

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

in widespread and stable expression of micro-dystrophin for at least two years, and led to a marked amelioration of dystrophic pathology.

As the carrying capacity of lentiviral vector has been considered to be limited to ~9 kb, it can package truncated versions of dystrophin gene, such as the micro- or mini-dystrophins. Recently, several modifications of lentiviral vector production methods have been successfully employed to generate relatively higher titers of vector stocks. Consequently, we have explored the ability to package the full-length dystrophin cDNA in a VSVG-pseudotyped lentiviral vector. Although the functional titer was still relatively low compared with smaller dystrophin cDNAs, it was able to deliver the full-length transgene into mdx myoblasts. These genetically corrected cells successfully expressed the 427 kDa of dystrophin protein, which could also be provided to dystrophin deficient myotubes in vitro. After intramuscular transplantation, these genetically modified myogenic cells supplied full-length dystrophin protein to mdx muscle fibers. Our candidate strategy with lentiviral vectors carrying full-length dystrophin cDNAs could be useful for ex vivo cell therapies.

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### P3.11 Adenoviral mediated MyoD transduction confers migratory potential to human primary fibroblasts

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MyoD is a master gene switch for conversion of mesoderm-derived cells to the myogenic lineage. We previously reported that MyoD gene delivery using the adenovirus Ad.CAGMyoD could efficiently convert primary skin fibroblasts into myoblasts. Here, we presented the surprising behavior of adenoviral MyoD-transduced primary fibroblasts in vitro. MyoD-expressing primary fibroblasts displayed rapid movement on the surface of culture dishes that was significantly faster than that of primary myoblasts, indicating that MyoD can also induce cell migration. We also confirmed that Ad.CAGMyoD-transduced primary fibroblasts have the ability to fuse with primary myoblasts. Finally, injection of these cells achieved the expression of the dystrophin protein to rescue the dystrophic mdx muscle. MyoD transduction of human skin fibroblasts using this method may serve as a new cell transplantation therapy for patients with muscular disorders.

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### P3.12 Concurrent administration of prednisolone and peptide conjugated PMOs is not contraindicated in the mdx mouse

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Duchenne muscular dystrophy (DMD) is a progressive, fatal muscle wasting disorder with a predictable course and limited treatment options. Advances in clinical care and management have almost doubled the life expectancy of affected boys over the last 2–3 decades, but do not address the primary etiology of DMD, the loss of dystrophin. Corticosteroids are effective in stabilizing muscle strength and prolonging ambulation, although the exact mechanism by which steroids slow the dystrophic process is unknown. Despite the limited therapeutic value of corticosteroids these drugs represent the best treatment option currently available, and a large proportion of DMD patients are treated with prednisone/prednisolone or deflazacort. Biological therapeutics are becoming available, and it will be important to establish whether these compounds can be safely administered to patients being treated with corticosteroids. Anti-sense oligomer-mediated splicing manipulation can bypass dystrophin gene lesions and is showing promise as a therapy for DMD. Intramuscular injection of RNA analogues, targeting exon 51, in DMD patients has resulted in specific exon exclusion and dystrophin expression in the treated muscle. We report that concurrent peptide-conjugated phosphorodiamidate morpholino oligomer and prednisolone administration is not contraindicated in mdx mice and that muscle physiology is improved by the combined treatment.

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### P3.13 Reprogramming nebulin pre-mRNA splicing by spliceosome-mediated RNA trans-splicing

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The nebulin gene (*NEB*) has 183 exons encoding transcripts up to 26 kb in length. Extensive alternative splicing of at least 41 exons have the potential to produce more than 3000 different splice variants in skeletal muscle. Mutations in *NEB* cause autosomal recessive nemaline myopathy, distal nebulin myopathy, and core-rod myopathy. Recent advances in RNA repair or reprogramming technologies have raised the possibility to develop RNA therapies for correction of mutations in *NEB* at the pre-mRNA level. Spliceosome-mediated RNA trans-splicing, i.e. splicing between two separate pre-mRNA molecules, allows the replacement of mutant exons with wild type ones at the pre-mRNA level. Targeted spliceosome-mediated RNA trans-splicing requires three components: the spliceosome, a target pre-mRNA, and a pre-trans-splicing RNA molecule (PTM). The spliceosome and target pre-mRNA are provided by the cells whereas the PTM RNA molecule is produced from an expression vector transfected into the cells. PTMs can be designed to carry out one of three forms of trans-splicing; 3'exon replacement, 5'exon replacement and internal exon replacement, depending on the trans-splicing domain, i.e. the domain responsible for recognition and splicing of the target pre-mRNA. We are developing PTMs for *NEB* 3'exon replacement and internal exon replacement, which we are testing in cell cultures. The target pre-mRNAs are produced from *NEB* minigene expression vectors. We have recently achieved successful 3'exon replacement by spliceosome-mediated RNA trans-splicing between a *NEB* minigene pre-mRNA and a PTM RNA in C2C12 and HeLa cells. This is the first evidence that *NEB* pre-mRNA splicing can be reprogrammed, and the results form the basis for development of RNA therapies for myopathies caused by mutations in *NEB*.

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