients.

GENE-EDITING CONSIDERATIONS

Gene-editing therapeutic strategies have gained traction since the emergence of CRISPR gene-editing technology, however their clinical translatability for systemic use remains untested for NMDs. In his talk, Charles Gersbach (Duke University) points out the advantages of CRISPR-based gene correction over gene replacement therapy [35], but also raises several outstanding issues that need further investigation. Advantages discussed include the potential to correct a spectrum of underlying mutations in the DMD gene directly, by the addition, removal or disruption of sequences within the gene. Correction strategies targeting the endogenous genetic locus will result in expression of a closer to a full length gene product than microdystrophin, while also removing concerns pertaining to episomal loss and over-expression toxicities. Editing of satellite cells *in vivo* further provides the promise of contributing a renewable pool of 'corrected' cells to existing muscle fibers. Issues that warrant further investigation include immune responses to Cas9 [36], durability of dystrophin restoration based on correcting just a relatively small subset of myonuclei and whether constitutive (or merely transient) expression of Cas9 is necessary. Dongsheng Duan (University of Missouri) reported on observations in canine models that local and systemic delivery of AAV CRISPR therapy induces a cytotoxic T cell response, unlike the response seen in microdystrophin gene replacement therapy [20]. This Cas9-induced response was shown to be independent of AAV serotype, promoter or bacterial origin of Cas9; and is thought to eliminate gene-edited fibers, thus affecting the overall durability of dystrophin expression. These findings suggest that immune responses to Cas9 may represent a critical barrier to the translation of CRISPR therapies and will require exploration of novel strategies to minimize and manage these responses.

ADDITIONAL GENE THERAPY CONSIDERATIONS

Qi Lu (Atrium Health/Wake Forest University) presented on strategies to achieve more balanced and homogenous transgene expression at lower doses. Current promoters in use result in unbalanced heart and skeletal muscle expression and high inter-fiber variability. This is speculated to result in uneven fiber correction and hence disorganized muscle contraction possibly resulting in further degeneration, thus reducing the overall efficacy and durability of the treatment. Qi Lu introduced a new promoter, MCK-Optimal, which combines different regulatory elements from MCK and other muscle-specific genes. The use of this promoter to drive FKRP expression results in homogenous glycosylated alpha-dystroglycan within single muscles at AAV dose of $1 \times e^{13}$vg/kg. Therefore, promoter optimization strategies can be used to reduce overall therapeutic dose and the subsequent toxicity and costs associated with AAV gene therapy.

Jeffrey Chamberlain presented data pointing to peculiarities in the relationship between AAV dosing levels, transduction thresholds and empty-to-full capsid ratios. At high vector doses, there appears to be a threshold level at which transduction levels can elicit a logarithmic increase. Unexpectedly, transduction levels can also be positively impacted by the presence of empty capsids, albeit a serotype-dependent phenomenon. AAV6 'empties' were shown to enhance muscle transduction but AAV9 'empties' can sometimes be inhibitory, depending on species and muscle type. The relationship between muscle transduction and total capsid concentrations was posited to aid in enabling the delivery of two-vector gene therapies with a minimal dose increase.

ETHICAL CONSIDERATIONS IN GENE THERAPY

The field of gene therapy is complex and fast changing, requiring constant evaluation of ethical issues pertaining to its clinical translation. Emma James (Encoded Therapeutics) led the discussion on issues related to ethical considerations for gene therapy clinical trials and covered topics such as: the impact on autonomy (including the complexity of potentially lifelong informed consent and assent for children participating in trials, with an inability to truly withdraw consent following a single administration therapy) understanding an evolving risk-benefit ratio, consent for autopsy, and the appropriateness of treatment prior to onset of symptoms [37]. As presented by Kevin Flanigan, a DMD patient as young as seven months of age has now

undergone AAV gene therapy (U7snRNA for treatment of exon 2 duplication at Nationwide Children's Hospital, ClinicalTrials.gov Identifier: NCT04240314). Kevin Flanigan also presented considerations pertaining to treating infants and young children with gene therapy prior to onset of symptoms. In these cases, it is difficult to assess the risk vs benefit ratio because clinicians are required to predict future disease severity and progression based on the patient's genotype alone. Are the risks of gene therapy worth taking if the resulting mutation will manifest in a mild disease phenotype? Kevin Flanigan strongly suggests that a confirmatory muscle biopsy should be taken to confirm accuracy of diagnosis before considering gene therapy. Given the emerging safety profile of gene therapies, however, it is of utmost importance that patients and families have a thorough and ongoing informed consent dialogue to understand how receiving gene therapies may affect their access to future therapies, how emerging data may affect the risk–benefit profile and required mitigation strategies, and the need for long-term follow-up not only to ensure participant safety, but also to explore and establish gene therapy safety and efficacy for the wider population and societal good [38–40].

OUTLOOK AND RECOMMENDATIONS

To fully realize the considerable potential of AAV mediated gene therapy, emerging toxicities (summarized in Table 2) need to be proactively addressed and managed based on a rational understanding of their mechanisms. In the general forward-looking discussion, several topics and concepts were identified which could accelerate safety related insights and the development of appropriate protocols and databases on a collaborative basis. Meeting chairs agreed that these initiatives would be most effective if initiated pre-competitively and supported broadly by the field and its various stakeholders.

STANDARD OPERATING PROCEDURES (SOPS), PROTOCOLS, AND REFERENCE STANDARDS

In order to create a meaningful data set that is large enough to draw actionable conclusions there is a need for comparable SOPs or common data elements (CDEs) for monitoring participants pre- and post-treatment. This meeting has begun the process of identifying clinical and laboratory parameters that are associated with the toxicities discussed. In this SOP/CDE a minimum dataset would be standardized that will be used to follow all gene therapy trials and thus make comparative safety monitoring possible. It does not exclude individual more extensive and specialized monitoring in specific trials (generating data that can lead to expansion of the SOP). The expected rise in gene therapy trials will also likely lead to an increase in the range of adverse events reported, thus advocating the need for a working group where clinicians and trial sponsors can discuss cases and make periodic updates to SOPs.

ASSAYS IN NEED OF STANDARDIZATION TO ENABLE SUCH COMPARISON OF RESULTS BETWEEN STUDIES INCLUDE FOR INSTANCE

Pre-dosing immune status is of paramount importance for assessing post-dosing immune responses, however current assays remain individualized and not directly comparable across trials. Thus standardization of assays for both neutralizing (NAb) as well as total binding antibodies, and their cut-off thresholds is necessary. The creation of reference standards to aid in reducing inter-laboratory variability for AAV titering is a high priority. Similarly, dose of capsid protein delivered and vector genomes (full, partial and empty capsids) is important to quantify in a standardized and directly comparable way. A set of standard immunomodulatory protocols and strategies for intervention should be developed and used as starting reference standard and a starting point for comparative studies and further development.

REPOSITORIES AND DATABASES

The SOP/CDEs developed (see above) should ideally form the basis for a de-identified database, in which reporting safety events across studies that can then be linked to a minimal dataset describing the vector, dose, administration, and co-medications. A collaborative database model which includes participation from trial sponsors and mediation by a neutral third-party stakeholder would serve as a powerful resource for aggregated analysis of safety data to reveal trends, susceptibilities, and drug class effects, and would allow sponsors to react in real time to safety signals generated in other trials. Alternatively, a voluntary adverse event reporting database for patients can also be explored.

CENTRALIZED COLLECTION OF SERUM AND DNA FROM PARTICIPANTS IN NMD GENE THERAPY TRIALS

Discussions from this meeting have made it clear that there are individual and likely predominantly genetic differences in susceptibility to safety events that would be important to identify in order to institute individualized risk assessment and management. Considerations include the collection and storage of biosamples from each patient dosed with AAV, including DNA for whole genome sequencing in every participant in an AAV gene therapy trial. The proposed genome sequence database can subsequently be linked with the individual safety responses ascertained using the SOPs and database discussed above. Analysis would occur on an iterative basis until the database has enough depth to identify risk genotypes that might emerge for certain toxicities, such as genetic variants in the complement pathway susceptibility alleles for TMA [41].

CONCLUDING REMARKS

Gene replacement therapy via AAV is a rapidly growing field, both in clinical practice as well as in clinical trials) with many hundreds of patients anticipated to undergo such therapy for a range of diseases in the coming years. The outcome of trials thus far demonstrates that this therapeutic approach carries a lot of promise, but can be linked to considerable clinical hazard in some patients, prompting a need to better understand the nature of these safety events and potentially identify at-risk individuals. Frequent and ongoing discussions amongst stakeholders regarding clinical observations and mitigation strategies are therefore recommended to minimize the potential risk to patients and increase chances of conducting a successful trial. In recognizing the importance of these discussions for the translation of genetic therapies in the NMD community, MDA will host a follow-on summit in 2023, allowing stakeholders to provide updates and progress in overcoming the challenges associated with gene therapy.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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