



6th International Round Table, Monaco, June 23rd, 2007

Clinical trials in Duchenne muscular dystrophy: from phase 1 trials to therapeutic benefits

Report by Olivier Danos, PhD, Necker Hospital, Paris

The 6th Round Table organised by the Association Monégasque contre les Myopathies and Duchenne Parent Project France was attended by 21 scientists and industry representatives and 10 DMD patient organisation representatives. The objective was to discuss the progress from early phase 1 trials in DMD to the development of therapies in clinical use.

Kathryn Wagner (Johns Hopkins School of Medicine, Baltimore) presented the status of a clinical trial using an inhibitor of myostatin. The rationale was to delay as much as possible the progressive muscle weakness and wasting that takes place in muscular dystrophies. Myostatin (GDF8) is an endogenous inhibitor of muscle growth. Its loss leads to increased regeneration and decreased fibrosis in the mdx mouse muscle. No effect on the heart has been observed.

A phase I/II clinical study in patients with different MD was described. This safety assessment study was a double-blind, placebo-controlled, randomized trial of a Myostatin neutralizing antibody (MYO-029) developed by Wyeth Pharmaceuticals. Multiple ascending doses (1, 3, 10, 30 mg/kg) were infused every 2 weeks for 24 weeks in 4 cohorts of 36 subjects equally divided between BMD, FSH, and LGMD. Subjects were followed up for 12 weeks and no severe adverse events related to the drug were observed.

Only short term safety assessed, but there is still concern in the community regarding long term safety of drugs that stimulate regeneration without altering degeneration.

The next step is to document efficacy by measuring muscle mass (MRI) and strength, as well as muscle function using timed function test.

Myostatin blockade is investigated at the level of biosynthesis, extra cellular accumulation or intracellular signal transduction. Companies involved in developing Myostatin inhibitors include Wyeth, Acceleron, PTC Therapeutics and Merck.

Samit Hirawat (PTC Therapeutics, USA) described a clinical trial of PTC 124 in Duchenne. patients. The definition of clinical endpoints in trials involving DMD patients was discussed.

PTC 124 is a molecule that interacts with the ribosome and triggers translational readthrough of stop codons. It is being investigated by the company as a treatment for different genetic diseases including DMD.

The trial involved two groups of DMD patients receiving three injections of low doses (n=6, 4, 4 and 8 mg/kg) or high doses (n=20, 10, 10 and 20 mg/kg) of the drug. Patients were observed for 21 days before

the injection during which a biopsy was performed and muscle cells were tested for their ability to re-express dystrophin in the presence of the drug (0.5 to 10 µg/ml). Injections were made over a period of 28 days after which a muscle biopsy was obtained (Extensor Digitorum Brevis). Immunofluorescence staining for dystrophin was documented in two patients of the high dose group. Western blots confirming the presence of full-length dystrophin were not available. Decreases in circulating levels of muscle enzymes (CK, AST, ALT) were measured.

Patrice Denèfle (Genethon, France) in collaboration with clinical investigators from **Hôpital Pitié-Salpêtrière** in Paris reported on an ongoing phase I/IIa clinical trial in Limb Girdle Muscular Dystrophy 2C (LGMD2C), using an AAV2/1 vector containing the γ -sarcoglycan cDNA under a desmin promoter. The vector was prepared in Harvard Medical School, using 293 cells. The project had obtained an Orphan Drug Designation from the EMEA in October 2004 and the trial was started on November 21st, 2006. Its primary objective was to assess clinical safety of a local vector administration. Secondary objectives included the study of the immune response both to AAV1 and to the transgene, the evaluation of histological changes secondary to vector intramuscular injection and the evaluation of transduction efficiency. Nine patients over 15 year old with a confirmed diagnosis of LGMD2C (homozygous Δ 525T mutation) are sequentially enrolled in three groups. Escalating doses of the AAV vector (3, 5 and 15 10⁹ vg in 100 µl) are injected in the carpi radialis muscle under open procedure. To date, 3 patients have been enrolled, and two treated. Patients were released from confinement 24 hours after injection with no vector detected in blood and urine. No adverse events were observed and a weak humoral response was measured. Positive histological data (immuno-cytochemistry, one month) were presented. Patients will be

followed up for two years. The next step is obviously to deliver the vector to an extended muscle area through perfusion. Preclinical studies are in progress using vectors prepared with the baculovirus technology.

Jan Verschuuren from Leiden University Medical Centre and his collaborators from Judith van Deutekom and Gert Jan van Ommen groups and Prosensa BV, reported on a clinical trial using 2'-O-methyl phosphorothioate antisense oligonucleotides (AON) for exon skipping in Duchenne Muscular Dystrophy. This trial addressed the situation of patients in which the skipping of exon 51 would be beneficial by creating an in frame “quasi-dystrophin” deleted from exons 48 to 51 (18% of patients in the Leiden database). The trial is a single dose, open, single center study on 4 DMD patients aged 10-13. Patients were prescreened for responsiveness to the PRO051 oligonucleotide on fibroblast cultures induced to differentiate into myocytes with MyoD. Patients were injected in the Tibialis Anterior and biopsied after 4 weeks. Evidence for PRO051 activity was obtained by analyzing the mRNA (RT-PCR) and the protein (IF and Western blot). Strong positive signals were obtained in all four patients. Based on this first remarkable success, further trials involving systemic delivery of the AONs are in preparation by **Prosensa**, and should start by the end of 2007. Prosensa has developed in house analytical capacities for AONs and it has partnered production with **Girindus** who runs a commercial scale solid phase facility in Cincinnati. Production and control up to the kilogram scale are possible.

Kate Bushby (Newcastle University) on behalf of the **Mdex Consortium**, described the preparation of a clinical trial using AON with a different chemistry (Morpholino), supplied by **AVI Biopharma (USA)**. Structurally, the difference between Morpholinos and standard nucleic acids is that the bases are bound to morpholine rings

instead of deoxyribose rings and linked through phosphorodiamidate groups instead of phosphates. As a result, they are non-charged and very stable in the cell, because they are not recognized by nucleases

The preclinical studies involve the comparison of available and novel sequences for exon 51 skipping. Sequences are selected for levels and longevity of the skipped product. The EDB muscle has been confirmed as a good target for the study, with a good correlation between MRI and biopsy findings. The project has received GTAC approval on 16/02/07 and an authorization for import of the AON on 21st June 2007. Eleven patients aged between 12 and 17 have been selected and are ready for approach. They will receive 9 injections (100ul, 0.09 to 0.9 mg) at 3mm intervals. Muscle biopsies will be obtained between 14-28 days post injection and analysed for skipping of transcript, restoration of dystrophin protein and restoration of DGC. The consortium is continuing optimisation of sequences for other target exons (46, 53).

Paul Morcos (Gene Tools, USA), further discussed the potential of morpholinos for the treatment of DMD, and described a process for cost-efficient (\$15000 per gram) preparation of large quantities of the products. Intellectual property issues may delay the use of the technology in DMD patients.

Clinical trials where muscle precursor cells are delivered were discussed by **Yvan Torrente (Fondazione IRCCS Policlinico of Milan, University of Milan)**. CD133+ cells were identified as muscle progenitors able to migrate through the blood vessels and participate to muscle regeneration in mice. A clinical trial was organized in which DMD patients received 2x10⁴ autologous CD133+ cells prepared from a Tibialis anterior biopsy into the left abductor digiti minimi muscle. Muscle force measurements suggested that functional progenitors were engrafted. A related approach is to use mesangioblast cells as progenitors, as

described recently in the GRMD model. These preclinical data are highly controversial and pros and cons were discussed by Torrente. A trial in preparation for 2008 will propose intra-arterial HLA-matched donor mesoangioblast transplantation in 3 pediatric DMD patients, with immunosuppression (cyclosporine).

Terry Partridge (Children's National Medical Center, Washington DC) discussed a number of issues related to the use of morpholino AONs for exon-skipping. It appears that morpholinos do not readily enter muscle cells upon systemic delivery. This could be in part due to a suboptimal access to the muscle fiber for AON distributed through the microvasculature. This access is likely to be much better when the AON is delivered intramuscularly, i.e. in direct contact with the fibres. Another point in discussion was the optimization of antisense sequences themselves. There are no rules to predict the activity of a given sequence and evaluations in cultured cells are often not consistent with observations made in vivo, as illustrated here in the GRMD dog model. Yet, given the right sequence, a widespread effect on dystrophin re-expression was observed in GRMD following 7 weekly injections of morpholino AONs.

Luis Garcia (Institut de Myologie, Paris) described preclinical studies of AAV-mediated exon skipping in both mouse and dog models of DMD. In mice, systemic administration of AAV2/1-U7 vectors resulted in widespread long-lasting dystrophin recovery including in the diaphragm and heart.

A similar situation was reported by **Irene Bozzoni (University of Rome La Sapienza)**, using U1 snRNA as antisense carrier delivered by the AAV vector.

In the GRMD dog, the team led by **Luis Garcia** obtained high levels of exon skipping with U7snRNAs carrying antisense sequences designed to mask determinants of exon 6 and 8 definition. After two months,

levels of dystrophin were almost normal in transduced fibres. Histological examination revealed that the dystrophin glycoprotein complex was restored and that spontaneous muscle damages were stopped. Muscle architecture was fully corrected and fibres displayed the hallmarks of mature and functional units. Muscle force as well as NMR imaging indices reflecting muscle structural and functional integrity were improved. This study documents for the first time the recovery of dystrophin at the scale of entire limbs in a large animal and thus represents a critical milestone for the development of clinical trials in human patients.

The immune response against AAV capsids was studied in mice by **Garcia**, who observed that it could be alleviated by a co-stimulation blockade protocol involving anti-CD40 antibodies and CTLA4/Fc fusion protein injections at the time of AAV-U7 administration.

In conclusion, full body treatment of DMD patients with AAV-U7 vectors would require: 1) vector production in the range of 10^{16} vg per patient and 2) the elaboration of an immunosuppressive protocol that would allow for at least a limited number of sequential vector administration.

Valuable data on the immunogenicity of capsids in human patients were obtained by **Amsterdam Molecular Therapeutics (AMT)** as a result of their clinical trial in LPL deficiency. This trial involved intramuscular injection of an AAV2/1 vector (AMT-010) to 8 patients. An increase in CPK around 10 weeks in one patient receiving the highest dose was analyzed as a sign of immune-mediated tissue destruction. Capsid-specific T cell response was detected by IFN- γ ELISpot on PBMC isolated from the patient at 4 weeks. Three other subjects show T cell response against AAV capsid and all have humoral response. A dominant immunogenic peptide has been characterized. This information will be very important for the design of future vectors and immunosuppressive treatments.

Carrie Miceli, (Microbiology, Immunology, & Molecular Genetics, UCLA) presented the design of high-throughput systems for the discovery of drugs able to modulate splicing and/or possibly synergize with AONs. A GFP reporter gene containing dystrophin introns 49 and 50, as well as exon 50, in which GFP is activated upon exon skipping was described. Compounds facilitating exon-skipping can be tested on a C2C12 derived cell line expressing the reporter construct. At present, 8 compounds have been identified that were consistent with induced skipping of exon 50 (no AON present). Five compounds were consistent with enhancement of AON-mediated skipping of exon 50. Another reporter construct based on luciferase is being developed for the screening of a larger library (10000 compounds).

Jamal Tazi (Institut de Génétique Moléculaire, Montpellier) discussed other approaches looking for drugs that target SR protein as modulators of splicing. The group has characterized a number of indole derivatives with such activities, which can induce exon 23 skipping in mdx. A luciferase based reporter system is being used for further screenings. A novel inhibitor of Nonsense Mediated Decay was also described, as a new class of compounds that could stabilize the mutated DMD pre-mRNA, and possibly enhance its availability for AON-induced exon skipping.

Robert Kotin (NIH, Bethesda) presented the state of the art in AAV vector scaled-up production using the baculovirus system. A number of improvements have been made to the original process, resulting in better vector yields and quality. Scalability is now demonstrated up to the 40 litre scale in stirred tank or single use bioreactors (up to 10^{15} vg). Production has been successful with AAV serotypes 1, 2, 4, 5, 6, and 8. A pilot 40 litre production of an AAV2/1-U7 vector is in progress. In theory, extrapolating

to the largest available bioreactors (over 10 000 litres) would yield up to 10e17 vg.

Sander van Deventer described the approach of **Amsterdam Molecular Therapeutics (AMT)** for AAV production using baculovirus. An efficient affinity chromatography procedure has been developed (60% yield). Five GMP production campaigns of 1-2 10e14 vg have been achieved to date.

Généthon is also using the baculovirus technology with 50 litre stirred tank bioreactors and plans to have a process ready to produce 2x10e15 vg by the end of 2007. Translation into GMP is believed to take another 18 months.

A general discussion was organized on the requirements and the competences needed for the organisation of clinical trials with DMD patients.

It was illustrated by a presentation by **Diana Escolar (Children's National Medical Center, Washington DC)** who described the experience of the CINRG network in the USA, and of **Kate Bushby** who presented the initiative of the TREAT-NMD EU network of excellence. The points in discussion were, from the medical stand point, the clinical trial infrastructure, and the validation of outcome measures, as well as the recruitment criteria. From the patients and families perspective, a number of issues were discussed such as time investment, risk, expectations and emotional investment. The fact that participation in early phases might prevent participation in advanced/higher benefit phases was clearly a concern.

In his concluding remarks, **Thomas Voit (Institut de Myologie, Paris)** conveyed the general feeling of cautious optimism shared by scientists, clinicians and parents. Truly exciting times are ahead of us with a great amount of experimental, clinical and regulatory / organisational work to be done. If not a complete cure for DMD, effective therapies are in sight.

Participants of the 6th Round Table

Scientists and clinicians:

Irene Bozzoni (PhD), University of Rome La Sapienza, Italy
Kate Bushby (MD, PhD), University of Newcastle, UK
Olivier Danos (PhD), Necker Hospital, Paris, France
Patrice Denèfle (PhD), Genethon, Evry, France
Diana Escolar (MD, PhD), Children's National Medical Center, Washington, USA
Luis Garcia (PhD), Myology Institute, Paris, France
Claudia Hirawat, PTC Therapeutics, South Plainfield, NJ, USA
Samit Hirawat (PhD), PTC Therapeutics, South Plainfield, NJ, USA
Eric Hoffman (PhD), Children's National Medical Center, Washington, USA
Robert Kotin (PhD), NIH, Bethesda, MD, USA
Carrie Miceli (PhD), Molecular Biology Institute, UCLA, Los Angeles, USA
Paul Morcos (PhD), Gene Tools, Philomath, OR, USA
Stan Nelson (MD), David Geffen School of Medicine, UCLA, Los Angeles, USA
Gerard Platenburg (PhD), Prosensa, Leiden, Netherlands
Terry Partridge (PhD), Children's National Medical Center, Washington, USA
Jamal Tazi (PhD), Molecular Genetic Institute, Montpellier, France
Yvan Torrente (MD, PhD), University of Milan, Italy
Sander van Deventer (MD, PhD), Amsterdam Molecular Therapeutics, Netherlands,
Jan Verschuuren (MD, PhD), Leiden University Medical Center, Netherlands
Thomas Voit (MD, PhD), Myology Institute, Paris, France
Kathryn Wagner (MD, PhD), Johns Hopkins Hospital, Baltimore, USA

DMD associations:

Filippo Buccella, President Parent Project Italy
Nick Catlin, National Coordinator Parent Project UK
Christine Dattola, President Duchenne Parent Project France
Pat Furlong, Executive Director Parent Project Muscular Dystrophy, USA
Rod Howell (MD), Chairman Scientific Advisory Committee MDA, USA
Luc Pettavino, President Association Monégasque contre les Myopathies, Monte Carlo
Benjamin Seckler (MD), President Charley's Fund, Egremont, MA, USA
Laurence Tiennot-Herment, President AFM, Evry, France
Joel Wood, Foundation to Eradicate Duchenne, Alexandria, VA, USA
Elizabeth Vroom, President Duchenne Parent Project Netherlands and UPPMD