



Abstracts, UK Neuromuscular Translational Research Conference 2010 Posters

Muscular Dystrophies

DMD – Molecular Therapy

P01 Poster

Current progress 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 have identified a PMO to skip dystrophin RNA exon 51 in DMD patients, restore the reading frame and enable expression of dystrophin protein. Here, we test 6 PMO doses to select an effective, well tolerated dose for subsequent registration.

Method: Open label, dose escalation study in ambulant DMD boys aged 5–15 years with relevant deletions, of 12 weekly administrations of AVI-4658; 14 week follow up with muscle biopsy to assess dystrophin expression. Clinical efficacy (including 6 minute 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. A 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 (ongoing at 20 mg/kg). No Drug Related SAEs or severe AEs reported so far. To date, maximum single dose is 900 mg and cumulative PMO dose exceeds 8100 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.

Conclusion: Study drug well tolerated to date. Dosing and follow up continue on schedule. These preliminary data bode well for safe long-term administration of AVI4658 in DMD boys, and suggests clinically meaningful dystrophin expression can be expected following systemic administration. Preliminary, laboratory data from the remaining cohorts are due in 2Q 2010.

P02 Poster

Multixon skipping in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is caused by the lack of dystrophin protein, most commonly as a result of frame-shifting mutations, both deletions and duplications, in the dystrophin gene. Selective removal of exons flanking an out-of-frame DMD mutation can result in an in-frame mRNA transcript that may be translated into an internally-deleted, BMD-like but functionally active dystrophin protein with therapeutic activity.

Antisense oligonucleotides (AOs) have been designed to bind to complementary sequences in the targeted mRNA and modify pre-mRNA splicing to correct the reading frame of a mutated transcript so that gene expression is restored. The rapid steady advances made in this field suggest that it is likely that AO-induced exon skipping will be the first gene therapy for DMD to reach the clinic. However, the different deletions that cause DMD would require skipping of different exons, and personalised molecular medicine may be required. As DMD deletions appear to be concentrated in the region around exons 45 and 55 (65% of all DMD mutations), multiexon skipping has been proposed as a means to treat the maximum number of patients with one formulation of AOs. We describe here studies in cultured human skeletal muscle cells to optimise the skipping of exon 45–55 block, using linked AOs tagged with hnRNP A1 binding sites, and polypyrimidine tract binding protein binding sites. This work will be extended in vitro in cultured DMD patient cells and in the humanised DMD mouse, a transgenic mouse that expresses full length human dystrophin.

P03 Poster

The characterisation of out of frame duplications in DMD patients

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Introduction: Duchenne muscular dystrophy (DMD) is an X-linked recessive disease caused by mutations in the dystrophin gene, leading to disruption of the reading frame. Out-of-frame deletions are the most common mutations (65%) but out-of-frame duplications are also frequent (at least 15% of all DMD mutations). Although there is no treatment at the moment, restoring the reading frame using antisense oligonucleotides (AOs) has been shown to be effective in early “exon skipping” trials in DMD boys with out of frame deletions. However, the application of AOs to the duplicated patients has its own unique problems. Firstly, these AOs not only recognise duplicated exons specifically, but also recognise normal exons. Also, the skipping could be problematic in patients with big duplications.

This study focuses on the characterization of splicing patterns in patients with duplications (inverted or non-inverted duplication, tandem duplication), which will help to evaluate the feasibility of exon skipping strategies to restore the disrupted reading frame in these patients.

Method: We used frozen sections, fibroblast or myoblast cell lines from 16 patients with proven duplications. We used several combinations of primers targeting the areas adjacent to the duplication and then the presence of the right products was confirmed by sequencing. Nested PCR was necessary for the patients with more than two duplicated exons.

Results: All the patients in this study showed non-inverted duplications and tandem.

Conclusion: Traditional PCR method has been used to characterize the duplications in 16 patients and this is the first step to design the AOs that will be tested as a potential therapy for the DMD patients.