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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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**Background:** Creatine supplementation increases muscle strength in healthy persons. The aim of our meta-analysis was to evaluate the efficacy and safety of creatine treatment in muscle disorders. **Methods:** We evaluated randomised controlled trials of creatine treatment in patients with hereditary and inflammatory myopathies. Primary outcome measures were changes in muscle strength determined by quantitative muscle testing and changes in activities of daily living (ADL). Secondary outcomes included changes in manual muscle testing, changes in lean body mass and adverse events. **Main results:** Thirteen trials including 303 participants fulfilled the selection criteria (treatment period: 3 weeks to 6 months, creatine dosage: 3–20 g daily). In muscular dystrophies, analysis of pooled data revealed a significant improvement in maximum voluntary contraction of about 8.5% with creatine supplementation as compared to placebo (95% confidence intervals (CI) 3.6–13.4). Manual muscle testing also showed a significant increase in strength after creatine treatment (weighted mean difference (WMD) 2%, CI 1.0–3.0). The lean body mass increased during creatine treatment (WMD 0.6 kg, 95% CI 0.02–1.25). Two trials in muscular dystrophies and one trial in inflammatory myopathies reported a significant effect of creatine in functional tests and ADL. In metabolic myopathies, meta-analysis showed no significant change in muscle strength or functional scores. One trial in glycogenosis type V reported a significant increase in muscle pain episodes during high-dose creatine treatment. Apart from this, no other trial reported any clinically relevant adverse event. **Conclusions:** There is evidence from randomised controlled trials that creatine treatment increases muscle strength, improves ADL and is well-tolerated in patients with muscular dystrophies and inflammatory myopathies. The available evidence does not support the use of creatine in metabolic myopathies.

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## DYSTROPHINOPATHIES: CLINICAL AND GENETIC; POSTER PRESENTATIONS

### P4.01

#### ncRNAs originating from the dystrophin gene as biomarker for assessing antisense therapy

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Mutations in the DMD gene lead to Duchenne Muscular Dystrophy, Becker Muscular Dystrophy and X-linked dilated cardiomyopathy phenotypes. Therapeutic approaches are now reality in DMD, nevertheless, clinical outcome measures may not always be sensitive enough to detect small changes in disease progression/regression and after short treatment periods. It is therefore imperative to identify enrichment endpoints, as biomarkers, able to document benefits of the treatment at early stages and at the individual level.

Recently, genome-wide approaches to monitor transcription have revealed a notable number of non coding RNAs with many regulatory functions. To address this issue we designed a novel gene-specific Gene Expression array covering the full DMD gene and used it to

search for non-coding transcripts in polyA+ RNAs from human brain, heart, skeletal muscle and skin.

We identified 13 sense and 2 antisense oriented transcripts. Six of these ncRNAs were validated through Northern blotting and fully characterized by RACE PCR and sequencing. Their length ranges from 1800 to 2800 bp, five were unspliced, but one was spliced in several isoforms. Compartmentalisation studies demonstrated that all the six ncRNAs are located in the nucleus. None of these has an open reading frame, suggesting that they belong to the long ncRNAs category.

CGH-DMD analysis in five DMD patients with dystrophin exons 45–50 deletion (thus eligible for exon 51 skipping antisense therapy) allowed us to define the deletion breakpoints therefore predicting the loss/maintenance at the genomic level of the ncRNAs region. One sense oriented ncRNA, deleted in only one DMD boy, was consistently missing in his myogenic cells.

Further studies are in progress in order to profile the expression of these ncRNAs in patients' myogenic cells before and after AONs treatment for exploring if these transcripts may be used as muscle transcriptomic biomarkers for monitoring the impact of novel treatment in dystrophinopathies.

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### P4.02

#### MLPA is a useful tool for analyzing fragmented DNA samples from dried umbilical cord

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In Duchenne muscular dystrophy, not only definitive diagnosis of patient but also carrier diagnosis is an important theme both on genetic counseling and health cares. In order to diagnose a client as non-carrier, the proband's gene mutation should be identified, but this is often difficult as patients unfortunately die. Good quality DNA can be obtained and analyzed if the patient's muscle biopsy remains in freezer. In Japan, there is a custom to keep dried umbilical cord as a memento. But, its DNA receives damage such as degradation, and the PCR analysis is difficult in a half of cases. In this study, we evaluated the performance of the multiplex ligation-dependent probe amplification (MLPA) method to detect deletion/duplication mutations using umbilical cord DNA.

MLPA analysis (MRC-Holland) was done using DNA samples extracted from the dry umbilical cord of 14 patients, and compared to the previous results of PCR analysis. As a result, the judgment of deletion mutation was possible in all samples including five samples in which previous PCR analysis was completely useless due to DNA fragmentation. Also, it seemed that the judgment of duplication mutation was possible in most samples except for some samples with a marked DNA degradation. In such samples, amount of MLPA products of each probe went so uneven that a quantitative judgment for duplication mutation became difficult.

In the MLPA, tandem two probes are hybridized to each exon. These probes are then ligated, amplified and detected. If the part where specific probes hybridize (around 60–80 bases) is kept even if DNA is fragmented, it is thought that MLPA works. However, when DNA fragmentation becomes remarkable, a quantitative duplication analysis becomes difficult. In conclusion, MLPA method shows better tolerance than PCR method to the use of fragmented DNA, making the process of giving carrier diagnosis more available.

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