Molecular Therapy
Perspective

Broader Implications of Progressive Liver Dysfunction and Lethal Sepsis in Two Boys following Systemic High-Dose AAV

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By virtue of their transformational power, the technologies at the core of the gene therapy community have brought widespread public attention to the unique burden of previously neglected orphan diseases. On June 23, a report from Audentes Therapeutics of the tragic death of two patients with X-linked myotubular myopathy (XLMTM)[1] brought into ever sharper focus an added dimension of this burden. XLMTM is recognized for its devastating prognosis in terms of standardized metrics, including disability-adjusted life years and healthy life expectancy. Highly publicized reports of the earliest results of Audentes' ASPIRO gene therapy trial revealed a spectacular therapeutic response. Both the specifics and the broader context of the unexpected adverse events illustrate why, for patients and their families, the stakes have never been higher as the limitations of initially promising therapies become fully known only through advanced phase clinical trials. Transparency to enable a better understanding of the clinical pathology will be essential to inform the risk-benefit calculus for XLMTM patients contemplating enrollment in future trials, but also for the broader community to enable rationale design of improved gene therapy vectors.

The specifics of Audentes' letter addressed to the "XLMTM Patient Community" can be briefly summarized: two of the three older patients in the AT132 (AAV8-MTM1) high dose cohort (3×10^{14} vg/kg) died following clinically similar courses, approximately 4–6 weeks post-administration, in the setting of progressive liver dysfunction and sepsis. The observation of serious adverse events (SAEs) of hepatobiliary disease included all three of the older patients in this dose cohort. Four of six patients previously treated at 1×10^{14} vg/kg had no liver SAEs, despite previous histories of hepatobiliary disease associated with XLMTM.

Understanding the broader context of these SAEs is critical to defining the pathway forward. It begins with the devastating disease first described by Spiro et al.[2] in 1966 and christened myotubular myopathy on the basis of the histological appearance of the centronuclear myocytes resembling fetal myotubes. The protein product of the X-linked gene (MTM1), myotubularin, is a ubiquitously expressed phosphatidylinositol 3-phosphate (PI(3)P) phosphatase.[3,4] The resulting lipid second messenger is involved in endosome trafficking, autophagy, desmin filament architecture, mitochondrial dynamics, muscle excitation-contraction coupling, and regulation of the ubiquitin-proteasome pathway.[5,6] The spectrum of XLMTM clinical phenotypes was first broadly characterized 20 years ago with an estimated 50% mortality by 18 months of age. Recent refinement[7] revealed improvement in survival to a median age of 12 years through long-term mechanical respiratory support but re-affirmed the extraordinary burden of disease, with 87% of patients non-ambulant with heavy dependence on gastrostomy tube feeding.

As with the majority of myopathies and muscular dystrophies, there remain major gaps in our understanding of XLMTM pathogenesis. Nonetheless, establishment of a canine model from a naturally occurring missense mutation[8] provided an opportunity to explore investigational therapies agnostic to the exact function of the gene product.[9,10] Unlike dystrophin, the 71.6 kDa molecular weight of myotubularin puts its 2 kb cDNA well within the cloning capacity of adeno-associated virus (AAV) vectors, facilitating a remarkably rapid progression of critical translational steps. The efficacy of systemic gene delivery using an AAV8 vector with the human desmin promoter driving MTM1 expression was unprecedented for a large animal disease model, with a dramatic survival advantage noted in the earliest treatment cohort. Audentes Therapeutics moved rapidly to license and commercialize the investigational therapy based largely on patent US20140249211A1, selecting doses of 1×10^{14} and 3×10^{14} vg/kg for systemic delivery. Publicly available documents describe the company's rationale for shifting from the baculovirus/sf9 insect cell platform used for the pivotal preclinical studies to the suspension 293 cell platform. In 2019, the company reported that "treated patients across both dose cohorts show significant reductions in ventilator dependence and the progressive attainment of developmental motor milestones," in support of previously announced plans to work "with the FDA to advance AT132 to registration as quickly as possible." Audentes announced plans for a large vector manufacturing facility in Sanford, NC. As of June 30, multiple news outlets reported that, in response to the deaths cited above, the US Food and Drug Administration (FDA) had placed a clinical hold on further development of AT132, with Audentes postponing

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plans to submit a Biologics License Application.

This tragic turn of events graphically illustrates the scope and magnitude of challenges in biologics development and regulation and, above all, the emotional turmoil faced by the most critical community of stakeholders. AT132′s rapid development to this point was driven, in large part, by the level of organization and commitment of the XLMTM community and the Joshua Frase Foundation, as illustrated by the identification of the canine model. What can be learned from these devastating SAEs to rapidly yet rationally mitigate the risk to XLMTM patients for whom the durability, efficacy, and safety of future treatment may hinge on seemingly minor adjustments in vector design?

In view of the reported timing, tissue-specificity, and dose-dependency, plausible mechanisms for lethal toxicity must emphasize hepatotoxicity from the vector capsid or transgene product, perhaps driven by adaptive and/or innate immune responses. Although the investigator brochure is not in the public domain, the 3×10^{14} vg/kg dose of AAV vector is the highest ever used in humans. This suggests that the authorized interval of close monitoring between patients injected within this cohort would have been at least 4 weeks, providing circumstantial evidence against an innate immune response as the primary etiology. Interpretation is further complicated by an aspect of the disease that is poorly understood, not seen in animal models, and rarely reported in other human myopathies: a significant proportion of XLMTM patients have underlying liver disease. In its extreme form, peliosis hepatis, there is risk of exsanguinating hemorrhage. Prior reports emphasize the morbidity/mortality of opposing outcomes: forensic examination of the liver at autopsy to rule out death by suspected caregiver abuse[11] or survival by virtue of emergency, living-related donor liver transplantation.[12] The ASPIRO trial was designed to mitigate this risk by excluding subjects with "clinically significant underlying liver disease" (ClinicalTrials.gov: NCT03199469), yet four of the first six patients enrolled were cited in the Audentes letter as having previous histories of hepatobiliary disease.

As with other AAV vectors under clinical investigation at high systemic dose for neuromuscular disease (AAV9 and Rh74), the AAV8 capsid serotype was initially identified and commercially developed on the basis of its high tropism for the liver. It is notable that, in a follow up study in the XLMTM (MTM1-N155K) canine model, theoretical concern was expressed about "potential off-target effects, such as in the liver," which might be addressed by selective tissue de-targeting with other capsids and/or promoters.[10] Furthermore, it was noted by these authors that the dose-response study was limited to 0.3×10^{14}, 2.0×10^{14}, and 5×10^{14} vg/kg, with the latter two doses showing dose-dependent increases in vg content in most tissues, but indistinguishable in terms of gait speed at 17 weeks, and no data for 1.0×10^{14}. However, there are no published reports of hepatotoxicity in the murine or canine XLMTM models following AAV8.-Des.MTM1 treatment.

The mutational spectrum in human XLMTM includes deletions, frameshifts, and early nonsense codons. Thus, as with the various canine models for Duchenne muscular dystrophy (DMD), the possibility remains that central immunological tolerance to the full length product of the canine MTM1 p.N155K allele might mask adaptive immunotoxicity.[13] In canine XLMTM, AAV8 had higher tropism for the left ventricle and liver than any other tissues, including skeletal muscle,[10] and, in the ASPIRO clinical trial, 3 of the first 10 patients had laboratory findings indicative of myocarditis or cholestasis.[14] Interestingly, myotubularin is the first identified member of a family of 14 paralogs or "MTMR"s. Although MTM1 is ubiquitously expressed, disruption of excitation-contraction coupling is unique to skeletal muscle, suggesting that cardiac myocytes are unaffected because they co-express additional isoforms, e.g., MTMR14, which is also involved in the trophic response to hemodynamic load. Perhaps substitution of an MTMR for MTM1 would eliminate the risk of adaptive immunotoxicity, as with utrophin for DMD in the setting of deletional/frameshift loss of dystrophin.[13]

The most serious of unanticipated adverse events in gene/cell therapy clinical trials can take years to decipher, integrating information from forensic pathology and hypothesis-driven mechanistic dissection.[15,16] Meanwhile, an added dimension of the burden for the XLMTM community is acknowledging the potential impact of their advocacy for alternative paths forward in the face of grave uncertainty, e.g., vector modification requiring new preclinical studies versus continued investigation of AAV8.Des.MTM1 at the 1×10^{14} vg/kg dose. A major factor in the risk tolerance of research subjects is the individualized perception of time, as can be framed by survey instruments within the stakeholder community, to weigh the high morbidity and lethal natural history of the disease against informed estimates of the risks of accessing the only potentially life-improving or saving investigational therapy on the immediate horizon. As soon as possible, the findings of clinical toxicology must be made public to accelerate the development of novel vectors with the expectation of a substantially improved therapeutic index. Meanwhile, the inclusion/exclusion criteria, informed consent, and data monitoring protocols must be overhauled and continuously updated to integrate the implications of new findings, with revised estimates of risks and potential benefits to XLMTM research subjects at specific vector doses.

One change that the entire field of gene therapy would benefit from is increased disclosure in cases of fatal SAEs, as in the current study. Companies, clinicians, and the FDA would all be able to apply the hard-won lessons learned from these negative studies to improve the design and testing of future constructs. We appreciate the sensitivity of disclosing detailed results of company-sponsored studies, but mechanisms to protect confidentiality while disseminating life-critical information can be developed and will also be critical on a global scale in the months ahead with the rapid evaluation of candidate COVID-19 vaccines against the risk of enhanced respiratory disease.[17]

CONFLICTS OF INTEREST

L.M. is a PhD thesis student working under the joint mentorship of J.B. and H.H.S. and has been evaluated for participation in a clinical trial for Fascioscapularhumeral muscular dystrophy. Y.G. is an undergraduate at the University of Pennsylvania and is currently enrolled in a clinical trial for an experimental therapy for Duchenne muscular dystrophy. J.B. has received grants from the Foundation Fighting Blindness, the National Institutes of Health, and Spark Therapeutics; non-financial support from the Center for Advanced Retinal and Ocular Therapeutics, University of Pennsylvania, and the FM Kirby Foundation; and has a provisional patent pending and a US patent licensed to Spark Therapeutics, for which she has waived financial interest. H.H.S. has received grants from the Muscular Dystrophy Association and National Institutes of Health and is an inventor on provisional and issued patents licensed to StrongHolt Therapeutics and 4MVac, for which he has served as a co-founder.

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