

expression throughout the body with improvement of muscle function and pathology (Ann Neurol 2009;65:667–76). To date, AO used for exon skipping in DMD animal models have not been directly applied to DMD patients having the same exon deletions. We recently experienced a DMD patient with exon 7 deletion, therefore, we evaluated the multi-exon skipping in cultured cells derived from the patient and compared the efficiency with cells from CXMDJ. We isolated fibroblasts from skin biopsies of CXMDJ and exon 7 deleted DMD patient, and converted them to myogenic cells by MyoD transduction. PMO cocktails with antisense sequences targeting exons 6 and 8 were administered to canine or human myogenic cells, respectively. Skipping of exons 6 and 8 were achieved with similar efficiency between canine and human cells, but skipping of exon 9 was different between these cells, which did not alter the reading frame. The similarity of exon sequences of targeted region, and the antisense design methodology targeting identical sequence, may assure similar skipping efficiency between dogs and humans. This study shows directing an AO to the same motif in both humans and dogs induce comparable exon skipping, provides a proof of concept for systemic multi-exon skipping therapy for the DMD patient with exon 7 deletion using the AO sequences optimized in CXMDJ.

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### P3.05

#### Checking exon-skipping events in candidates for clinical trials of morpholino

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Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused by abnormalities in the dystrophin gene. DMD, which manifests as a severe muscle weakness phenotype, results from an out-of-frame deletion(s) in the dystrophin gene. In contrast, BMD, which results from an in-frame deletion(s) in the dystrophin gene, causes a milder muscle weakness. Antisense-mediated exon-skipping, which changes out-of-frame deletions to in-frame deletions, is a promising therapeutic approach for DMD. Morpholino, a type of antisense molecule, is a powerful tool because it has higher affinity for the target nucleic acid sequences and greater resistance to degradation than conventional nucleic acids. It is important to confirm whether the morpholino, which has been tested in animal models, is effective in cells from clinical trial candidates. Fibroblasts from DMD patients with deletions of exons 45–50, 48–50, 49–50 and 50 of the dystrophin gene were isolated, induced to differentiate into the myogenic lineage by MyoD expression, and transfected with antisense morpholino; then, the exon-skipping event was checked by RT-PCR and sequence analysis. One of the two types of antisense molecules did not induce exon-skipping, which was the result that we anticipated. We also evaluated the quantity of dystrophin in morpholino treated cells by Western blotting analysis. Additionally, the contractile response of morpholino treated cells and non-morpholino treated cells were compared in order to analyze dystrophin function after inducing by KCL depolarization. The result shows the morpholino treated cells were more contractile than non-morpholino treated cells.

In this study, we developed an in vitro system that can easily screen the effectiveness of antisense sequences. This system can be used to identify patients who are good candidates for the therapy.

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### P3.06

#### Evaluation of the truncated products of exon and multiple exon skipping in DMD therapy

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Duchenne's muscular dystrophy (DMD) is a severe muscle wasting disorder affecting 1/3500 male births. Lack of dystrophin in skeletal muscle compromises the integrity of the muscle membrane and thus muscle fibres are prone to contraction induced injury. Further rounds of muscle degeneration and regeneration leads to the replacement of muscle fibres with non contractile fatty/fibrotic tissue. These alterations lead to progressive muscle wasting, weakness and death in late adolescence.

Gene therapy for DMD have been hampered by a number of factors (e.g. gene transfer efficiency, immune responses). A promising therapeutic approach for DMD is antisense-mediated exon skipping; antisense oligonucleotides (AONs) targeting specific exons to restore the DMD reading frame. The resultant truncated forms of dystrophin, restore the integrity of the muscle cell membrane and improve muscle pathology. An ideal therapy could target multiple exons, thereby treating many more patients whilst still producing a partially functional truncated dystrophin protein product. Some of these AONs are currently in clinical trial for single exon skipping.

To evaluate the therapeutic value of these therapies, different inframe truncated human dystrophins have been generated; these represent the dystrophins created by skipping different single exons or skipping multiple exons currently being investigated. The truncated dystrophins were electro-transferred into mdx mice muscle with a subset of constructs have been used to generate transgenic mice. Truncated dystrophins, akin to multiple exon skipping products, localise to the sarcolemma and re-establish the dystrophin associated protein complex. Further histological and functional data will be presented.

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### P3.07

#### Preclinical safety of AVI-4658, a phosphorodiamidate morpholino oligomer (PMO) being developed to skip exon 51 in Duchenne muscular dystrophy

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**Background:** AVI-4658 is a PMO that skips dystrophin exon 51, restores the reading frame and enable dystrophin expression in selected DMD patients, proven by a single IM dose study in the UK. To enable clinical trials in the US, three 12-week GLP studies in animals were performed. Published data suggests the older phosphorothioate antisense oligonucleotides have dose limiting toxicities. **Objective:** The current studies were designed to assess the safety of AVI-4658 (and PMO in general). **Method:** (1)mdx mice were dosed IV with 0, 12, 120 or 960 mg/kg (the maximum feasible dose (MFD)), or subcutaneously at 960 mg/kg; wild type C57 mice at 0 and 960 mg/kg (i.e., 7 groups) with AVI-4658. (2) A second identical study with AVI-4225, the PMO to skip exon 23 of in the dystrophic mouse and restore dystrophin (looking for mechanistic toxicity), was also performed. (3) Cynomolgus monkeys were dosed IV with 0, 5, 40 or 320 mg/kg (MFD) and 320 mg/kg subcutaneously. A 28 day recovery period was included in all studies. **Results:**

In mice, both AVI-4658 and AVI-4225 were well tolerated at doses including 960 mg/kg/injection, with no adverse effects. Findings were generally limited to the kidney, and were generally reversible, as shown in the 28 day recovery groups. No evidence of kidney function change was detected. In cynomolgus monkeys, AVI-4658 was also well tolerated at all doses including 320 mg/kg/injection, with no adverse effects. Findings were similar to those seen in the mouse studies. *Conclusion:* AVI-4658, the first PMO for DMD, was extremely well tolerated at all doses in dystrophic mice, normal mice and primates. In addition, AVI-4225, which restores dystrophin in mdx mice, also led to no adverse effects. Based on this preclinical package, and encouraging safety and dystrophin expression results from a concurrent UK clinical study, US clinical studies are anticipated.

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### P3.08

**Induction of dystrophin in DMD patients by antisense oligonucleotide AVI-4658 restores the dystrophin glycoprotein complex**  
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We have recently performed a proof of principle single-blind, controlled, two-doses escalation study of a morpholino splice-switching oligonucleotide (AVI-4658) which induced skipping exon51 in dystrophin mRNA in seven patients with DMD (Kinali et al., 2009). The morpholino was injected into one extensor digitorum brevis (EDB) muscle while the contralateral muscle received saline. In all patients exon 51 skipping was demonstrated at the RNA level, and the higher-dose of AVI-4658 resulted in increased dystrophin protein expression in all treated muscles. Although the intensity of dystrophin immunolabelling was not uniform, it increased up to 42% of that of healthy muscle.

We now report the expression of proteins from the dystrophin-associated glycoprotein complex (DAGC). The DAGC proteins were studied by immunofluorescence with antibodies to dystrophin (dys1, dys2, dys3 and Mandys106), a-sarcoglycan, b-dystroglycan, nNOS, and dystrobrevin, and compared with b-spectrin, utrophin and neonatal myosin and other markers of immaturity such as laminin a5.

The increased detection of dystrophin with intact C-terminus detected by dys2 in the treated muscle was accompanied by more intense immunofluorescent labelling of the DAGC. In addition, we show clear sarcolemmal restoration of nNOS expression in a patient with Del 48–50; the sarcolemmal nNOS immunostaining was however relatively weak in the patient with Del 45–50. This has therapeutic implications as nNOS expression counteract vasoconstriction and focal ischemia following exercise. The nNOS binding site of dystrophin has been mapped to the spectrin repeats 16/17 of the rod domain, encoded by exons 41–46 (Lai et al., 2009). Our results indicate that the administration of AVI4658 induces exon skipping and the restoration of functional dystrophin as indicated by restored sarcolemmal expression of proteins of the DAGC. Further analysis of the effect of regeneration and DAGC protein quantification is in progress.

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### P3.09

**The effect of combining activin receptor type IIB inhibition and prednisolone treatment in mdx mice**

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Activin receptor type IIB (ActRIIB) is a signaling receptor for ligands involved in suppressing muscle growth. Blocking ActRIIB signaling increases muscle mass and function. RAP-031, a soluble fusion protein comprised of a form of ActRIIB extracellular domain fused to a murine Fc, also increases muscle mass and improves muscle weakness in mdx mice. There is currently no cure for Duchenne muscular dystrophy (DMD); however, glucocorticoids, such as prednisolone, slow disease progression and are commonly used for the management of disease. To study the effect of combined glucocorticoid/RAP-031 regime in a dystrophy model, mdx mice received either: vehicle/vehicle (VEH/VEH), prednisolone (5 mg/kg, 5×/week, po)/vehicle (PRED/VEH) or prednisolone/RAP-031 (10 mg/kg, 1×/week, sc; PRED/RAP). At 2 weeks, PRED/VEH gained body weight at a rate that was 8.5% less ( $p < 0.01$ ) than VEH/VEH. In contrast, PRED/RAP and VEH/VEH gained body weight at similar rates. NMR analysis established the body weight effect as being due to decreased lean mass gain in PRED/VEH ( $p < 0.01$ ) compared to VEH/VEH whereas PRED/RAP gained lean mass similarly to VEH/VEH. At study day 9, VEH/VEH and PRED/VEH had comparable absolute forelimb grip strength while PRED/RAP mice had significantly greater grip ( $p < 0.05$  compared to VEH/VEH and PRED/VEH). Prednisolone alone does not affect absolute grip strength, but strength improvement is evident if grip strength is normalized to body weight. PRED/VEH had 8.2% greater body weight-normalized grip strength compared to VEH/VEH ( $p < 0.05$ ). Importantly, the normalized grip strength improvement is retained in PRED/RAP (VEH/VEH =  $p < 0.05$ , PRED/VEH = ns). RAP-031 improves mdx muscle function by increasing overall lean mass or by preventing lean mass loss, as is the case when given with prednisolone. In contrast, prednisolone improves muscle function independent from a lean mass increase. These data support the use of ACE-031, the human version of RAP-031, for the treatment of DMD.

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### P3.10

**Lentiviral vector mediated delivery of full-length dystrophin for gene therapy of muscular dystrophy**

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Duchenne muscular dystrophy (DMD) is an inherited severe muscle wasting disorder, and there is currently no effective treatment. DMD causes respiratory and/or cardiac failure and results in death at about 20 years of age. Lentiviral vectors are an efficient gene delivery tool for skeletal muscle fibers and myogenic progenitor cells. The integration ability of lentiviral vectors is a huge advantage for targeting DMD myogenic cells to express dystrophin permanently, which cannot be achieved with non-integrating vectors. We have shown that stable transduction of myogenic stem cells in vivo using lentiviral vectors could be of benefit for treating dystrophic muscles in mdx mice. Injection of micro-dystrophin expressing lentiviral vectors into neonatal mdx muscles resulted