

P04 Poster
Lentivirus-mediated stem cell therapy for Duchenne muscular dystrophy

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Duchenne muscular dystrophy is a genetic disorder characterized by loss of dystrophin leading to progressive muscle fibre degeneration, finally resulting in failed muscle regeneration. One possible therapeutic approach is to deliver viral vectors that can transduce skeletal muscle and replace dystrophin. Satellite cells (SCs), stem cells residing underneath the basal lamina of myofibres, play a central role in skeletal muscle regeneration. Long-term therapeutic benefit could only be achieved by restoring dystrophin protein expression not only in muscle fibres but also in SCs, thereby maintaining healthy muscle fibres throughout life. Lentiviruses hold great potential as a gene therapy tool for skeletal muscle, as they can stably integrate their genomes into dividing and non-dividing cells, and provide long-term expression. However, low transduction efficiencies in muscle and early promoter silencing *in vivo* have been discouraging. Here, we show that primary SC cultures can be transduced with lentiviral vectors requiring moderate MOIs. Lentiviral transduction does not affect SC myogenicity, and transgene expression is maintained for at least 3 weeks in culture. Interestingly, transduction of single myofibres *in vitro* revealed GFP expression in both associated SCs and the myofibre syncytium. Next, we will compare muscle-specific (e.g. Desmin) and silencing-resistant (e.g. 2AUCOE) promoters with our results of a strong viral promoter, and determine the level and longevity of transgene expression in SCs and myofibres *in vitro* and *in vivo*. Finally, we will investigate the potential of transduced SCs engrafted into immunodeficient mice to repair and regenerate skeletal muscle *in vivo*.

P05 Poster
Induction of dystrophin in Duchenne muscular dystrophy patients by antisense oligonucleotide AVI-4658 restores the dystrophin-associated 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 exon 51 in dystrophin mRNA in seven patients with DMD [1]. The morpholino was injected into one extensor digitorum brevis (EDB) muscle while the contralateral muscle received saline. No adverse effects resulted from injection of AVI-4658; 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 (DGC) in the dystrophin-positive fibres

Methods: The DGC proteins were studied by immunofluorescence with antibodies to dystrophin (dys1, dys2, dys3 and Mandys106), α -sarcoglycan, β -dystroglycan, nNOS, and dystrobrevin, and compared with β -spectrin, utrophin and neonatal myosin and other markers of immaturity such as laminin α 5. Further assessment of the effect of regeneration, quantification by immunoblotting and quantification of immunofluorescence is in progress.

Results: The increased detection of dystrophin in the treated muscle was accompanied by more intense immunofluorescent labelling of the DGC. Intensities were variable and differences due to different affinities of the antibodies were taken into account.

Conclusion: We show that dystrophin expression induced by AVI-4658 is followed by restoration of the DGC in these fibres, further indicating that the shortened dystrophin produced in these patients is functional.

Reference(s)

[1] Kinali M, et al. *Lancet Neurol.* 2009; 8: 918–928.

P06 Poster
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. DMD is caused by mutations in the DMD gene leading to a lack of dystrophin protein in skeletal muscle resulting in a breakdown of the integrity of the muscle cell membrane. The resultant muscle fibres are highly prone to contraction induced injury. Consequently the progressive rounds of degeneration and regeneration of the muscle lead to the replacement of muscle fibres with non contractile fibrotic tissue and fatty infiltrates. These alterations lead to progressive muscle wasting, weakness and death in late adolescence. Gene therapy strategies for the delivery of dystrophin to skeletal muscle have been hampered by a number of factors.

A promising alternative therapeutic approach for DMD is antisense-mediated exon skipping using antisense oligonucleotides (AONs) targeting specific exons to restore the DMD reading frame. The products of these therapies are truncated forms of dystrophin, which should restore the integrity of the muscle cell membrane and elevate the degeneration of muscle fibres. 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.

In order to evaluate the therapeutic value of these therapies, several different forms of truncated human dystrophin were cloned into the pCI plasmid. These truncated forms represent the dystrophins created by skipping different single exons or skipping multiple exons currently being investigated by various labs. The truncated dystrophins were electro-transferred into *mdx* mice muscle and their expression was assessed. Truncated dystrophin resulting from skipping exon 45 to exon 55 is expressed in mice muscle and correctly localises to the sarcolemma.

P07 Poster
Translation related clinical trials in duchenne muscular dystrophy (DMD) in the UK

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A number of studies have moved to the bedside in the form of phase I/II/III clinical trials in DMD:

ANTISENSE OLIGONUCLEOTIDE (AO) AVI-4658: The MDEX Consortium in collaboration with AVI BioPharma USA is conducting AO trials in London and Newcastle. A phase I/Ib proof of concept IM study