

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

in 7 DMD boys, funded by DoH is complete [1]. A phase I/II systemic, open-label, dose escalating safety study in 19 DMD boys in six cohorts and jointly funded by MRC UK and AVI-BioPharma is ongoing. Preliminary data from this study will be presented at the meeting. ATALUREN (PTC124): This is a phase 2b efficacy and safety study in DMD with Nonsense-Mutations. It is an international, multicenter, randomized, double-blind, placebo-controlled, dose-ranging study. It recruited 174 subjects from 37 centres, including 21 in Newcastle, Oswestry and London. This study is complete and patients enrolled to the open-label extension arm with 96 weeks further treatment. DMD HEART PROTECTION: This is a double-blind randomised multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy in DMD without echo-detectable left ventricular dysfunction. It is funded by BHF with centres in Newcastle, London, Oxford, Oswestry and Birmingham. The study will recruit 140 patients for a five year treatment period. Primary outcome will be measured through echocardiography. These and other planned clinical studies mark the beginning of a new era for clinical trials in DMD which will hopefully open the way forward for improved treatment and survival of affected boys.

Reference(s)

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

P08 Poster Exploring emotional impact in a proof-of-principle single-blind, controlled, two-doses escalation intramuscular study of a morpholino splice-switching oligonucleotide (AVI-4658) trial to induce dystrophin restoration in children with Duchenne muscular dystrophy

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Objective: Previous researchers have noted depressive reactions in neuromuscular patients taking part in proof of concept trials. We developed a tool to assess risk of adverse emotional reactions and tested it in eight children with DMD who participated in our recently completed proof-of-concept study [1].

Methods: The emotional risk tool had 11 items and quantified (1) family expectations, psychosocial stress, function, emotional reactivity, psychiatric history; (2) current child psychiatric status. Assessment included interviews/questionnaires with parents and children at trial entry and completion (when emotional impact as assessed).

Results: The mean child age was 12.9 years (6 were using wheelchairs). Mean total risk score was 3.13 (2.3). One family with the highest risk score (7/11) revealed unreasonable expectations from the trial and withdrew participation. Five families returned follow-up impact questionnaires. On an 11-point Lykert scale (10 highest impact) the mean child emotional impact score was 2.0 (2.3): the two children with the highest scores (4 & 7/10) reported an increase in anxiety symptoms but their parents did not note deterioration in psychiatric status. The mean reported impact on mothers was 1.9 (2.4). Child and parent impact was significantly associated with total risk scores (especially so with psychosocial family stress and to a lesser extent with family communication difficulty at study entry).

Conclusion: Emotional impact from the trial was reported for a minority of children and was predicted by our risk tool. Impact scores were associated with only limited change in child psychiatric adjustment.

Reference(s)

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

DMD – Clinical Management/Networks

P09 Poster A Novel Ankle foot orthoses/footwear combination to aid walking in Duchenne muscular dystrophy

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This pilot study will investigate the use of a novel AFO/footwear combination that supports dynamic equinus in ten boys with Duchenne muscular dystrophy (DMD). To maintain independent walking, boys with DMD use equinus gait for optimal alignment of the ground reaction force through the hip and knee joints. As the disease progresses, there is reduced ability to compensate for muscle weakness. This new orthotic strategy is based on understanding DMD gait and the impact of ankle foot orthoses on lower limb biomechanics during walking. It has been trialled with good effect in one boy with DMD. This project for ten boys with DMD will examine user opinion on the novel ankle foot orthosis, quantify its effect on walking parameters using three dimensional movement analysis and measure changes in activity levels.

P10 Poster Parental stress levels in parents of children with muscular dystrophy

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It has been suggested that children and adults with conditions, including Duchenne muscular dystrophy, myotonic dystrophy and congenital myotonic dystrophy, would particularly benefit from the input of a clinical psychologist to help families develop effective management strategies [1]. In Shropshire a clinical psychologist was integrated into the Neuromuscular Service in September 2008 and the effectiveness of psychological intervention is being assessed through prospective audit. As there is currently no baseline information on stress levels in parents of children with muscular dystrophy that use the service, a research project which aims to assess this is currently being developed. It hopes to establish whether there are differences in the levels of stress experienced by parents whose children have been diagnosed with a specific neuromuscular condition, for example Duchenne muscular dystrophy or congenital myotonic dystrophy; and also whether gender differences exist between parents. We intend to use a measure of 'Childhood Illness-Related Parenting Stress' the Paediatric Inventory for Parents (PIP) (Streisand, Braniecki, Tercyak & Kazak, 2001) which measures levels of stress in parents of children with a critical illness. It consists of 42 questions based upon difficult events which parents of children who have (or have had) a serious illness sometimes face. Using the 5 point scale (1 = Never, 2 = Rarely, 3 = Sometimes, 4 = Often, 5 = Very often), parents are asked to indicate a) how often the event occurred in the past 7 days, and (b) how difficult it was/or generally is for them to cope with (1 = Not at all, 2 = A little, 3 = Somewhat, 4 = Very much, 5 = Extremely). Data will be analysed with a One-way analysis of variance (ANOVA) and the results will allow us to determine whether there are significant differences for (a) condition and (b) gender. The findings will help guide the delivery of care provision and service development.

Reference(s)

Streisand R, Braniecki S, Tercyak KP, Kazak AE (2001) Childhood illness-related parenting stress: The Pediatric Inventory for Parents. *Journal of Pediatric Psychology*, 26(3), 155–162.

Yoshimura I, Sasaki A, Akimoto H, Yoshimura N (1989) A case of congenital myotonic dystrophy with infantile autism. *Brain Development*, 21, 379–384.