

P25 Poster
Utrophin luciferase knock-in mouse model for *in vivo* assessment of drug efficacy in preclinical trials for utrophin upregulation

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Duchenne muscular dystrophy (DMD) is a severe muscle wasting disorder caused by mutations in the cytoskeletal protein dystrophin. By pharmacologically upregulating the dystrophin-related protein utrophin, our aim is to develop a therapy for DMD by reconstructing the dystrophin-associated protein complex. BMN-195 (SMT C1100) – the lead compound identified from our recent small compound screening programme – has recently entered Phase I trials in humans. Preclinical screening for utrophin upregulation in the *mdx* mouse is complicated by large variations in background levels of utrophin. In order to circumvent this problem and expedite the preclinical screening process we have generated a new mouse model in which a luciferase reporter has been knocked into one utrophin allele. The reporter is under the control of the endogenous utrophin regulatory region including both promoters A and B. The other allele remains intact to provide all the therapeutic utrophin necessary to compensate for the lack of dystrophin. Quantification of the level of luminescence being emitted from this model *in vivo* during a drug trial will enable assessment of drug efficacy without having to sacrifice the animal and terminate the trial. Variation in endogenous utrophin between littermates can be assessed prior to trial enrolment. The ability to prolong the length of promising trials and prematurely terminate those where efficacy is low should dramatically improve the throughput of *in vivo* preclinical drug trials for utrophin up-regulation in the *mdx* mouse.

P26 Poster
Rescu of severely affected dystrophin/utrophin deficient mice by morpholino-oligomer mediated exon skipping

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Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the dystrophin gene that result in the absence of functional protein. Antisense-mediated exon skipping is one of the most promising approaches for the treatment of DMD because of its capacity to correct the reading frame and restore dystrophin expression which has been demonstrated *in vitro* and *in vivo*. In particular, peptide-conjugated morpholino oligomers (PPMO) have recently been shown to induce widespread high-levels of dystrophin expression in the *mdx* mouse model.

In this study, we have investigated for the first time the therapeutic potential of PPMO in the utrophin/dystrophin double-knockout mouse (dKO) which is a much more severe and progressive mouse model of DMD. Repeated intraperitoneal injections of a PPMO targeted to exon 23 of dystrophin pre-mRNA in dKO mice induce a near-normal level of dystrophin expression in all muscles examined, except for the cardiac muscle, resulting in a considerable improvement of their muscle function and dystrophic pathology. PPMO treatment strikingly prevented kyphosis and contractures in dKO mice and remarkably improved their motility. Treated dKO mice showed almost return to normalcy for most of the examined parameters as well as an extended lifespan, suggesting great potential for PPMO in systemic treatment of the DMD phenotype.

P27 Poster
Chronic long term administration of phosphorodiamidate morpholino oligomer profoundly ameliorates activity, muscle strength and phenotype in dystrophic *mdx* mice

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DMD is characterized by premature termination of dystrophin translation and the administration of properly designed antisense oligonucleotides (AOs) can restore the correct reading frame in the dystrophin transcript. This approach in humans can potentially convert the DMD to the milder Becker Muscular Dystrophy phenotype. Due to the nature of this approach, chronic administration of AOs for all the life of the patient would be necessary. The phosphorodiamidate morpholino oligomer (PMO) is one of the most promising AO chemistries thanks to the high affinity to the sequence target and the resistance to endonucleases which reduce the number of administrations and allow a long lasting exon skipping.

In this study *mdx* mice were systemically treated with 2 different dosages distributed in 20 injections in a time of 12 months: a low dose which represents a clinically applicable amount of PMO and a high dose to verify the eventuality of toxic effects.

PMO was systemically injected in 6 weeks old animals. Mice were sacrificed 4 and 12 months after the beginning of the treatment. Skeletal muscles showed widespread dystrophin expression and significant histological improvement. Creatine kinase assay, *in situ* force measurement of muscle strength and open-field behavioural activity monitoring test showed a substantial amelioration of the dystrophic phenotype. Biochemical assays demonstrated no toxic effects after long term PMO administration.

Our results support the clinical feasibility of this approach with naked PMO.

P28 Poster
Muscular dystrophy begins early in embryonic development deriving from stem cell loss and disrupted skeletal muscle formation

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By examining embryonic myogenesis in two functionally related skeletal muscle dystrophy mutants (*mdx* and *cav-3^{-/-}*) we establish that the pathology of Duchenne muscular dystrophy (DMD) and limb-girdle muscular dystrophy type 1C (LGMD-1c) originates in the disruption of the embryonic cardiac and skeletal muscle patterning processes. Myogenesis is severely disrupted and occurs earlier in *mdx* (DMD model) than in *cav-3^{-/-}* (LGMD-1c model) and includes developmental delay; myotube morphology and displacement defects; and aberrant stem cell behaviour. These data are consistent with the milder phenotype of LGMD-1c, and the earlier (E9.5) embryonic expression of dystrophin. Stem cell defects (hyperproliferation and apoptosis of Myf5⁺ and attrition of Pax7⁺ myoblasts) occur in both *cav-3^{-/-}* and *mdx*, from E15.5 and E11.5, respectively, both mutants have cardiac defects. Several *mdx* embryo pathologies have reciprocity with *cav-3^{-/-}* mutants and caveolin-3 protein is elevated in *mdx* embryos. In double mutant (*mdxcav-3^{-/-}*) embryos where caveolin-3 is reduced below WT levels, phenotypes are severely exacerbated: intercostal muscle fibre density is reduced by 71%, and Pax7⁺ cells are depleted entirely from the lower limbs and severely attenuated elsewhere. These data establish a key role for dystrophin in early muscle formation and demonstrate that caveolin-3 and dystrophin are