Gene replacement rescues severe muscle pathology and prolongs survival in myotubularin-deficient mice and dogs

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We previously reported in *Science Translational Medicine* (*STM*) unprecedented results (1) from gene replacement experiments in *MTM1*-mutant animal models of X-linked myotubular myopathy (XLMTM, OMIM 310400) (2). This devastating congenital muscle disorder results from deficiency of myotubularin, a phosphatidylinositol-3-phosphatase required for skeletal muscle function, growth and ultrastructural organization (3-6). The overall median survival of XLMTM patients is only 29 months (7) and the majority of patients who survive beyond two years require ventilator support (8). While intensive medical support extends the life of young patients, there is no effective treatment. Of therapeutic approaches tested in animal models of XLMTM, gene replacement targets the underlying cause of this monogenic disorder (9). Analogous to human XLMTM patients, mice engineered with a targeted deletion in the *Mtm1* gene demonstrate marked muscle weakness and shortened lifespan (4). A naturally occurring *MTM1* missense mutation in dogs results in a similar phenotype with reduction of muscle strength and lifespan (10). As we reported in *STM*, myotubularin-deficient mice and dogs both responded to systemic and quasi-systemic administration of a recombinant serotype 8 adeno-associated virus (AAV8) carrying full-length murine *Mtm1* or canine *MTM1* coding sequence. Both mouse and dog models responded within a few short weeks with rapid and sustained increases to near normal levels of muscle strength and prolonged survival.

As sometimes occurs in science, initial failures can lead to subsequent successes. In our case, systemic AAV8-Mtm1 vector injections in myotubularin deficient mice resulted in body-wide muscular improvement. This result encouraged us to test first the functionality of an AAV8-MTM1 vector by intramuscular delivery in three *MTM1*-mutant dogs. We first noted robust and rapid muscle size enlargement and strength gains in the injected muscles. We next administered the vector using regional limb perfusion to three additional *MTM1*-mutant dogs. The idea was to isolate, via a tourniquet, venous blood flow to the entire hind limb musculature, infuse the AAV8-MTM1 vector and then compare findings with the non-infused opposite limb. The first *MTM1*-mutant dog responded as predicted: large gains of strength and muscle mass only in the infused limb. The non-treated contralateral limb remained in a weakened state. Subsequently, we attempted to replicate these findings using regional hindlimb infusion in two other *MTM1*-mutant dogs. The result was an "experimental failure" but a "therapeutic success". Regional limb infusion resulted in systemic distribution of the vector in skeletal musculature with subsequent body-wide expression of the *MTM1* transgene. These two treated *MTM1*-mutant dogs remain in the canine colony today and appear healthy and robust 3 years after AAV-MTM1 vector infusion.

To our knowledge, ours was the first report of successful body-wide gene replacement in a congenital muscle disease that resulted in amelioration of severe muscle pathology and prolongation of life in both rodent and canine models. Why did this gene replacement approach rescue severe muscle pathology and prolong survival in myotubularin-deficient animals when similar approaches in dystrophin-deficient animal models failed to achieved such dramatic improvement? A number of characteristics of XLMTM might provide a few clues. First, the protein product of *MTM1*, myotubularin, is a low abundance enzyme in contrast to dystrophin, an abundant cytoskeletal protein, whose structural role in myofiber maintenance is dependent on sustained expression at high levels (11,12). Second, the entire *MTM1* gene coding sequence easily meets current packaging capacity for AAV without the need for generation of mini or micro-versions, which is the case for dystrophin, that might impair the function of the gene product (13). Third, the architecture of muscle tissue in XLMTM generally remains intact without replacement by fat and scar tissue typically seen in DMD. Together, these differences may point to the idea raised by Dr. Pierson (14) suggesting that non-dystrophic myopathies may provide a more approachable tissue landscape for gene replacement therapy compared to degenerative processes typically seen in muscular dystrophies.

Our findings raise additional questions to address in animal models of XLMTM. Topics include identifying a safe dose-response for simple intravenous systemic infusion of AAV. Ideally, a wide dosing range between a minimally effective low dose and a log-scale increase to a high dose of AAV8-MTM1 would provide a margin of safety when translated into a dosing for human patients. Another topic of importance concerns the question of effectiveness in older patients with advanced disease. Our recent data established that a single systemic treatment with AAV8-*MTM1* resulted in long-term survival and rescued muscle function in both XLMTM mice and dogs. Strikingly, the phenotype of adult mice with severe and advanced disease was also corrected by a single intravenous infusion of AAV8-Mtm1. These observations in the murine and canine models have now led to a clinical trial development program for systemic AAV8-MTM1 gene therapy in human XLMTM patients. We hypothesize that modest levels of myotubularin will suffice to sustain long-term functionality of striated muscles throughout the body, including the vital respiratory muscles. While systemic disease progression was halted after AAV8-MTM1 infusion, and to some extent reversed in young MTM1-deficient dogs, we hypothesize that the disease can be reversed in adult full-sized dogs. Thus, the next most pressing research question to address is simple: can advanced disease be ameliorated in the dog as we have observed in knockout mice? Because dogs are similar in size and closer physiologically to patients compared to mice, experiments in dogs are exceptionally informative for clinical trial design. We are currently using the canine system to test this hypothesis and to optimize dosing while assessing potential safety concerns for full-sized animals that can no longer ambulate and have impaired breathing. Unlike dystrophic muscles undergoing replacement with fat and scar tissue, myotubularin-deficient muscles are hypotrophic but do not become infiltrated with fat and connective tissue. Thus, reversal of the disease is possible even in advanced cases, as seen in *Mtm1*-knockout mice with end-stage disease that completely recovered after a single dose of AAV8-Mtm1. If AAV8-MTM1 can reverse advanced disease in dogs as it has in mice, the future for effective patient therapy appears even brighter.

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Footnote

Conflicts of Interest: The authors are inventors of a patent on gene therapy for myotubular myopathy and are paid members of the scientific advisory board for Audentes Therapeutics.

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