

Duchenne muscular dystrophy (DMD), is caused by mutations in the dystrophin gene. We are developing methods to deliver therapeutic genes to muscles throughout the body to either replace the missing dystrophin gene or to help compensate for the lack of dystrophin. We show that shuttle vectors derived from adeno-associated virus type 6 (rAAV6) are able to deliver genes to muscles throughout the body of adult mice when injected directly into the bloodstream. rAAV6 delivery results in highly efficient gene expression in skeletal and cardiac muscle that persists for the lifespan of the mouse. To accommodate the limited cloning capacity of rAAV vectors we have designed a variety of different micro-dystrophin vectors, and a recently modified micro-dystrophin with alterations in the hinge domains increases functionality. We have begun testing these AAV vectors in wild type and dystrophic dogs, and in wild type non-human primates. These studies revealed a cellular immune response directed against the AAV capsid proteins, but which could be blocked by short-term immune suppression, leading to long-term dystrophin expression. We have observed that delivery of AAV6 vectors into various veins and arteries of the dog results in efficient gene transfer to downstream muscles, but does not lead to whole body gene transfer. Instead, it appears that vector will need to be delivered into multiple vascular sites to target muscles body wide. These results suggest that a combination of intravascular AAV delivery coupled with transient immune suppression could lead to an effective therapy for DMD.

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O.14

Exon exchange approach to repair Duchenne dystrophin transcripts

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Trans-splicing strategies for mRNA repair involve engineered transcripts designed to anneal target mRNAs in order to interfere with their natural splicing, giving rise to mRNA chimeras where endogenous mutated exons have been replaced by exogenous replacement sequences. A number of trans-splicing molecules have already been proposed for replacing either the 5' or the 3' part of transcripts to be repaired. Here, we show for the first time the feasibility of RNA surgery by using a double trans-splicing approach allowing the specific substitution of a given mutated exon. As a target, we used a minigene encoding a fragment of the mdx dystrophin gene enclosing the mutated exon (exon 23). This minigene was cotransfected with a variety of exon exchange constructions, differing in their annealing domains. We obtained accurate and efficient replacement of exon 23 in the mRNA target. Adding up a downstream intronic splice enhancer DISE in the exon exchange molecule enhanced drastically its efficiency up to nearly 50% of repair. These results demonstrate the possibility to fix up mutated exons, refurbish deleted exons and introduce protein motifs, while keeping natural untranslated sequences, which are essential for mRNA stability and translation regulation. Conversely to the well known exon skipping, exon exchange has the advantage to be compatible with any type of mutations and more generally to a wide range of genetic conditions. In particular, it allows addressing disorders caused by dominant mutations.

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NEW THERAPEUTIC TARGETS FOR NEUROMUSCULAR DISORDERS 2; ORAL PRESENTATIONS

O.15

24 week follow-up data from a phase I/IIa extension study of PRO051/GSK2402968 in subjects with Duchenne muscular dystrophy

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Objective: To evaluate the efficacy and safety after 24 weeks of treatment with PRO051/GSK2402968 in boys with Duchenne muscular dystrophy (DMD). **Background:** DMD patients suffer from progressive muscle degeneration due to mutations in the DMD gene and resulting absence of functional dystrophin in the muscle cell wall. PRO051/GSK2402968 is an antisense oligonucleotide compound which induces exon 51 skipping during pre-mRNA splicing and produces novel dystrophin expression in a subpopulation of DMD patients. **Design/methods:** 12 DMD patients (11 ambulatory, 1 non-ambulatory at study entry) completed a dose-escalation Phase I/IIa study (Netherlands Trial Register #124) and entered an open-label extension study. All subjects received weekly subcutaneous injections of 6 mg/kg of PRO051/GSK2402968 in the extension study, regardless of earlier dose. All subjects were at stable steroid doses during the study. Study visits were performed at baseline and 4 weekly thereafter. **Results:** All patients completed 24 weeks of treatment and reported treatment-emergent AEs. Mild proteinuria was detected in all boys at some point during continued treatment, confirmed as greater than the upper limit of normal in 2 boys in subsequent 24 h collection. There were no severe treatment-related AEs. Two serious AEs (not treatment related) were reported. The most common AE was local injection site reaction, none were considered severe. A raised GGT level was observed in one patient. The mean (SD) 6MWD in patients increased from baseline by 36.8 (59.8) m ($n = 10$) in this heterogeneous population. Two boys, aged 9 and 11 observed a 115 m increase over the 24 week period. **Conclusion:** PRO051/GSK2402968 6 mg/kg administered weekly by subcutaneous injection was generally well tolerated across 24 weeks of treatment in DMD patients with mutations/deletions correctable by exon 51 skipping. Renal and hepatic function warrant further monitoring. Encouraging gains in the 6MWD were observed in some boys.

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O.16

Current progress and preliminary results with the systemic administration trial of AVI-4658, a novel phosphorodiamidate morpholino oligomer (PMO) skipping dystrophin exon 51 in Duchenne muscular dystrophy (DMD)

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Objective: AVI BioPharma in collaboration with the MDEX consortium, UK, have identified a PMO to skip dystrophin RNA exon 51 in patients with DMD, restore the reading frame and enable expression

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.

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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.

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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.

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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 phosphorothioate 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