TITLE: Systemic MV Gene Therapy Close to Clinical Trials for Several Neuromuscular Diseases

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Systemically delivered AAV gene therapies close to clinical trials for several neuromuscular diseases.

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While there have been a number of gene therapy clinical trials using adeno-associated viral (AAV) vectors, the majority were safety studies involving local intramuscular delivery; to date only one has involved systemic delivery, AAV-SMN for spinal muscular atrophy type 1 (Table 1). Likewise, many of the studies in large animals have focussed on local or regional delivery (e.g. Qiao et al. [5] and Le Guiner et al. [6]) or have examined the effects of systemic delivery in young puppies (e.g. Yue et al.[7]). In this issue of *Molecular Therapy*, Mack and colleagues [8] employed systemic intravenous delivery of an AAV serotype 8 (rAAV8) vector expressing the canine myotubularin (cMTM1) gene in the p.N155K canine model of X-linked myotubular myopathy (XLMTM). Skeletal muscle and cardiac expression was ensured by a muscle-specific desmin promoter. Importantly, they treated juvenile dogs with clinical signs of the disease and showed a clear dose-response to systemic treatment in terms of levels of expression of cMTM1 and the functional consequences of such expression. The effect of AAV mediated gene therapy was to prolong life substantially with doses of 4×10^{14} vector genomes per kilogram (medium dose) or greater (high dose). Other outcome measures included global neurological function, spinal reflexes, forelimb extensor and flexor strength, hindlimb extensor and flexor strength, gait speed, stride length, peak inspiratory flow and histopathology, all of which improved in the medium and high dose groups.

XLMTM is a consequence of mutations in the MTM1 gene and the associated deficiency of myotubularin, a 3-phosphoinositide phosphatase enzyme. Most XLMTM patients develop severe muscle weakness leading to respiratory failure and death, typically under 2 years of age. The study by Mack and colleagues [4] not only sets a potential dose for human clinical trial but also carefully documents the differences in vector biodistribution and expression of myotubularin between different skeletal muscles in the canine model. Indeed, the muscle maps of these features are in themselves works of art.

This study follows up a regional delivery trial of the same vector in the same model [5]. The previous study had shown remarkable results from a regional infusion of AAV8-cMTM1, leading to a halt in the progression of the disease, dramatically improved muscle strength, and a dramatically extended survival. That the regional infusion had a body-wide effect was a surprise and the authors speculated that the widespread delivery might have been due to an inefficient tourniquet or the presence of substantial amounts of the AAV that could enter the circulation after release of the tourniquet. From this previous study, it appeared that relatively small levels of myotubularin expression are sufficient for rescue of the pathological phenotype, and this was confirmed in the present study.

Few studies are without flaws. Randomisation lead to a preponderance of females in the control groups, while experimental groups were predominately male and this may have affected the statistical analysis. However, as the authors note, the study was not sufficiently powered for robust statistical analysis, a common problem with breeding mutant dog colonies. Although the authors report no difference between affected homozygous females and hemizygous males, there are

different hormonal profiles between the two sexes that might influence aspects of the manifestation of disease. Estrogen has implicated in muscle membrane stability (e.g. [6]) and testosterone has a well-known influence on muscle fibre size (e.g. [7]). Such differences might translate into functional differences between the treated and control groups. Thus, the reader might wish to focus on the comparisons between the different treatment groups, which show a clear dose response for many of the outcome measures performed with the dogs.

Dog models have proven invaluable in the development of gene therapies for haemophilia [8] and are likely to prove so for XLMTM, Duchenne muscular dystrophy (DMD), and other neuromuscular disorders. Quite recently, body-wide but patchy expression of a highly engineered microdystrophin was demonstrated in a dog model of Duchenne muscular dystrophy (DMD) treated with intravenous AAV as juveniles [9]. Importantly the DMD dog study also did not report any safety concerns. Of course, it is important to recognise where there are differences between animal models and the human condition. Although the dog model of XLMTM shows many features of the human condition, it develops respiratory problems in the later stages of the disease whereas infants with XLMTM present with respiratory failure at birth. Consequently, the dramatic amelioration of the disease seen in the murine and canine models of XLMTM may not precisely replicated by a similar improvement in man.

The current study lays the foundation for a human clinical trial of systemic AAV-MTN in myotubular myopathy. In parallel, there are a number of groups that propose to undertake human clinical trials of systemic AAV microdystrophin delivery for the treatment of DMD, although none are registered to date on https://clinicaltrials.gov/. Enthusiasm for such trials follows the presentation at the October 2016 Annual Congress of the World Muscle Society given by Jerry Mendell for the results of the AAV-SMN1 treatment of SMA type 1[10]. The presentation reported a good safety profile and an improved clinical outcome, especially in those infants treated at the earliest ages.

All of the above information points towards a likely rapid increase in the number of clinical trials using systemic delivery of AAV-mediated gene therapies, and the next few years could see a resurgence of interest and potentially promising results for gene therapy of neuromuscular disorders.

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Table 1

Clinicaltrials.gov identifier	**Trial**	**Route**	**Associated publications**
NCT00428935	Safety Study of Mini-dystrophin Gene to Treat Duchenne Muscular Dystrophy	Intramuscular	[1]
NCT00494195	Gene Transfer Therapy for Treating Children and Adults With Limb Girdle Muscular Dystrophy Type 2D (LGMD2D)	Intramuscular	[2]
NCT01344798	Clinical Study of AAV1-gamma-sarcoglycan Gene Therapy for Limb Girdle Muscular Dystrophy Type 2C	Intramuscular	none
NCT01519349	Follistatin Gene Transfer to Patients With Becker Muscular Dystrophy and Sporadic Inclusion Body Myositis	Intramuscular	[3, 4]
NCT02122952	Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1	Systemic (IV)	ongoing
NCT02354781	Clinical Intramuscular Gene Transfer of rAAV1.CMV.huFollistatin344 Trial to Patients With Duchenne Muscular Dystrophy	Intramuscular	ongoing

A summary of human clinical trials of AAV based gene transfer in neuromuscular diseases**.**