

of dystrophin protein. We have tested 6 PMO doses to guide selection of an effective, well tolerated dose for further clinical evaluation and subsequent registration. *Method:* Open label, dose escalation study in ambulant patients with DMD aged 5–15 years with relevant deletions, of 12 weekly IV administrations of AVI-4658; 14 week follow up with muscle biopsy to assess dystrophin expression. Clinical efficacy (including 6 min walk and North Star assessment), skeletal muscle, pulmonary and cardiac function is being assessed. Safety assessment includes adverse events, physical examinations and laboratory tests – including hematology, coagulation studies, chemistry and anti-dystrophin antibodies. Plasma pharmacokinetics and urinary elimination assessed at 1st, 6th and 12th doses. DSMB guided dose escalation decisions (across 6 doses: 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/kg). *Results:* Study fully enrolled 19 patients by Dec 2009. All doses well tolerated. No Drug Related SAEs or severe AEs reported during 12 weeks of dosing at any dose. Maximum single dose 900 mg; cumulative PMO dose exceeds 10,000 mg. Biopsies from first 4 cohorts showed exon 51 skipping at 2 and 4 mg/kg and 1 patient with 20% increase in number of dystrophin positive fibres. Preliminary plasma PK showed short half life (1.7–3.9 h), dose proportional increase in Cmax and AUC across doses with no accumulation. *Conclusion:* Study drug well tolerated. All dosing completed. These preliminary data bode well for safe long-term administration of AVI-4658 in patients with DMD, and suggests clinically meaningful dystrophin expression can be expected following systemic administration. Preliminary laboratory data from the remaining patients at 4, 10 and 20 mg/kg are due in 2Q 2010.

doi:10.1016/j.nmd.2010.07.140

O.17 Efficient bypass of mutations in dysferlin deficient patient cells by antisense-induced exon skipping

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Mutations in DYSF encoding dysferlin cause primary dysferlinopathies, autosomal recessive diseases that mainly present clinically as Limb Girdle Muscular Dystrophy type 2B and Miyoshi Myopathy. The size of the dysferlin coding sequence is above the limited packaging size of AAV vectors, so alternative therapeutic strategies must be addressed for patients. A gene therapy approach for Duchenne Muscular Dystrophy was recently developed based on exon-skipping strategy. Numerous sequences are recognized by splicing protein complexes and, when specifically blocked by antisense oligonucleotides (AON), the corresponding exon is skipped. We hypothesized that this approach could be useful for patients affected with dysferlinopathies. To confirm this hypothesis, exon 32 was selected as a primary target for exon skipping strategy due to the report from Sinnreich and colleagues, which described a very mild and late-onset phenotype associated to a natural skipping of exon 32 in a 70 years old woman. Based on this observation, we used AON to exclude exon 32 by exon-skipping. Four different AON were tested in myoblasts generated from control and patient MyoD-transduced fibroblasts, either as synthetic oligonucleotides or after expression from lentiviral vectors. These approaches led to a high efficiency of exon 32 skipping correlated with an amelioration of both membrane fusion and membrane repair mechanism. To test the gain of function

in vivo, we are developing a KI mouse model (non sense codon in exon 32) in which AON will be injected. Finally, these encouraging results could pave the way for an antisense induced therapy in dysferlinopathies.

doi:10.1016/j.nmd.2010.07.141

O.18 Intravenous injection of SMN1-expressing self-complementary AAV9 rescues severe type I SMA mice

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Spinal muscular atrophy (SMA) is the most common genetic disease leading to infant mortality. This neuromuscular disorder is caused by loss or mutation of the telomeric copy of the “survival of motor neuron” (*Smn*) gene, termed SMN1. Loss of SMN1 leads to reduced SMN protein levels, inducing degeneration of motor neurons (MN) and progressive muscle weakness and atrophy. To date, SMA is still incurable notably due to the lack of method to deliver therapeutically active molecules to the spinal cord. Gene therapy, consisting of reintroducing SMN1 in MNs is an attractive approach for SMA. We performed intravenous injections of self-complementary (sc) AAV9 vectors encoding human SMN1 to restore the SMN protein in type I SMA mice. This treatment restored complete mouse survival, increasing life expectancy from 16 to over 200 days. It also dramatically improved the weight loss phenotype and allowed complete correction of motor symptoms. This study demonstrates the feasibility of a post-natal systemic gene therapy for the rescue of a fatal SMA phenotype, and paves the way for a clinical trial in humans.

doi:10.1016/j.nmd.2010.07.142

DUCHENNE MUSCULAR DYSTROPHY AND OTHER MUSCLE DISORDERS; POSTER PRESENTATIONS

P3.01 Antisense RNA/ethylene-bridged nucleic acids chimera induces exon 45 skipping and restores dystrophin expression in myocytes of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease, succumbing in their twenties and caused by mutations in the *dystrophin* gene. Nearly two thirds of DMD cases are found to have exon deletion mutations in the *dystrophin* gene. Patients with out-of-frame deletion mutations show severe DMD, while in-frame mutations show mild Becker muscular dystrophy (BMD). It is supposed that transformation of an out-of-frame mutation into an in-frame mutation is a way of DMD treatment. We have long proposed that induction of exon skipping by antisense oligonucleotides is the most plausible therapy for DMD. Our previous studies have demonstrated antisense phosphothioate oligonucleotides induced exon 19 in human dystrophin mRNA and restored muscle dystrophin expression in vitro and in vivo. Taken it into consideration that a 2'-O, 4'-C-ethylene-bridged nucleic acid (ENA) is

highly nuclease-resistant and thermodynamically stable, we reported that an antisense RNA/ENA chimera is 40 times as effective as corresponding conventional phosphorothioate oligonucleotides in inducing exon 19 skipping. To develop the broad therapeutic applicability of this exon skipping therapy, it is necessary to identify the specific antisense oligonucleotides that can skip exons located in the deletion hot spots in the *dystrophin* gene. We focus to induce skipping of exon 45 that is located in one of the deletion hot spots. We designed many antisense RNA/ENA chimeras against exon 45 and examined their ability to induce exon skipping. Here, we identified the best antisense RNA/ENA chimera could induce exon 45 skipping in cultured myotubes from DMD patients with deletion mutations neighboring exon 45. The production of functional dystrophin in the treated myotubes was also confirmed. It is proposed that antisense RNA/ENA chimera can be applied to treat a broader spectrum of DMD cases.

doi:10.1016/j.nmd.2010.07.143

P3.02

AAV-U7snRNA mediated multi exon-skipping for Duchenne muscular dystrophy

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Most cases of Duchenne muscular dystrophy (DMD) are caused by mutations that disrupt the dystrophin mRNA reading frame. In many cases, skipping of a single exon could restore the reading frame, giving rise to a shorter but still functional quasi-dystrophin protein. It has previously been proposed to use small nuclear RNAs, especially U7snRNA, to shuttle antisense sequences designed to mask key elements involved in the splicing of targeted exons.

Our present project focuses on the optimisation of U7snRNA constructs to complete rescue of dystrophin by exon-skipping in DMD patients. In particular, we are investigating the multi exon-skipping of exons 45–55, which could rescue up to 63% of DMD patients with a deletion.

In order to achieve this multi-skipping, we have first developed U7snRNA constructs targeting every exon between 45 and 55. Each construct has been inserted into lentiviral vectors for *in vitro* analysis in myoblasts from DMD patients. After transduction of these cells with lentiviral vectors encoding the various U7 constructs, specific skipping of the targeted exon was confirmed by RT-PCR. In parallel, we demonstrated the efficacy of these constructs *in vivo* in transgenic mice carrying the entire human DMD locus (*hDMD mice*) after intramuscular injection of AAV vectors encoding the U7snRNAs. Based on *in vitro* and *in vivo* results, the U7snRNA constructs inducing the most efficient skipping for each targeted exon were selected and combined into multi-skipping vectors. These AAV vectors are currently being tested in DMD myoblasts and in *hDMD mice* and efficient skipping of up to four exons has been demonstrated thus far.

These very encouraging results provide evidence that efficient multi exon-skipping can be achieved *in vitro* and *in vivo* using AAV vectors encoding multiple U7snRNAs. These new constructs offer therefore very promising tools for clinical treatment of DMD.

doi:10.1016/j.nmd.2010.07.144

P3.03

Nanoparticles are effective vehicles for systemic delivery of 2'OMePS antisense oligonucleotides in exon skipping-mediated dystrophin restoration

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We used a novel PMMA/N-isopropyl-acrylamide+ (NIPAM) cationic nanoparticle (ZM2-NPs) for systemic delivery of 2'OMePS AONs in the mdx animal model. Six weeks-old mdx mice were intraperitoneally treated: group 1 received 225 µg of naked M23D AON and group 2 received of 225 µg of M23D AON conjugated with 2.5 mg of ZM2 NPs. Four non-injected mdx mice were used as controls. The mice were injected weekly for 7 weeks (7.5 mg/kg/injection) and sacrificed 1 week (4) and 12 weeks (4) after the final administration. In treated animals sacrificed 1 week and 12 weeks after final administration we observed restored dystrophin protein synthesis in arrector pili skeletal and cardiac muscles (only for 1 week sacrificed animals), allowing protein localization in up to 40% of muscle fibers. The mdx exon 23 skipping level was up to 20% and 8% respectively. We measured the dystrophin transcript amount in all animal groups by four exon specific real time PCR assays (ESRAs). In non-treated mice, we found that the dystrophin transcript amount ranged 20–30% in all muscles of younger mice (7 weeks of age), whereas it was lower in skeletal muscles (10–20%) and higher in the heart (up to 30%) in older mice. In ZM2-AON or naked AON treated animals sacrificed 1 week after final administration, the dystrophin transcript amount did not vary significantly. Differently, in 12 weeks/sacrificed ZM2-AON treated animals, the transcript level was significantly higher in skeletal muscles but reduced in the heart. In mice treated with naked AONs the amount of transcript was higher also in the heart. In conclusions, ZM2-AON complexes are able to induce dystrophin restoration, which still persists in skeletal muscles, though at low level, 12 weeks after final administration. The basal dystrophin transcription amount in the heart seems to be age-related, having a different behavior during AON therapy.

doi:10.1016/j.nmd.2010.07.145

P3.04

Skipping of exons 6 and 8 of the DMD gene has been achieved in myogenic cells from an exon-7 deleted DMD patient: direct application of antisense sequences found in study with canine muscular dystrophy

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Exon skipping approach using anti-sense oligonucleotide (AO) is a promising therapeutic approach for Duchenne muscular dystrophy (DMD). The multi-exon skipping could theoretically rescue up to 63% of DMD patients. We have previously tried a systemic delivery of phosphorodiamidate morpholino oligomer (PMO) AO targeting exons 6 and 8 of the DMD gene in canine X-linked muscular dystrophy in Japan (CXMDJ), which harbors a point mutation within the 3' splice site of intron 6. We successfully achieved recovery of dystrophin