Microdystrophin and follistatin combinatorial gene delivery to treat a severe mouse model of Duchenne muscular dystrophy

T.D. Colgan,[1] K.T. Murphy,[2] T. Kennedy,[2] D. Ham,[2] T. Naim,[2] A. Chee,[2] M.N. Nguyen,[3] H. Qian,[1] J.S. Chamberlain,[4] G.S. Lynch[2] and P. Gregorevic,[1,4,5] [1]Laboratory for Muscle Research & Therapeutics Development, Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3994, Australia, [2]Basic and Clinical Myology Laboratory, The University of Melbourne, Melbourne, VIC 3010, Australia, [3]Experimental Cardiology, Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3004, Australia, [4]Department of Neurology, The University of Washington School of Medicine, Seattle, WA 98105, USA and [5]Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800, Australia.

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder that results in ambulatory reduction of affected children and premature death from cardiac and/or respiratory failure. DMD is caused by a variety of mutations that result in the loss of, or the production of, an aberrant dystrophin protein. As DMD is a single gene disorder, gene therapies have been pursued with the intention of restoring dystrophin expression in order to ameliorate the dystrophic pathology. Gregorevic *et al.* (2006) demonstrated the efficacy of a recombinant adeno-associated virus serotype 6 (rAAV6) systemic delivery of microdystrophin (rAAV6:microdystrophin). Though the truncated dystrophin gene increased muscle strength and longevity of treated dystrophin$^{-/-}$:utrophin$^{-/-}$ (double knockout: *dko*) mice when compared to their untreated littermates, wild-type levels of strength and lifespan were not obtained. Alternate strategies that increase muscle mass and strength have subsequently been pursued. Follistatin binds and inhibits TGF-β ligands myostatin and activin, which are negative regulators of muscle mass. Follistatin has been recently confirmed to mediate increases in muscle growth and strength after systemic delivery (Winbanks *et al.*, 2012). We tested the hypothesis that co-delivery of follistatin with microdystrophin would increase muscle strength and ameliorate the dystrophic pathology to a greater extent than either gene delivered in isolation.

All experiments were conducted in accordance with the code of practise for the care and use of animals for scientific purposes, as stipulated by the NHMRC. To assess the effectiveness of the chosen gene therapy, healthy, mildly affected and severely affected mice were administered intramuscularly with rAAV6:FST317 (follistatin). While wild-type and mildly affected *mdx* mice responded with increased myofibre diameter, severely affected *dko* mice concluded the study with an increase in myofibre number; an unexpected result. Mice were treated systemically to determine whole body physiological effects. Systemic administration of follistatin was seemingly associated with an increase in *dko* mice unable to reach the experimental endpoint. To circumvent this obstacle, follistatin expression was delayed using an inducible DNA element. The combined delivery of inducible follistatin and microdystrophin to *dko* muscles increased myofibre diameter, paralleling the response of *mdx* mice to an intramuscular delivery of follistatin. Functional assessment of diaphragm and skeletal muscle was also performed subsequent to an intraperitoneal injection of the anaesthetic sodium pentobarbital (60 mg/kg). Delayed combinatorial delivery was identified to increase the maximum force producing capacity of hindlimb muscles above that of microdystrophin treatment alone, providing crucial evidence supporting the hypothesis.

Delaying follistatin expression in concert with a dystrophin-replacement strategy is an effective and promising method of increasing muscle strength in severely dystrophic mice. These results highlight factors to consider when developing therapeutics, including that the efficacy of potential interventions is influenced by the severity of the disease model. Our findings also emphasise the potential for a combinatorial gene therapy to increase patient's quality of life, whereby dual treatment of both microdystrophin and delayed follistatin expression could increase both mobility and independence of patients with DMD.

Gregorevic P, Allen JM, Minami E, Blankinship MJ, Haraguchi M, Meuse L, Finn E, Adams ME, Froehner SC, Murry CE, Chamberlain JS. (2006) *Nat Med*, **12**, 787-789.

Winbanks CE, Weeks KL, Thomson RE, Sepulveda PV, Beyer C, Qian HW, Chen JL, Allen JM, Lancaster, GI, Febbraio MA, Harrison CA, McMullen JR, Chamberlain JS, Gregorevic,P. (2012) *J Cell Biol*, **197**, 997-1008.