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The absence of dystrophin in Duchenne Muscular Dystrophy (DMD) muscle cells results in increased membrane permeability and subsequent intracellular calcium overload. The dysregulation of calcium homeostasis that characterizes the DMD models is exacerbated by the increased activity of the Sodium/Hydrogen Exchanger 1 (NHE1). NHE1 overactivity leads to an increased influx of sodium, which in turn switches the Sodium/Calcium Exchanger (NCX) into reverse mode, resulting in an increased calcium influx. Selective NHE1 inhibitors can be used to reduce the sodium influx and thereby revert the NCX to normal mode with a subsequent decrease in the cellular calcium load. This observation has led to the hypothesis that the use of specific NHE1 inhibitors could improve the calcium homeostasis and alleviate pathology in DMD muscle. The current study seeks to demonstrate the efficacy of a specific NHE1 inhibitor that has a good safety and potency profile in several pre-clinical studies. During the first part of the study, the drug action will be verified using in vitro pH, sodium-flux and calcium-flux fluorescent assays. Isolated muscles from *mdx* and control mice will be enzymatically and physically disaggregated and single muscle fibres will then be used for the in vitro assays. Fibres isolated from *mdx* mice will be treated with the drug in order to unravel its impact on intracellular H^+, Na^{2+} and Ca^{2+} concentrations. For the second part of the study, *mdx* mice treated chronically with drug in chow will be monitored in vivo by manganese enhanced magnetic resonance imaging (MEMRI) for calcium uptake in both skeletal and cardiac muscles. The imaging study will be supported by functional muscle testing and tissue analysis. The proposed studies with a first prototype NHE1 inhibitor are an important step towards potential clinical trials for dystrophinopathies with this class of compounds.

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G.P.355

Adiponectin, a powerful brake on skeletal muscle inflammation and injury

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The hormone adiponectin (ApN) is decreased in metabolic syndrome, where it plays a key pathogenic role. ApN exerts anti-inflammatory effects on skeletal muscles in mice exposed to acute (lipopolysaccharide injection) or chronic inflammation (obesogenic diet). Here we investigated whether ApN could be sufficiently potent to counteract a severe degenerative muscle disease. To this end, *mdx* mice (a model of Duchenne muscular dystrophy, where dystrophin is absent and a severe inflammation worsens the disease) were crossed with transgenic mice overexpressing ApN in order to generate *mdx*–ApN mice. Circulating ApN was markedly diminished in *mdx* mice. Replenishment of ApN strikingly reduced muscle inflammation, oxidative stress, and enhanced the expression of myogenic differentiation markers in *mdx*–ApN mice. Moreover, *mdx*–ApN mice exhibited higher global muscle force and endurance along with decreased muscle damage as quantified by curtailed extravasation of Evans Blue Dye in myofibers. These beneficial effects of ApN were also recapitulated in human myotubes in vitro. ApN seems to mediate its protective effects via the AdipoR1 (ApN main muscle receptor) and AMPK-SIRT1-PGC-1α signalling pathway, which leads to a downregulation of NF-κB activity and the expression of inflammatory genes along with an upregulation of utrophin in muscle fibers. Utrophin, an endogenous homolog of dystrophin, will thus help rescue the dystrophic phenotype. Adiponectin proves to be an extremely powerful hormone capable of protecting the skeletal muscle against inflammation and injury, thereby offering new therapeutic interests for dystrophinopathies.

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Preclinical data for a novel Toll-like receptor antagonist in *mdx* mice support its clinical development as a potential treatment for disease-related muscle inflammation in Duchenne muscular dystrophy

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Immunologically triggered inflammatory processes play a central role in the pathogenesis of Duchenne muscular dystrophy (DMD). Toll-like receptors (TLRs) are key components of the innate immune system involved in regulating inflammation and adaptive immunity. Data have shown that TLR 7 is significantly over-expressed in DMD patients at all stages of the disease, suggesting TLR activation is an early trigger of muscle inflammation. Targeting of TLRs is a novel therapeutic approach for DMD that has the potential to reduce chronic muscle inflammation and slow disease progression across a broad patient population regardless of dystrophin mutation type. IMO-8400 is a novel oligonucleotide-based antagonist of TLRs 7, 8 and 9 that was generally well tolerated and demonstrated anti-inflammatory activity in patients with autoimmune disease. To evaluate in vivo activity in a DMD model, we conducted multiple studies in *mdx* mice. Across studies, treated mice received one of several TLR antagonist regimens by i.p. injection for 5 weeks, and were assessed on measures of muscle function, muscle histology, biochemical response, and cytokine gene expression. Treatment resulted in significant reductions in muscle inflammation, serum creatine kinase, and gene expression of IL-6. An increase in the specific force of isolated extensor digitorum longus muscle was also observed. Detailed results from these preclinical studies will be presented. DMD is a genetic neuromuscular disorder in which immunologically triggered inflammatory processes play a central role in disease pathogenesis. TLRs are key drivers of inflammation and are over-activated in DMD patients at all stages of the disease. A TLR antagonist improved disease-associated measures in *mdx* mice. Collectively, these data support advancing the TLR antagonist drug candidate IMO-8400 into clinical development as a potential non-steroidal anti-inflammatory treatment for DMD patients.

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G.P.357

Early expression of ΔCH1 dystrophin isoform reverses or prevents muscular dystrophy in the Dup2 mouse

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We recently demonstrated that truncation of the dystrophin reading frame upstream of exon 5 induces alternate translation initiation from an internal ribosome entry site (IRES) which is active in the absence of exon 2 but not in the presence of a duplicated exon 2. The resulting N-truncated dystrophin (delCH1) is highly functional as demonstrated both in patients expressing it and in our DMD mouse model carrying a duplication of exon 2 (Dup2) in which expression of the delCH1 protein reduces muscle deficiency despite lacking half of the actin-binding domain 1. To assess the properties of delCH1, we used bioinformatic prediction tools and in vitro studies using a recombinant delCH1 to evaluate actin binding and stability. Our data show that delCH1 is stable and seems to bind actin. We next investigated whether this isoform is functional in