

**P3.48****Losartan improves muscle strength and ameliorates fibrosis in the dy2J/dy2J mouse model of merosin deficient congenital muscular dystrophy**

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Merosin deficient congenital muscular dystrophy (MDC1A) is the most common form of the congenital muscular dystrophies. In contrast to healthy muscle, dystrophic muscle is characterized by failure of regeneration and abundant proliferation of connective tissue with progressive loss of muscle function. Previous studies have shown that TGFbeta pathway is involved in enhancing fibrosis in muscular dystrophy. Losartan, an angiotensin 2 type 1 receptor antagonist, a medication most commonly used for treatment of hypertension, has anti-fibrotic properties by TGFbeta pathway inhibition. We evaluated the effect of 12 weeks oral Losartan treatment on fore and hind limb muscle strength, TGFbeta serum level, quantitative Sirius red staining of muscle fibrosis, and fibrosis (fibronectin & vimentin) and regeneration (myogenin & myo-D) markers in the dy2J/dy2J mouse model of merosin deficient congenital muscular dystrophy. Losartan treatment was associated with significant improvement in both fore and hind limb muscle strength. Significant quantitative reduction in muscle fibrosis was demonstrated on Sirius red staining associated with reduced expression of fibronectin & vimentin fibrosis markers and decreased serum TGFbeta level. While myo-D remained unchanged, the level of myogenin regeneration marker significantly decreased in the treated mice. We conclude that Losartan treatment was associated with prominent and statistically significant histological and clinical improvement in the dy2J/dy2J mouse model of congenital muscular dystrophy. This commercially available medication with a low side effect profile should be considered as a potential candidate for clinical trials in children with merosin deficient congenital muscular dystrophy.

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**P3.49****The soy isoflavone genistein promotes muscle regeneration and function acting on cell cycle and apoptosis in mdx mice**

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Soy isoflavones have been reported to have antioxidant bioactivities and to inhibit several proinflammatory pathways known to be involved in Duchenne muscular dystrophy (DMD) pathogenesis. Moreover genistein has been shown to enhance cell proliferation promoting G1/S phase transition through the induction of cyclin D1 in different in vitro experimental models. The exhaustion of muscle regeneration in DMD muscle is one of the main targets of current therapeutic approaches. We tested whether genistein could have a beneficial effect on muscle function, morphology and biochemical pattern in mdx mice.

Five-week old mdx mice received for 5 weeks genistein (2 mg/kg i.p. daily) or vehicle. Genistein treatment (1) increased forelimb strength ( $p < 0.05$ ) and strength normalized to weight ( $p < 0.05$ ); (2) reduced serum creatine-kinase levels ( $p < 0.01$ ); (3) increased cyclin D1 expression ( $p < 0.05$ ); (4) showed an antiapoptotic effect,

modulating the expression of BAX and Bcl-2 ( $p < 0.05$ ); (5) reduced muscle necrosis ( $p < 0.01$ ) and enhanced regeneration ( $p < 0.05$ ) with an augmented number of myogenin-positive satellite cells and myonuclei.

Our results suggest that this isoflavone might have a beneficial effect on muscle function and morphology in mdx mice promoting G1/S phase transition in muscle cell and inhibiting apoptosis. Further studies are needed to investigate the biochemical substrates of such encouraging preliminary results taking into account that this supplement could be easily introduced in the daily diet of patients with DMD.

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**P3.50****Therapeutic drug screen using dystrophin deficient zebrafish**

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Zebrafish animal models of human diseases are powerful tools for screening a large number of chemical compounds for therapeutic efficiency. The zebrafish sapje mutant and sapje like (*sapc/100*) are excellent models of human Duchenne Muscular Dystrophy (DMD) and each shows disturbed muscle structure and a severe reduction of birefringence (the ability to refract polarized light). The birefringence is a result of dorsal skeletal muscle deterioration and weakness that ultimately results in lethality of the most sapje mutants at 10 dpf. However, a few dystrophin deficient fish can survive over 30 days.

To identify potential therapeutic chemicals for treating DMD, we undertook a chemical screen of a small, commercial molecular library using the sapje fish. Embryos from mating heterozygous sapje fish were cultured in fish water containing pools of the chemical library. To examine the effects of chemicals for the recovery of the muscle phenotypes, birefringence assays were examined at 4 dpf. Fish treated with some pooled chemicals in the chemical library that were genotypically confirmed as sapje mutants showed no defects in birefringence compared to the control, suggesting that we may have successfully identified compounds that are effective for the recovery of phenotypes caused by a dystrophin gene mutation. Individual chemical tests indicated that some chemicals in the small molecular library are capable of extending the lifespan of the embryos of sapje fish and can reduce muscle weakness. The candidate chemicals were also effective in the second allele of dystrophin deficient zebrafish, sapje like (*sapc/100*).

Our results of this screen using the zebrafish model of DMD may lead to new candidate molecules and pathways towards better therapeutic strategies for DMD.

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**P3.51****Results of a Phase 2b, dose-ranging study of ataluren (PTC124®) in nonsense mutation Duchenne/Becker muscular dystrophy (nmDBMD)**

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**Introduction:** DBMD is an X-linked disorder causing severe, progressive muscle loss in children. Patients (~13%) have DBMD due to a nonsense (premature stop codon) mutation in the gene for dystrophin, a protein needed for muscle stability. Ataluren is an investigational drug designed to promote ribosomal readthrough of premature stop codons in mRNA, leading to production of full-length, functional protein. **Methods:** This Phase 2b, randomized, double-blind, placebo-controlled, dose-ranging study assessed the efficacy and safety of ataluren in males  $\geq 5$  year with nmDBMD documented by dystrophin gene sequencing. Patients received high-dose ataluren; low-dose ataluren; or placebo orally for 48 week. The primary endpoint was change in 6-min walk distance (6MWD). **Results:** The study enrolled 174 subjects (median [range] age = 8 [5–20] year, steroid use = 123/174 [71%], median [range] baseline 6MWD = 356 [75–554] m) at 37 sites in 11 countries. The mean change in 6MWD at week 48 was similar in the high-dose ataluren and placebo groups (RANCOVA/ANOVA  $p$  values = .476/.947). When comparing the low-dose ataluren and placebo groups, ataluren-treated patients showed a mean change in 6MWD at week 48 that was ~29 m (~8%) higher (RANCOVA/ANOVA  $p$ -values = .149/.040) and had a longer time to 10% worsening in 6MWD (log-rank  $p$ -value = .039). Ataluren was well tolerated. **Conclusions:** Coupling genetic diagnosis with a mutation-specific therapeutic approach, ataluren is designed to enable full-length, functional protein production in patients whose disorder results from a nonsense mutation. Patients receiving low-dose ataluren experienced better 6MWD outcomes than patients receiving high-dose ataluren or placebo, potentially consistent with preclinical data suggesting that excessive exposure impedes premature stop codon readthrough. This trial comprises one of the largest prospective studies ever performed in DBMD and provides important longitudinal data in this disease.

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### P3.52

#### **FOR-DMD: double-blind randomized trial to optimize steroid regime in Duchenne Muscular Dystrophy (DMD)**

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Although research into molecular treatments for DMD is at an exciting stage, the use of corticosteroids and good quality care currently remain a high priority in prolonging survival and increasing quality of life. The benefits of steroids in DMD were first suggested in 1974. However, lack of long-term study and concerns about side effects have led to inconsistency of use, regime and dosage in clinical practise. Patients and families ask for more information to guide practice within different centres and countries.

This is a multi-centre, double-blind, parallel group study comparing three steroid regimens in wide use in DMD: prednisone 0.75 mg/kg/day; deflazacort 0.9 mg/kg/day; prednisone 0.75 mg/kg 10 days

on/10 days off. Primary objective is addressing the hypothesis that daily steroids will be of greater benefit than intermittent steroids. A second hypothesis is that daily deflazacort will be associated with a better side effect profile than daily prednisone.

The trial will randomize 300 boys aged 4–7 years to the three regimes. It is expected that about 40 sites across 11 countries will participate. All boys will complete a minimum of 3 years and a maximum of 5 years of treatment period. The primary outcome variable will be a three-dimensional outcome including: time to stand from lying; forced vital capacity; subject/parent global treatment satisfaction. Secondary outcomes will include tolerance, adverse events, secondary functional outcomes, quality of life, and cardiac function. The study protocol includes standardized regimens for prevention/treatment of predictable side effects and standards of care for management of DMD.

This study will determine the relative efficacy and sustainability of these regimens over a longer period than has previously been addressed. The trial addresses the current chaos in prescribed treatment regimes; its results will have direct impact on the current and future management of DMD, providing the evidence base for rational clinical practice.

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### P3.53

#### **The small molecule BMN 195 upregulates utrophin in human myoblast and myotube cell-based assays**

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Duchenne Muscular Dystrophy (DMD) is the most prevalent genetically inherited neuromuscular disorder, which affects approximately 1 in 3500 young males. DMD is a severe muscle degenerative disease caused by the absence of dystrophin, for which there is currently no effective treatment. Utrophin shares a high degree of sequence identity with dystrophin and also associates with members of the dystrophin-associated protein complex (DAPC). Studies in the mdx mouse, a dystrophin negative model of DMD, have established that the elevation of utrophin levels in dystrophic muscle fibers can restore sarcolemmal expression of DAPC members and alleviate the dystrophic pathology. Knowledge of the utrophin-A promoter has initiated the search for small molecules that could stimulate utrophin transcription in muscle cells.

The small molecule BMN 195 (formerly C1100) was previously identified by high throughput screening as a potential utrophin upregulator. In the present study, we used cell-based assays in several muscle cell types to assess the activity of the compound to increase utrophin mRNA as well as protein levels. Using these cell-based assays, we showed that BMN 195 is able to increase endogenous utrophin expression in muscle cells.

A Phase 1 study in healthy volunteers to assess the safety, tolerability and pharmacokinetics of BMN 195 is ongoing.

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### P3.54

#### **Creatine for treating muscle disorders: meta-analysis of randomised controlled trials**

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