TREAT-NMD
Neuromuscular Network

International Conference

Bringing down the barriers

in translational medicine in inherited
neuromuscular diseases

NATIONAL INSTITUTES
OF HEALTH

Brussels, Belgium
17-19 November 2009

– Book of abstracts –

TREAT-NMD/NIH International Conference

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Wageningen Academic
P u b l i s h e r s

Table of contents

Welcome from the European Commission

Dear Delegates,

The European Commission issued in 2005 a call for research proposals in rare inherited neuromuscular disorders, within the context of its Sixth Framework Programme for Research and Technological Development. The aim of this call was to ensure a long-lasting collaboration amongst first-class researchers in this field in order to foster translational research to benefit as much as possible the patients.

As a result of this call, the European Commission granted 10 million Euro (around 14.5 million US Dollar) to support the network of excellence 'TREAT-NMD' for a period of 5 years. This multidisciplinary consortium is composed of 24 partners, gathering basic and clinical academics, patient representatives, industries and managers from all over Europe. Interaction with the wider international neuromuscular research community is ensured through a broad 'Club of Interest'.

TREAT-NMD eventually started in 2007 and is setting up a 'TREAT-NMD Coordination Centre', a key step to long-lasting integration of all levels of European research in neuromuscular diseases. Activities include the development of a clinical and therapeutic platform, improved data and sample collection and availability, the development and use of disease models, as well as promising preclinical and clinical research.

It is my pleasure to welcome you to this TREAT-NMD international conference, co-sponsored by the National Institutes of Health. Gathering international experts around an exciting programme, this conference will update you on the state of the art in research on neuromuscular diseases.

I wish all participants a fruitful conference, strengthening the experts' network and bringing knowledge forward.

Dr. Ruxandra Draghia-Akli
Director,
Health Directorate,
DG Research,
European Commission

SIXTH FRAMEWORK PROGRAMME   COOPERATION

Welcome from the Conference organisers (TREAT-NMD and NIH)

Dear Delegates,

On behalf of the Organising Committee, TREAT-NMD and the NIH, we welcome you to Brussels for our first International Conference entitled 'Bringing Down the Barriers in Translational Medicine in Inherited Neuromuscular Diseases'. We are pleased to be able to bring you this conference in Brussels, the home of the European Commission, who have funded the establishment of TREAT-NMD, and many other initiatives, to improve the quality of life for all its citizens. In partnership with the NIH we are ensuring that we recognise the need to look beyond Europe and address these issues globally through international collaborations for the benefit of all.

We have assembled a stimulating programme to discuss progress in translational medicine and map the future collaborative agenda to ensure care and therapy development is driven forward between clinicians, scientists, patients, and industry for the benefit of the neuromuscular community.

Participants of the conference sessions have been working hard in the lead-up to this meeting to prepare the key information on the current 'state of the art' in their respective areas, and they will present this via expert panel discussion sessions and participation from the conference delegates in general.

Our aim has been to bring together the experts, opinion leaders, and the neuromuscular community to tackle the key issues that need to be addressed if we are to see new and promising therapies and treatments rapidly delivered to patients all around the world.

We hope you enjoy your time in Brussels and we look forward to your active participation in the conference over the next few days.

Best wishes.

Katie Bushby (TREAT-NMD)
Volker Straub (TREAT-NMD)
Stephen Lynn (TREAT-NMD)
John Porter (NIH)

Conference programme

Tuesday 17th November

9:00   Registration and setting up of posters

14:00   Opening of conference (Kate Bushby and John Porter)

14:05–14:30   **Keynote lecture**
DMD: the long march from the parts to the whole
Gert-Jan van Ommen (*Leiden University, the Netherlands*)

14:30–16:00   **Making clinical trials in neuromuscular diseases a reality**
Chairpersons: Kate Bushby (*TREAT-NMD Coordinator, Newcastle University, UK*) and
John Porter (*NIH/NINDS, USA*)

This session starts the conference with an exploration and discussion of the models that would best guide therapy development programmes, including the industry standard of label-based design, and will address novel partnering models to ensure that appropriate expertise and funding is recruited to projects. We will also discuss the important and controversial issue of triaging both repositioning candidates as well as novel therapeutics for neuromuscular disease – seeking input from drug developers, academics, advocacy groups, and patients. In a focussed discussion session, the views of patient organisations representing small patient numbers, as well as the larger patient organisations and the FDA, will be discussed.

Presentations:
14:30–15:05   Moving forward with TACT (TREAT-NMD Advisory Committee for Therapeutics) (Cristina Csimma)
15:05–15:20   The STAR initiative; partnering between the CMTA, academia and government to develop therapies for CMT (Michael Shy)
15:20– 5:30   Attaining symbiosis for therapy development efforts in neuromuscular diseases (John Porter)

15:30–16:00   **Panel session:**   Cristina Csimma (*Virdante Pharmaceuticals, USA*)
Michael Shy (*Wayne State University, USA*)
Thomas Voit (*Institute of Myology, France*)
Petra Kaufmann (*NIH/NINDS, USA*)
Elizabeth McNeil (*FDA, USA*)
Anne Rutkowski (*Cure-CMD, USA*)
Plavi Mittal (*Jain Foundation, USA*)
Sharon Hesterlee (*MDA Venture Philanthropy, USA*)
Gunnar Buyse (*University of Leuven, Belgium*)

16:00–16:30   Afternoon tea and coffee (with poster viewing)

16:30 – 18:00   **Target and candidate identification**
Chairpersons: Jon Tinsley (*Summit, UK*) and
Lee Sweeney (*University of Pennsylvania, USA*)

We will review the kinds of cell assays one can use for initial screens to identify potential therapeutic starting points for possible future development. For this, one needs to consider a number of related themes such as:
- What cell types are to be used? e.g. primary cells, immortal cell lines, stem cells.
- Any trade off between throughput vs. target relevance? e.g. compound collection size, assay complexity, disease relevance.
- Are the assay end points appropriate for screening?

Once 'hits' have been identified using the screening assay the next requirement is to confirm the hit can manipulate the cellular processes believed to gain a therapeutic advantage in order to develop a candidate for consideration in animal models i.e. the concept of hit validation *in vitro*. Questions that need to be asked when considering this include:
- Is the 'hit' mechanism known?
- Is there a valid *in vitro* therapeutic endpoint? e.g. increased target RNA, relocalisation of target protein.
- Is the 'hit' toxic in cells?

It is only after iteration and refinement of the therapeutic repetitively through the assay and *in vitro* validation that a compound should be considered a therapeutic candidate and moved to testing *in vivo* for proof of concept.

Presentations:

16:30–17:00	HTS for NMD targets: first step in the search for critical therapeutics (John Babiak)
17:00–17:15	Drawbacks and possible solutions for the use of myoblasts to screen dystrophin exon-skipping antisense oligonucleotides (Jenny Morgan)
17:15–17:30	Where's the target? Drug discovery and target identification applied to motor neuron diseases (Rebecca Pruss)
17:30–17:45	SMN function and high throughput screening for SMA (Gideon Dreyfuss)

17:45–18:00	**Panel session:**	John Babiak (*PTC Therapeutics, USA*)
		Rebecca Pruss (*Trophos, France*)
		Gideon Dreyfuss (*University of Pennsylvania, USA*)
		Jenny Morgan (*University College London, UK*)

18:00–20:00	Drinks reception and poster session 1

Wednesday 18th November

09:00–10:30	**Animal model assessment**	
	Chairpersons:	Markus Ruegg (*University of Basel, Switzerland*) and
		Joe Kornegay (*University of North Carolina, USA*)

Testing of candidate drug and biologic therapeutics in animal models is an essential step to provide the proof-of-concept needed for moving new treatments into clinical trials. Although animal models are, by definition, specific to single neuromuscular diseases, it is critical to collectively learn from the experiences in individual diseases. We will describe common lessons from the shared experience and pitfalls from a diverse range of therapy development efforts in neuromuscular disease.

Topics to be addressed include:
- Bringing adequate rigor to animal efficacy testing – efficacy data largely drive investments made in drug development and patent applications. How do we ensure these investments are based on the strongest possible data?
- In search of the 'ideal' animal model for therapeutic testing – recognizing that an animal model will never be perfect, what do we need in an animal model to meet the potentially conflicting demands of academic intellectual curiosity and industrial emphasis on simplicity and high-throughput.
- Rodent versus 'large animal' models – for some neuromuscular diseases, there are no defined genetic animal models. For others, multiple models have been characterized. What are the roles for the different animal models and, in particular, for rodent versus 'large animal' models?
- Outcome measures to define whether a candidate is a 'go' or a 'no-go'– how many measures to use and what kind?
- Ensuring consistency from lab-to-lab and company-to-company in neuromuscular therapy development – what are the issues in standardizing the different animal models and achieving standard operating procedures in therapy testing?
- Ensuring consistency from lab-to-lab and company to company in neuromuscular therapy development – is there a place for independent efficacy testing in core facilities?

Presentation:

09:00–09:30	Lost in translation: lessons for ALS from the SOD1 mouse (Michael Benatar)

09:30–10:30	**Panel session:**	Michael Benatar (*Emory University, USA*)
		Annamaria De Luca (*University of Bari, Italy*)
		Miranda Grounds (*University of Western Australia*)
		Kanneboyina Nagaraju (*Children's National Medical Centre, USA*)
		Charlotte Sumner (*Johns Hopkins University, USA*)
		Shin'ichi Takeda (*National Centre of Neurology and Psychiatry, Japan*)

10:30–11:00	Morning tea and coffee (with poster viewing)

| 11:00–12:30 | **Therapeutic misconception and ethical considerations** |
| | Chairperson: Simon Woods (*Newcastle University, UK*) |

It is absolutely necessary to conduct clinical trials involving adults and children with rare diseases. It is also mandatory that participation in clinical trials is based upon voluntary and adequately informed consent. However there is a growing body of evidence that those with parental decision making responsibility may be unable to distinguish between research and treatment, the so-called 'therapeutic misconception'.

In this session we will explore the possible implications of this evidence for the design and conduct of clinical trials for neuromuscular diseases through a formal debate and interactive panel discussion. It is perhaps uncontroversial that those responsible for regulating research and for providing ethical approval have the responsibility to ensure the safety and well-being of vulnerable children. During this session we will explore the extent of this responsibility and its implications for parents, researchers, and regulators. It will be proposed that where parents have unrealistic expectations of benefit it is unlikely that they meet the conditions for a legal and ethical consent. With this in mind, the panel will debate the following motion:

> *'Parents who express hope in the possibility of therapeutic benefit from clinical trial participation should not be allowed to consent for their children to enter trials'*

The audience will be invited to vote on the motion before and after the debate. This session involves people with NMDs, patient advocates, a clinician, an ethicist, a parent and a lawyer. During the debate the panel will:
- deconstruct the concept of the 'therapeutic misconception';
- invite audience participation and questions;
- provide expert commentary;
- conclude with constructive advice using examples of good practice with a view to their dissemination throughout the wider community.

Panel session: Elizabeth Vroom (*United Parent Project MD, the Netherlands*)
Volker Straub (*TREAT-NMD Coordinator, Newcastle University, UK*)
Pauline McCormack (*Newcastle University, UK*)
Lynn Hagger (*University of Sheffield, UK*)
Maryze Schoneveld van der Linde (*ENMC, the Netherlands*)

| 12:30–14:00 | Lunch and poster session 2 |

14:00–14:30	**Keynote lecture**
	New treatments for hereditary neuromuscular diseases
	Kenneth Fischbeck (*NIH/NINDS, USA*)

14:30–16:00	**Developing novel, disease targeted therapies and systemic delivery**
	Chairpersons: Serge Braun (*AFM, France*) and
	Kenneth Fischbeck (*NIH/NINDS, USA*)

We will highlight novel gene directed therapies for neuromuscular diseases: expected therapeutic benefits, delivery issues, and regulatory challenges. Exon skipping is taken as a model approach for gene-specific therapy. Invited speakers will present the latest results of cutting-edge pre-clinical and clinical trials.

Presentations:
14:30–14:40	New results from Prosensa's exon skipping trial (Judith Van Deutekom)
14:40–14:50	Limb perfusion gene delivery (Jon Wolff)
14:50–15:00	New results from AVI's exon skipping trial (Francesco Muntoni)
15:00–15:10	New results from systemic AAV total body delivery (Luis Garcia)

15:10–16:00	**Panel session:**	Judith van Deutekom (*Prosensa, the Netherlands*)
		Jon Wolff (*Roche, USA*)
		Hong M. Moulton (*Oregon State University, USA*)
		Luis Garcia (*Institute of Myology, France*)
		Annemieke Aartsma-Rus (*Leiden University, the Netherlands*)
		Francesco Muntoni (*University College London, UK*)
		Dominic Wells (*Imperial College London, UK*)
		Alessandra Ferlini (*University of Ferrara, Italy*)
		Brian Kasper (*Nationwide Children's Hospital, USA*)

16:00 – 16:30   Afternoon tea and coffee (with poster viewing)

16:30 – 18:00   **Registry development for clinical trials**
Chairpersons: Hanns Lochmüller (*Newcastle University, UK*) and
                 Jacqueline Jackson (*Indiana University, USA*)

As clinical trials become a reality in an increasing number of neuromuscular conditions, the need for registries containing well-defined, up-to-date and locatable patient cohorts is becoming ever more pressing. Many therapies require a precise knowledge of the patient's particular genetic mutation. Other clinical items are important as potential inclusion criteria for a trial. At the same time, data collected must be streamlined, simple and in many cases self-reportable by patients or carers in order to achieve maximum uptake. Ethical issues around consent, data ownership and data protection must also be carefully considered, and the benefits to patients and families of signing up for the registry clearly defined.

We will draw on the success of the TREAT-NMD global registries for DMD and SMA, which are already being used by pharmaceutical companies to provide trial feasibility information, and extend this with experience from other disease groups, using the example of the National Research Roster for Huntington Disease. The importance of registries from a regulatory perspective in terms of post-marketing/approval studies and pharmacovigilance will also be considered. The overall aim of the session will be to draw on lessons learned and summarise best practice for registry development for other neuromuscular conditions.

Presentations:
16:30–16:50   Huntington disease and SMA (Jackie Jackson)
16:50–17:10   Regulatory and industry perspective (Per Nilsson)
17:10–17:30   TREAT-NMD patient registries (Hanns Lochmüller)

17:30–18:00   **Panel session:**   Per Nilsson (*Actelion, Switzerland*)
                                      Christophe Béroud (*INSERM, France*)
                                      Vanessa Rangel Miller (*DuchenneConnect, USA*)
                                      Maryze Schoneveld van der Linde (*ENMC, the Netherlands*)

20:00   Gala dinner at the Concert Noble, Aarlenstraat 82 B-1040, Brussels

Bus transportation will be provided from the Crowne Plaza to the Concert Noble departing at 19:00. The evening will be a special and festive party with tasty dishes, drinks and live music from the 'Jazz collective' and DJ Pipa.

Thursday 19th November

09:00–10:30   **Clinical outcome measures**
Chairpersons: Michael Rose (*King's College London, UK*) and
                 Julaine Florence (*Washington University, USA*)

The correct choice of outcome measures for a clinical trial can be critical to its success. Making these choices can be a time consuming and lengthy process and if the process is repeated for every trial may result in a large duplication of effort. Even when the choices have been made there are many practical issues that need to be addressed in order to successfully implement an outcome measure for a trial. The panel participants represent the many stakeholders involved in outcome measures including clinicians and academics who have created, assessed, chosen and implemented outcome

measures for trials, industry partners, regulators and patient representatives. We will highlight the factors that may influence the choice of outcome measures and the tools that are being developed to help with making the choice. We will draw on examples from trials planning and implementation for SMA, DMD, Charcot Marie Tooth, myotonic dystrophy, inclusion body myositis and peripheral nerve disease.

Presentations:

09:00–09:15	How should we choose outcome measures for clinical trials and studies? (Michael Rose)
09:15–09:30	Implementing and optimising the clinical outcome measure (Julaine Florence)
09:30–09:45	Quality assessment of outcome measures (Jeremy Hobart)

09:45–10:30   **Panel session:**   Leone Atkinson (*PTC Therapeutics, USA*)
Joanne Auld (*King's College London, UK*)
Carole Bérard (*Hospices Civils de Lyon, France*)
Michael Benatar (*Emory University, USA*)
Anne Connolly (*Washington University Saint Louis, USA*)
Michelle Eagle (*Newcastle NHS Trust, UK*)
Jeremy Hobart (*Peninsula Medical School, UK*)
Anna Mayhew (*Newcastle University, UK*)
Eugenio Mercuri (*Catholic University Rome, Italy*)
Ingemar Merkies (*University Hospital Maastricht, the Netherlands*)
Elizabeth McNeil (*FDA, USA*)
Mary Reilly (*University College London, UK*)
Reza Sadjadi (*King's College London, UK*)
Benedikt Schoser (*Ludwig Maximilians University Munich, Germany*)
Michael Shy (*Wayne State University, USA*)
Jean Louis Thonnard (*Universite Catholique de Louvain, Belgium*)
Elizabeth Vroom (*United Parent Project MD, the Netherlands*)

10:30–11:00   Morning tea and coffee (with poster viewing)

11:00–12:30   **Effects of long-term treatment and combination therapeutics**
Chairpersons: Rudolf Korinthenberg (*University Hospital Freiburg, Germany*) and
Robert Griggs (*University of Rochester, USA*)

We will focus upon the future of long-term treatment. Most approaches currently in development do not cure a disease; however they will slow its progression. Determining long-term net benefit requires different outcomes measures than the ones generally used in short term studies. In addition, of the drugs already used, benefit for long-term treatment has not been established. Using Pompe disease and Duchenne Muscular Dystrophy as examples basic guidelines for the use of these drugs should be created in the interim period.

Presentations:

11:00–11:10	Long-term corticosteroids in DMD: Implications for new treatments (Robert Griggs)
11:10–11:15	Long-term side effects of drugs in development for NMDs (Rudolf Korinthenberg)
11:15–11:25	Post-marketing studies - using Pompe disease as a paradigm (Bruno Eymard)
11:25–11:40	Combination of old and new approaches - ending with a forward look and guidelines (Francesco Muntoni)

11:45–12:30   **Panel session:**   Bruno Eymard (*Institute of Myology, France*)
Francesco Muntoni (*University College London, UK*)
Kate Bushby (*TREAT-NMD Coordinator, Newcastle University, UK*)
Annemieke Aartsma-Rus (*Leiden University, the Netherlands*)

12:30–13:00   **Closing keynote lecture**
Breaking down the barriers between stakeholders
Volker Straub (*TREAT-NMD Coordinator, Newcastle University, UK*)

13:00   Closing remarks followed by lunch

Biographies of keynote speakers

Gert-Jan van Ommen (Leiden University Medical Centre)

Prof. dr. Gert-Jan B. van Ommen, PhD, (1947) is head of the Department of Human Genetics of Leiden University Medical Centre (LUMC) and founder of the Leiden Genome Technology Centre (LGTC), a principal genomics facility in the Netherlands. He has as major research interests neuromuscular and neurodegenerative diseases (with a focus on Duchenne Muscular Dystrophy, DMD, and Huntington Disease); development and application of genome research and diagnostic technology for disease study, diagnosis, therapy and prevention, including the societal aspects of genetic advances. He is past president of HUGO, the European Society of Human Genetics and the Dutch Society of Human Genetics and Editor-in-chief of the European Journal of Human Genetics. He is member of several National, EU and HUGO committees in the fields of Genetics, Innovative Health Care, Genomics, Bioinformatics, Biobanking, Ethics and IP issues. He is Director of the Centre for Medical Systems Biology, CMSB, one of four Centres of Excellence of the Netherlands Genome Initiative. It is a joint initiative of Leiden University Medical Centre, Leiden University, Free University Amsterdam and its Medical Centre, Erasmus MC Rotterdam and TNO Leiden, aiming to improve diagnosis, therapy and prevention of common diseases and rare variants thereof.

Kenneth Fischbeck (NIH/NINDS)

Dr. Fischbeck received A.B. and A.M. degrees from Harvard University and an M.D. degree from Johns Hopkins. After a medical internship at Case Western Reserve University and a neurology residency at the University of California in San Francisco, he did postdoctoral research on muscular dystrophy at the University of Pennsylvania. In 1982 he joined the faculty in the Neurology Department at the University of Pennsylvania Medical School. In 1998 he came to the NINDS as Chief of the Neurogenetics Branch. He received the Cotzias Award from the American Academy of Neurology and was elected to the Institute of Medicine of the National Academy of Sciences. His laboratory is studying the mechanisms of hereditary neurological and neuromuscular disorders, particularly the polyglutamine expansion neurodegenerative diseases.

Volker Straub (TREAT-NMD Coordinator, Newcastle University)

Professor Volker Straub is joint co-ordinator of TREAT-NMD, executive board member of the World Muscle Society and executive board member of the Institute of Human Genetics at Newcastle University. Together with Hanns Lochmüller, Volker was responsible for setting up the German muscular dystrophy network, MD-NET, of which he was joint coordinator until 2008. Within the neuromuscular research group at Newcastle, Volker has a long-standing interest in the pathogenesis of muscular dystrophies, with research using zebrafish and mouse models. His current research also involves the application of contrast enhanced MRI.

Biographies of Conference session co-chairs

Making clinical trials a reality

Kate Bushby (TREAT-NMD Coordinator, Newcastle University)

Professor Kate Bushby has overseen the expansion of the Newcastle Muscle Centre since 1999 to its position today as a leading international neuromuscular centre. Together with Volker Straub, Katie is joint coordinator of the TREAT-NMD network. She is Research Director of the ENMC and is a member of the Scientific Advisory Committee of the AFM. In the UK she is Deputy Director of the MRC Centre for Neuromuscular Diseases at University College London and Newcastle, and Clinical Director of the NCG diagnostic and advisory service for rare neuromuscular disorders (limb-girdle muscular dystrophies).

John Porter (NIH/NINDS)

Dr. John Porter is Program Director at the National Institute for Neurological Disorders and Stroke (NINDS). He manages a portfolio of research grants that focuses on diseases affecting the motoneuron (spinal muscular atrophy and spinal bulbar muscular atrophy), axon (inherited and acquired peripheral neuropathies), neuromuscular junction (inherited and acquired myasthenia gravis and slow channel syndrome), and skeletal muscle, (myotonic dystrophy and congenital, Duchenne/Becker, Emery-Dreifuss, facioscapulohumeral, limb girdle, and oculopharyngeal muscular dystrophies). He currently serves as Executive Secretary for the interagency Muscular Dystrophy Coordinating Committee and is on advisory boards for Translational Research in Europe-Assessment and Treatment of Neuromuscular Diseases (TREAT-NMD), the MRC Centre for Neuromuscular Diseases, the Muscular Dystrophy Association (MDA) Translational Research Advisory Committee, the Fields Centre for FSHD and Neuromuscular Research, and the Jain Foundation. Dr. Porter received his undergraduate degree in Biology from the College of William and Mary and his PhD in Anatomy from Medical College of Virginia and completed postdoctoral training in systems neurophysiology at the University of Alabama at Birmingham. Prior to joining the NIH, Dr. Porter was Professor of Neurology at Case Western Reserve University. His 20+ year academic research career focused upon extraocular muscle biology in health and disease, including the mechanisms responsible for its novel responses to a variety of neuromuscular disorders.

Therapeutic target and candidate identification

Jon Tinsley (Summit plc)

Jon completed his undergraduate and postgraduate education at Leeds and Birmingham University in the field of molecular virology and cell biology, receiving his PhD in 1991 where he continued in academic research for several years. This included a number of years in the laboratory of Dame Prof. Kay Davies where he played a major role in elucidating the function and therapeutic potential of utrophin. Following on from this work, Jon received a prestigious MRC Non-clinical Senior Fellowship to develop proteomic and transcriptomic technologies in neurological diseases. Jon moved into the Biotech arena with Oxagen, as Head of Biology working on target identification and drug discovery projects yielding a number of compounds progressing into preclinical development. Jon joined VASTox (now Summit plc) as Head of Biology in the spring of 2005 tasked with the role of developing the biology capability, particularly the *in vivo* and *in vitro* screening platforms for internal drug discovery programs. Jon has been programme director for a number of projects successfully out-licensed which includes the utrophin upregulation programme licensed to BioMarin in 2008. Jon's current position is Senior Director of R&D.

Lee Sweeney (University of Pennsylvania)

Dr. Sweeney's research program addresses the molecular basis of cellular movement and force generation. His approach encompasses investigations on single molecules, single cells and whole organisms. At the level of the single molecule, the work examines the basic design and function of the molecular motor, myosin. These studies combine protein engineering with biochemical and structural analyses. At the level of isolated cells (cultured myocytes), the research program has two aspects: (1) investigation of the role of various proteins either in the generation of force, or in the transmission of force across the cell membrane, and (2) the process of assembly of the contractile apparatus. Studies at the whole animal level involve gene transfer into muscle (both germ line and somatic cell). Somatic cell gene transfer (utilizing viruses) allows the assessment of acute alterations in cell structure and function following viral-driven expression of a single protein. In response to acute changes in properties, feedback pathways intrinsic and extrinsic to the muscle cell signal alterations in the muscle gene expression program that result in an adaptive response. This new approach allows critical evaluation of principles of muscle cell design as well as evaluation of possible causes of and treatments for muscle diseases. Currently, Dr. Sweeney is studying two diseases, Duchenne Muscular Dystrophy and hypertrophic cardiomyopathy, with this approach.

Animal model assessment

Markus Rüegg (University of Basel)

Prof. Markus A. Rüegg has a track record as researcher in basic research addressing the development and function of the neuromuscular junction and mechanisms of neuromuscular diseases. Has authored more than 60 publications in major journals such as Nature, Nat. Cell Biol., Neuron, J. Cell Biol., EMBO J. and is inventor on 4 patents. He is an invited speaker to EMBO and ENMC Workshops, Gordon Research Conferences, to the World Muscle Society and several additional major meetings in the filed. He is also co-founder of MyoContract. He is the scientific representative of the University of Basel on the TREAT-NMD Governing Board.

Joe Kornegay (University of North Carolina)

Dr. Joe N. Kornegay is Professor in the Departments of Pathology and Laboratory Medicine and Neurology, and Investigator, Gene Therapy Centre, School of Medicine, University of North Carolina-Chapel Hill. After receiving his veterinary degree from Texas A&M University in 1973, Dr. Kornegay spent three years in private practice in Ohio and Texas, followed by six years in residency (neurology and pathology) and graduate (Masters and PhD) training at the University of Georgia College of Veterinary Medicine. Upon completion of this training, he served on the faculty of the College of Veterinary Medicine at North Carolina State University for 11 years before moving to the College of Veterinary Medicine at the University of Missouri. At Missouri, Dr. Kornegay principally served as an administrator, first as a department chair and later as dean. He moved to his current position at the University of North Carolina-Chapel Hill School of Medicine in 2006. For the past 25 years, Dr. Kornegay has studied a spontaneous canine disease termed *golden retriever muscular dystrophy (GRMD)*, which serves as an animal model for Duchenne Muscular Dystrophy (DMD) of humans.

Therapeutic misconception and ethical considerations

Simon Woods (Newcastle University)

Dr Simon Woods is a bioethicist at the Policy, Ethics and Life Sciences Research Centre (PEALS). He is a member of a number of research and clinical ethics committees and has an active international research profile in medical ethics and ethics related to developments in the life sciences. He has published widely in the field and provides professional development training to health professionals and members of ethics committees. Within the TREAT-NMD project Simon chairs the Project Ethics Council and is a leader of the ethics workpackage.

Developing novel, disease targeted therapies and addressing the challenges of systemic delivery

Serge Braun (AFM)

Serge Braun, PharmD, PhD is Scientific Director of AFM, the French Muscular dystrophy Association. He has 10 years experience in the University (working on the pathogenesis and treatment of different genetic and acquired neuromuscular diseases) and 10 years in the biotechnology sector (as VP Research at Transgene, Strasbourg, France; a leading gene therapy biotech company; also as co-founder of Neurofit, a contract research organization specialized in preclinical testing of both the central and the peripheral nervous system). He was Vice-President of Alsace BioValley, member of the tri-national biocluster. He is also scientific expert or member of the Scientific Board of different state Institutions, non-profit associations, Venture Capitalists, biotechs and bioclusters.

Kenneth Fischbeck (NIH/NINDS)

Dr. Fischbeck received A.B. and A.M. degrees from Harvard University and an M.D. degree from Johns Hopkins. After a medical internship at Case Western Reserve University and a neurology residency at the University of California in San Francisco, he did postdoctoral research on muscular dystrophy at the University of Pennsylvania. In 1982 he joined the faculty in the Neurology Department at the University of Pennsylvania Medical School. In 1998 he came to the NINDS as Chief of the Neurogenetics Branch. He received the Cotzias Award from the American Academy of Neurology and was elected to the Institute of Medicine of the National Academy of Sciences. His laboratory is studying the mechanisms of hereditary neurological and neuromuscular disorders, particularly the polyglutamine expansion neurodegenerative diseases.

Registry development for clinical trials

Hanns Lochmüller (Newcastle University)

Professor Hanns Lochmüller joined the Newcastle Muscle Centre in 2007 from Munich. Together with Volker Straub, Hanns was responsible for setting up the German muscular dystrophy network, MD-NET, of which he was Coordinator (jointly with Volker Straub) until 2008. He is the scientific coordinator of EuroBioBank, a European network of biobanks for rare disorders. Before coming to Newcastle University, Hanns held the post of Consultant and subsequently Professor of Neurology and Molecular Neurogenetics at Ludwig-Maximilians-University in Munich. He has a long-standing interest in the molecular genetics of the inherited myopathies and neuromuscular junction disorders, and his research focuses on the further study of animal models of these disorders as a means to understanding their pathophysiology, as well as to develop the means to monitor disease progression and therapeutic interventions.

Jackie Jackson (Indiana University)

Ms. Jackson has Bachelor of Science Degrees in Psychology and Biology. She has been a Research Manager in the Department of Medical and Molecular Genetics at Indiana University School of Medicine for nearly 25 years. She has been responsible for the development and direction of numerous research studies including studies of Huntington Disease, Parkinson Disease, Alzheimer Disease, Alcoholism, Spinal Muscular Atrophy, Charcot-Marie-Tooth and others. The studies she manages vary in scope ranging from the collection of family history information to the facilitation of neuropathology and collection and dissemination of a multitude of biological specimens. Indiana University maintains the National Research Roster for Huntington Disease Patients and Families a registry which has been ongoing for over 30 years. Indiana University also maintains data from the Huntington Disease in Venezuela Study. Ms. Jackson is a 25 year member of the Huntington Disease Venezuelan Collaborative Research Team responsible for finding the Huntington Gene. Indiana University currently participates in the TREAT-NMD global database for Spinal Muscular Atrophy and plans to contribute data on Charcot-Marie Tooth in the near future as well.

Clinical outcome measures

Michael Rose (Kings College London)

Michael Rose is consultant in adult neurology at King's College Hospital, London, United Kingdom and runs a regional muscle disease clinic service there. He was a Medical Research Council research fellow investigating mitochondrial muscle disease and spent a year in Rochester, New York gaining specialist training in muscle disease. He is a founder member of the Muscle Study Group and was involved in the conduct of their trials in inclusion body myositis. He has been involved in the planning stages of several NMD trials including those for Duchenne Muscular Dystrophy, spinal muscular atrophy, polymyositis and periodic paralysis. Michael is co-editor of the Neuromuscular Diseases Cochrane Review Group. He was clinical trials mediator for the European Neuromuscular Centre and is a current member of the ENMC Research Committee. He is a partner in the TREAT-NMD network responsible for the establishment, expansion and maintenance of the Registry of Outcome Measures for NMD and is keen to promote the systematic review of outcome measures for NMD clinical trials. He continues researching into muscle disease with particular interest in quality of life issues.

Julaine Florence (Washington University)

Dr. Florence is Research Associate Professor and Director of Clinical Studies with the Neuromuscular Division, Department of Neurology, Washington University Medical School, St. Louis, MO, USA. The focus of her career has been on the design, implementation and optimization of clinical outcome measures in therapeutic trials for individuals with Neuromuscular Disorders. Julaine also has a joint appointment in the Program in Physical Therapy, Washington University Medical School and has been involved in rehabilitation services with the neuromuscular disease clinic at Washington University since its inception.

Effects of long-term treatment and combination therapeutics

Robert 'Berch' Griggs M.D. (University of Rochester)

Dr. Griggs is Professor of Neurology, Medicine, Pathology and Laboratory Medicine and Pediatrics at the University of Rochester School of Medicine and Dentistry. Dr. Griggs is an internist/neurologist specializing in neuromuscular diseases with a focus on experimental therapeutics. He has directed an NIH-funded training program in the Experimental Therapeutics of Neurological Disease since 1989. He is currently President (2009-2011) of the American Academy of Neurology. Since 1998, Dr. Griggs has chaired the Executive Committee of the Muscle Study Group (MSG), an international consortium of investigators focused on developing new treatments for neuromuscular disease. He is the Principal Investigator of the NIH-funded Consortium for the Investigation of Neurological Channelopathies (CINCH) in the Rare Disease Network. He is the Principal Investigator of the NINDS-funded HYP HOP trial of dichlorphenamide in periodic paralysis and Co-Principal Investigator (with Dr. Kate Bushby) of FOR DMD, a NINDS-funded trial designed to determine the optimum corticosteroid regimen for Duchenne Muscular Dystrophy.

Rudolf Korinthenberg (University of Freiburg)

Prof. Rudolf Korinthenberg, MD is head of the Department of Neuropaediatrics and Muscular Disorders at Children's Hospital, University Hospital Freiburg. He received his paediatric and neuropaediatric training at University Hospital Münster/Westfalia and holds his actual position since 1990. He has published on a broad range of topics in child neurology, and during the last 18 years has concentrated on clinical scientific work in neuromuscular disorders. He has conducted several German multicentre trials in NMD as principal investigator, and he is head of the Trials Organisation Centre of MD-Net and The Clinical Trials Coordination Centre of TREAT-NMD.

TREAT-NMD services for clinicians and scientists conducting research and academic trials

Through the TREAT-NMD infrastructure, clinicians and basic scientists have access to a wide range of trial and research-related tools and services.

TREAT-NMD Advisory Committee for Therapeutics (TACT)

TACT is an expert body set up by TREAT-NMD to provide transparent guidance and advice on the trial-readiness of potential new therapies for neuromuscular diseases. PIs and researchers working on new therapies with promising preclinical results can contact TACT for advice on the steps to be taken to move into clinical trials and an unbiased appraisal of their therapy for this step.
www.treat-nmd.eu/TACT

Registry of Outcome Measures (ROM)

The TREAT-NMD Registry of Outcome Measures is a freely accessible and regularly updated online resource for information on existing outcome measures. It contains detailed summary information about outcome measures, including a description, availability information, contact details for providers, and references to related documents including manuals and training videos.
www.treat-nmd.eu/ROM

Standards of care guidelines

Variations in care standards between and even within countries not only impact on quality of life but also make comparison of trial results from different centres a challenge. TREAT-NMD has worked with international specialist groups to draw up international consensus documents on standards of care. Standards of care for SMA are available in in multiple languages on the TREAT-NMD website and those for DMD will be made available after publication in the Lancet Neurology in January 2010. A similar process is currently ongoing for CMD.
SMA care standards: www.treat-nmd.eu/sma-care
DMD care standards: www.treat-nmd.eu/dmd-care

The Care and Trial Site Registry

We encourage all clinicians interested in neuromuscular trials or with expertise in neuromuscular patient care to register with the TREAT-NMD Care and Trial Site Registry (CTSR), a database of information on clinical sites set up to facilitate the selection of centres with the expertise to take part in clinical trials. Companies have already made use of the CTSR to assist in their site selection for upcoming trials.
www.treat-nmd.eu/ctsr

Biobanks

EuroBioBank is the first operating network of biobanks in Europe providing human DNA, cell and tissue samples as a service to the scientific community conducting research on rare diseases. It is the only biobank network dedicated to rare disease research in Europe. A total of approximately 170,000 samples are available to researchers worldwide via the online catalogue.
www.eurobiobank.org

Standard operating procedures for animal models

As the result of international collaborations between animal model specialists worldwide, a set of SOPs for various experimental protocols on animal models have been drafted and made available on the TREAT-NMD website for the use of researchers working in this area across the world.
www.treat-nmd.eu/animalmodels

Regulatory affairs database

The regulatory affairs database is a valuable source of advice to investigators involved in clinical trial planning. It contains details of national legislation from countries across Europe. US regulations are planned to be incorporated in future. European regulations and guidelines (e.g. from ICH and EMEA) are also available.
www.treat-nmd.eu/regulatoryaffairs

Patient registries

The TREAT-NMD patient registries were set up primarily with future trials and therapies in mind. The global registries for DMD and SMA are recognised as the leading resource for trial planning and recruitment in these diseases at an international level and are already being used by pharmaceutical companies for this purpose. The registries are open to enquiries from academic colleagues, and clinicians are invited to make use of the registries for their own research questions.
www.treat-nmd.eu/patientregistries

www.treat-nmd.eu

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Poster abstracts – session 1

Tuesday 17ᵗʰ November from 18:00 - 20:00

Preclinical studies in models of NMD – methodology and characterisation

PSM-M&C-01 – Magnetic resonance imaging of cardiac function in mouse models for muscular dystrophy associated cardiomyopathy

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Cardiomyopathy is a major cause of death in muscular dystrophy (MD) and a number of studies have shown increased intracellular calcium levels in muscular dystrophy. Manganese Enhanced Magnetic Resonance Imaging (MEMRI) can be used to monitor intracellular calcium handling. MEMRI utilizes the divalent manganese ion which enters the cardiomyocyte through the L-type calcium channel and potentially other routes and acts as a contrast agent on T1 weighted images. Using MEMRI we have non-invasively assessed manganese uptake in the hearts of the dystrophin deficient *mdx* mouse, a model for DMD, and the delta-sarcoglycan deficient *Scgd-/-* mouse, a model for LGMD2F. Following MRI to measure global cardiac function, manganese chloride was infused to achieve a dose of 190 nmol/g body weight. A single mid-ventricular short axis slice was obtained using an ECG gated gradient echo (FLASH) sequence. Images were taken before the start of the manganese infusion and every 5 minutes thereafter for up to 90 minutes. Manganese uptake into the myocardium was quantified by changes in signal intensity from baseline. Preliminary data suggests that initially the rate of uptake and signal intensity are significantly increased in both *mdx* and *Scgd-/-* when compared to controls. This study demonstrates for the first time that *in-vivo* myocyte calcium influx is abnormally increased in muscular dystrophy cardiomyopathy and potentially a major factor in the development of left ventricular dysfunction. MEMRI also provides a non-invasive system to monitor therapeutic intervention in mouse models of muscular dystrophy.

PSM-M&C-02 – Mild exercise improves skeletal muscle performance in a mouse model of limb girdle muscular dystrophy 2B

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Defects in the dysferlin gene results in limb girdle muscular dystrophy (LGMD2B) and distal Miyoshi muscular dystrophy. The loss of dysferlin leads to adult onset of muscle weakness, high serum creatine kinase levels and a prominent inflammatory infiltrate in skeletal muscle. We sought to determine the effects of different exercises in AJ mice. AJ mice (n=40) were subjected to mild exercise interventions at 17 weeks age. They were divided into Untreated (NT; n=10), Horizontal Treadmill (HT; n=10), Downhill Treadmill (DT; n=10) and Voluntary Wheel (VW; n=10) groups. Treated mice underwent 48 exercise sessions with baseline, midpoint and endpoint measures. Muscle function was assessed using grip strength and rotarod, muscle force, and histology for inflammation, degeneration and regeneration. All intervention groups were compared to the no intervention group. No differences were observed in forelimb grip strength. However, VW showed better hindlimb grip strength ($P<0.01$). Furthermore, HT and VW groups showed a significant improvement in rotarod function ($P<0.05$), while DT group showed a strong trend of improved performance ($P=0.076$). Serum Creatine Kinase levels were not different between groups. The fiber size distribution curve in quadriceps was slightly larger in HT and VW. H&E and moma staining analysis showed a decreased inflammatory infiltrate and higher degree of regeneration in the HT and VW. In conclusion, mild exercise improves muscle function in A/J mice. The improvement in muscles strength seen at midpoint coincides with known histological presentation of muscle weakness in AJ mice before and after the onset of symptoms.

PSM-M&C-03 – Accelerate preclinical phase of new therapeutic treatment development

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TREAT-NMD Activity 07 focuses on those aspects of preclinical studies for DMD which subsequently affect the quality and relevance of therapeutic efficacy assessment. Four aspects are considered: (1) development of high-throughput screening setups in cellular systems and *in vitro* models to find drug candidates for DMD; (2) recommendation of appropriate animal models for use in preclinical efficacy studies of potential new treatments, in order to overcome the debate as to whether all currently used animal models correctly mimic the human disease and to establish guidelines detailing how to evaluate them. This work package assessed the available animal models by in-depth comparison of the human and animal disease states. The results of this work and the recommendation of the mdx mouse as the model of choice for DMD are published as a review paper in Neuromuscular Disorders 19 (2009); (3) eelection of readout parameters to assess the efficacy of treatment in the animal models, with the aim to increase comparability of preclinical data and facilitate prioritization of different treatment options prior to clinical trials. Our team currently supports and coordinates a discussion addressing this topic; (4) creation of a set of standardized experimental protocols to evaluate the selected parameters. To this end, two workshop meetings were held; one in USA (2007) and one in Switzerland (2008) with experts in the field from Europe, USA and Australia. The outcome of the meeting is a collection of standardized experimental protocols to assess the main endpoints used in DMD animal models. Protocols are available under http://www.treat-nmd/research/preclinical/SOPs.

PSM-M&C-04 – Urinary level of prostaglandin D2 metabolites in animal models of Duchenne Muscular Dystrophy

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Hematopoietic prostaglandin (PG) D synthase (H-PGDS) is responsible for the production of PGD2 in mast cells, Th2 lymphocytes, and antigen-presenting cells during inflammatory and immune responses. Recent studies showed that H-PGDS appeared in necrotic muscle fibers in patients with Duchenne Muscular Dystrophy (DMD) or polymyositis (Okinaga *et al.*, Acta Neuropathol., 104: 377, 2002). PGD2 production in necrotic muscle fibers is considered to be closely related with the pathologic variation in DMD and polymyositis. We investigated the correlation between the pathologic variation and the level of urinary PGD2 metabolites. When [3H]-PGD2 was infused intravenously into normal Wistar rats, 80% of the infused radioactivity was excreted into the urine and bile within 4 hours. Urinary metabolites were separated by reverse-phase HPLC and analyzed by tandem mass spectrometry. The urinary level of a PGD2 metabolite, tetranor-PGDM (Song *et al.*, J. Biol. Chem., 283: 1179, 2008) was about 3 times higher in mdx mice, a model of DMD, than in wild-type mice. Administration of H-PGDS inhibitor, HQL-79 to mdx mice for 5 days significantly decreased the level of urinary tetranor-PGDM and reduced the volume of the necrotic muscle fibers (Mohri *et al.*, Am. J. Pathol., 174: 1735, 2009), suggesting that urinary tetranor-PGDM is useful as a clinical indicator for DMD pathology.

PSM-M&C-05 – An *in vitro* cell model to further elucidate mechanisms of muscle fibrosis and test possible anti-fibrotic agents

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Innovative treatments for neuromuscular disorders are to be tested *in vitro* and *in vivo* before they can proceed into clinical trials. We isolated primary fibroblasts from DMD and control muscle biopsies and induced transdifferentiation to myofibroblasts by TGF-β1 treatment. We compared proliferating activity and soluble collagen production, as well as transcript and protein levels of decorin, myostatin, TGF-β1, MMP-1 (interstitial collagenase), MMP-2 (gelatinase), MMP-3 (stromelysin), MMP-7 (matrilysin), and the MMP inhibitors TIMPs 1 to 4, in fibroblasts and myofibroblasts. Principal differences included: significantly greater soluble collagen production; significant upregulation of decorin, myostatin and MMP-7 transcripts and proteins; and significant downregulation of MMP-1 and TIMP-3 transcripts and proteins, in untreated DMD fibroblasts compared to controls. TGF-β1 transdifferentiation significantly lowered decorin and myostatin, and significantly increased TGF-β1 transcript and protein; significantly increased MMP-1 and TIMP-3, and significantly lowered MMP-7 transcript and protein in DMD cells compared to pretreatment. The several differences between DMD and control fibroblasts show that DMD fibroblasts have a profibrotic phenotype, accentuated by TGF-β1 treatment. Dystrophin absence itself could exert a direct influence on ECM homeostasis by allowing leakage of cellular components to the extracellular space, or abnormal cellular uptake of extracellular growth factors, cytokines, or enzymes to influence muscle fibroblasts either directly by altering adhesion properties or indirectly by interactions with molecules released into the ECM by muscle or inflammatory cells. The transdifferentiation of muscle fibroblasts may serve as a simplified model of fibrosis to further elucidate mechanisms of muscle fibrosis and test possible anti-fibrotic agents.

PSM-M&C-06 – Phenotyping neonatal models of neuromuscular degeneration as a prelude for drug candidate evaluation.

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Phenotyping neonatal models of neuromuscular degeneration as a prelude for drug candidate evaluation. The present study addresses methodological approaches and common interpretational challenges relevant to neonatal models of neuromuscular diseases using Spinal Muscular Atrophy (SMA) (SMNdelta7) and Duchene's Muscular Dystrophy (DMD) (mdx) as examples. While indices of neonatal well-being are important endpoint for any drug screening, we sought *in vivo* non-invasive measures of muscle function to evaluate therapeutics that could potentially improve motor function. We will present the latest validation data for the hind limb suspension test (a.k.a. the tube test), a novel non-intrusive behavioral test for the evaluation of neonatal neuromuscular function (El-Khodor *et al.*, 2008). The neonatal tube test has been used successfully in ongoing drug screening efforts in the SMNdelta7 and mdx models. We show that this test can be used as a diagnostic test in the SMA mice. Although the first detectable muscle pathology appears at 3 weeks of age in the mdx animals, the tube test detected a significant motor deficit in the mdx animals as early as postnatal day 8. The present study will also detail PsychoGenics battery of straightforward, easy-to-perform, rapid and moderate-throughput tests of survival, motor function and indices of neonatal well-being in the SMNdelta7 and mdx mice.

PSM-M&C-07 – The immediate consequences of treadmill exercise on adult dystrophic mdx mice

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Despite suffering the same genetic defect as DMD patients the dystropathology in sedentary adult mdx mice is very mild. It is well documented that both voluntary exercise and treadmill exercise can increase dystropathology (myofibre necrosis) in adult mdx mice. The exact events leading to myofibre necrosis are unknown; excess inflammation and increased levels of oxidative stress are however strongly implicated. The aims of this study were to: (1) establish a short and repeatable 30 minute treadmill protocol and to profile the timecourse of indicators of muscle damage immediately after treadmill exercise; (2) evaluate the extent of dystropathology in mdx mice after a single exercise session in comparison to 4 weeks of treadmill exercise and conclude if a single exercise session is an appropriate protocol to screen pre-clinical drugs in adult mdx mice. Histological analysis consistently showed the highest level of myofibre necrosis in the quadriceps in comparison to other muscles. Transient elevations in blood serum creatine kinase were also a consistent marker of muscle damage peaking immediately after exercise. Gene expression of inflammatory cytokines showed a significant increase in IL-6 and Il-1α and decreased TNF mRNA. A more precise understanding of the sequence of molecular and cellular changes which lead to myofibre necrosis immediately after exercise can identify biomarkers to rapidly evaluate the efficacy of pre-clinical drug interventions in adult mdx mice. We have used this procedure to examine the beneficial effects of an anti-inflammatory drug (cV1q – a mouse specific TNF antibody) and an anti-oxidant (N-acetylcysteine) on adult mdx mice.

PSM-M&C-08 – Preclinical drug trial efforts for muscular dystrophy: methods and end points

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Promising therapeutic interventions for muscular dystrophy are rapidly increasing, leading to an increased demand for pre-clinical testing in mouse models of muscular dystrophy. Pre-clinical efficacy and toxicity assessments are critical steps in moving potential therapies from the bench to patient bedside. Currently, there are no dedicated preclinical facilities for muscular dystrophies. We have established a state-of-the-art facility at Children's National Medical Center (CNMC), Washington DC to maintain several mouse models of myopathy (mdx-23, mdx-52, mdx4cv, Calpain- 3 KO, dysferlin- deficient, EMD null and MHC class I model of myositis) and to evaluate therapeutic interventions in these models. We have developed several sensitive functional (grip strength, rota-rod, *in vitro* force contractions), behavioral (open field activity), imaging (echocardiography, MRI and optical imaging), biochemical (serum creatine kinase) and histological (EBD dye uptake, fibrosis and H&E) end points that are useful not only to assess drug efficacy but also to evaluate the phenotype of myopathic mouse models. So far, our pre-clinical drug testing facility has tested over 30 therapeutic interventions and more are in the pipeline. We will present these methods, their sensitivities and discuss their ability to detect a significant difference upon therapeutic intervention. These efforts would significantly help to accelerate the pace of human muscular dystrophy clinical trials.

PSM-M&C-09 – Effect of a mild exercise regime on disease parameters in the mdx mouse model

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Duchenne Muscular Dystrophy (DMD) is a severe, progressive, muscle wasting neuromuscular disorder, characterized by the lack of functional dystrophins. To assess the therapeutic effects of possible treatments in more detail and to determine which levels of dystrophin restoration are required for improved muscle function in mdx mice, we have set up several functional tests for monitoring muscle strength and condition. These tests consist of grip strength, rota-rod and 2 and 4 limb hanging wire tests. Where possible, standardized operating procedures (from the TREAT-NMD website) were implemented. Since there is some debate on the effect of exercise on disease progression, we first assessed whether there is a difference in histology and in previously identified fibrotic and immunologic RNA biomarker levels between exercised and sedentary mice. Male mdx mice (n=5) underwent a 12 week functional test regime starting at the age of four weeks. Mice performed different functional tests on consecutive days on a weekly basis. Creatine kinase levels were determined once a week. After sacrifice the percentage of fibrotic/necrotic areas throughout different skeletal muscles and heart were determined using a computer-automated image analysis system. We found no indications for differences in fibrosis between the exercised and sedentary mice in heart and skeletal muscle, nor did the amount of fibrosis vary between different locations within muscles. Gene expression levels of disease-related genes did not differ between the groups. Based on these results the functional test regime was found not to affect the natural disease progression in mdx mice.

PSM-M&C-10 – MALDI reveals membrane lipid profile reversion in MDX mice

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Duchenne Muscular Dystrophy (DMD), the most common and severe X-linked myopathy, is characterized by the lack of dystrophin, a sub-sarcolemmal protein necessary for normal muscle functions. In a previous study of the lipid content of skeletal muscles of dystrophic (mdx) mice, the animal model for DMD, by in situ Matrix-Assisted Laser Desorption-Ionization Mass Spectrometry (MALDI-MS), an inversion of the phosphatidylcholine PC34:2/PC34:1 ion peaks intensity ratio was observed between destructured (abnormal fiber morphology) and structured (normal fiber morphology). A possible treatment for this dramatic disease is to introduce an exogenous nitric oxide (NO) donor into the organism, leading to an increase of utrophin and a regression of the dystrophic phenotype. In the present work, after confirmation by tandem mass spectrometry of the structure of these two phospholipids, their intensity ratio inversion was used to evidence a restoration of membrane lipid composition very similar to those of wild-type mice after the treatment of mdx mice with molsidomine, a NO donor. This was associated with the observation by immunohistology of an increase of the regeneration process in the mice.

PSM-M&C-11 – Muscle hypertrophy and contractures in a myostatin heterozygote null GRMD dog

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Myostatin is a negative regulator of muscle growth. There has been increasing interest in inhibiting myostatin to promote muscle growth in a range of conditions, including Duchenne Muscular Dystrophy (DMD). To provide further justification for use of myostatin blockade as a treatment for DMD, we have initiated a set of experiments to define the phenotype of dogs produced by breeding obligate female carriers of the golden retriever muscular dystrophy (GRMD) canine model of DMD with male myostatin-null whippet dogs. The genotype of the first litter (F1 generation) included three females and three males. In subsequent phenotypic studies, we have focused mostly on the three males: GRMD Normal/Myostatin Normal (Racer), GRMD/Myostatin Heterozygote Null (Dash), and GRMD/Myostatin Normal (Flash). Characteristic signs of GRMD, including gait abnormalities and stunting, developed in the two dystrophic dogs within the first six weeks of life. The phenotype of these two dogs was similar until about 4 months of age, when the myostatin-heterozygote null dog was noted to have more difficulty at gait. Over the subsequent three months, the dog's gait became even more compromised, with associated contractures and hypertrophy of some muscles. The phenotype of this dog has been characterized with several tests, including MRI and force measurements. On MRI, there was selective apparent hypertrophy of flexor muscles, with associated atrophy of extensors. Force measurements showed a similar selective involvement of extensors and sparing of flexor muscle function. The imbalance between extensor and flexor muscle involvement seemingly contributed to the contractures in these dogs.

PSM-M&C-12 – Structural and functional evaluation of branched myofibers in mdx mice

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Myofibers with structural malformations have been reported in diseased and damaged muscle; however the basis for these malformations and the impact of these myofibers on overall muscle function is not fully understood. In dystrophic (dy/dy) mice, the proportion of malformed myofibers decreases after prolonged muscle activity, suggesting they contribute to injury susceptibility in dystrophic muscle. We assessed the occurrence, morphology, and function of malformed myofibers enzymatically isolated from young (2 months) and old (9 months) mdx and age-matched control mice. In mdx, there were visible malformations in 6% and 65% of EDL myofibers of young and old mice, and in 5% and 11% of FDB myofibers in young and old mice, respectively. Despite these visible malformations, cytoskeletal architecture was normal. Age-matched controls did not display altered morphology. In mdx FDBs, an assessment of global EC coupling revealed that the peak amplitude of Ca(2+) release in malformed portions of mdx myofibers was significantly decreased compared to the normal regions of the same myofibers. Osmotic challenge, an indirect measure of susceptibility to membrane stress, revealed a greater incidence of spontaneous Ca(2+) sparks in the malformed portions of myofibers. We also used MRI to determine the 3D architecture of hindlimb muscles. High resolution T1-weighted, multi-echo T2-weighted (for T2 mapping), and spin echo diffusion tensor (to noninvasively track myofibers) MR images were acquired on a 7T Bruker Biospec MR system. With this approach, we compare myofiber morphology and muscle architecture (PCSA, fiber length, and pennation angle) of intact mdx and control muscles *in vivo*.

PSM-M&C-13 – Modelling of SMA in mice: novelties and limits

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Mimicking SMA in mice to evaluate potential therapeutics is a great challenge. Several models have been developed introducing the human SMN2 transgene onto the null Smn-/- background showing that disease severity in mice depends on a fine tuning of SMN protein expression. We addressed this challenge by generating new SMN2;Smn-/- lines on a pure C57BL/6N background with transgene copy number ranging from 1 to 4. While 1-2 copies of SMN2 were insufficient and 4 copies fully rescued Smn-/- mice, we report here the establishment of Smn-/- mice carrying three copies of SMN2. Though indistinguishable from normal littermates until four days of age (P4), SMA mice rapidly developed muscle weakness and motor defects. At P15 (median survival), motor neuron and axonal loss were evident, correlated with a reduction in compound muscle action potential amplitude, and in nerve conduction. Although similar to wild type pups at P1, marked differences in breathing patterns were evident in SMA pups at P7 with smaller ventilation volume, longer breath duration and greater apnea frequency and duration. Defects in neuromuscular junction maturation were observed as early as P8 in the diaphragm. Whether these defects account for respiratory insufficiency and premature death of SMA mice remains to be explored. Though still severely afflicted, this new SMA mouse is the longest surviving SMN2;Smn-/- model on pure C57BL/6N background described to date. As one additional copy of SMN2 fully rescued the phenotype, it appears unlikely that a milder phenotype can be produced by manipulating SMN2 copy number alone.

PSM-M&C-14 – Non invasive assessment of skeletal muscle function in mouse using 1H-MR imaging and 31P-MR spectroscopy

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Although Magnetic Resonance (MR) techniques are able to provide key information related to muscle function, invasive procedures used for muscle stimulation and force output measurement in animal models preclude from a total non invasive assessment and repeated investigations in the same animals. We describe a new experimental setup allowing a strictly non-invasive investigation of muscle function in contracting mouse gastrocnemius muscle using 1H-MR imaging and 31P-MR spectroscopy. This setup allows prolonged anesthesia with control of the body temperature, transcutaneous muscle stimulation, force, MR Imaging and Spectroscopy measurements. We investigated 8 mice through two fatiguing stimulation protocols (6 minutes of repeated isometric contractions at 1.7 Hz) repeated over a 7-day period. T2-weighted imaging (T2WI) demonstrated that transcutaneous stimulation mainly activated the gastrocnemius muscle. Moreover, changes in isometric force and energy metabolism were highly reproducible. In addition diffusion WI allowed to check fibers integrity. The setup described in the present study is suitable for repeated MR assessment of mouse skeletal muscle function, allowing mechanical performance, energy metabolism, anatomy and physiology to be accessed strictly non-invasively in contracting gastrocnemius muscle. Longitudinal studies become thus readily feasible and each mouse can serve as its own control, thereby reducing inter-individual variability, workload and costs. This major advance in the exploration of skeletal muscle function opens up new perspectives for assessment of animal models of NMD.

Preclinical studies in models of NMD – therapeutic targets

PSM-TT-01 – Developing AMPA receptor aptamers as new drug candidates for ALS

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In finding new treatment for amyotrophic lateral sclerosis (ALS), one of the important therapeutic strategies is to develop inhibitors of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. This is because excessive activity of AMPA receptors, generally termed as excitotoxicity, is thought to link to the selective death of motor neurons. We are interested in developing AMPA receptor inhibitors that are both potent and water soluble, the properties superior to all existing inhibitors. Using systematic evolution of ligands by exponential enrichment (SELEX), we have successfully identified three classes of aptamers with nanomolar affinity against AMPA receptors. In the class of competitive aptamers, we found one aptamer with an IC50 value of 30 nM, rivaling any other exiting AMPA receptor inhibitors. Furthermore, this aptamer is broadly active in all AMPA receptor subunits (i.e. GluR1-4), but has no unwanted activity in kainate or NMDA receptors, the two other glutamate receptor subtypes. We have also identified two other classes of noncompetitive aptamers that are differentially selective to conformations of GluR2, a key AMPA receptor subunit that mediates excitotoxicity: one class uniquely inhibits the open-channel whereas the other inhibits the closed-channel conformation. Our results suggest the possibility of developing aptamers that are nanomolar affinity, water-soluble and highly selective to both an AMPA receptor subunit and a unique receptor conformation. These aptamers are excellent water-soluble, nanomolar affinity templates for design of better inhibitors as drug candidates for a potential new ALS therapy.

PSM-TT-02 – Up-regulation of TGF beta signaling in MDC1A

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MDC1A is a devastating form of muscular dystrophy that is caused by mutations in the LAMA2 gene encoding for laminin-a2, an integral component of the extracellular matrix laminin211 protein complex. Lama2Dy-w mice, the murine model for MDC1A, exhibit accelerated muscle degeneration with little to no regeneration, increased inflammation, and fibrosis, and have a severely reduced lifespan. It has been demonstrated that genetic/pharmacological inhibition of apoptosis increases lifespan and improves postnatal growth and myofiber histology in Lama2Dy-w mice. However, while promising, these treatment strategies lead to only partial recovery. Treated mice remain significantly smaller than healthy control mice and still retained poor muscle regenerative capacity with increased fibrosis. Members of the Transforming Growth Factor-β (TGF-β) superfamily have been shown to enhance fibrosis and are known to inhibit proliferation and differentiation of muscle satellite cells. Our results indicate increases in TGF-β1, 2 and 3 transcripts in Lama2Dy-w muscles relative to control muscles. Down-stream targets of TGF-β implicated in fibrosis such as periostin1, proteoglycan4 and Serpin E1 are also expressed at higher levels. In addition, we see a significant increase in mRNA expression of Cyclin Dependent Kinase (CDK) inhibitors such as p21 and p16. Increased TGF-β signaling has been suggested to inhibit proliferation by inducing expression of these and other CDK inhibitors. These preliminary results suggest that TGF-β signaling might contribute to the dystrophic pathology in MDC1A and inhibition of this pathway could potentially improve the pathology.

PSM-TT-03 – Activin receptor type IIB inhibition improves strength and function of dystrophic muscle

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Absence of the membrane stabilizing protein dystrophin, the defining characteristic of Duchenne Muscular Dystrophy, increases susceptibility to contraction-induced muscle damage which manifests into progressively weakening muscle. One strategy to prevent the decline in muscle function is by promoting muscle fiber hypertrophy. Inhibition of activin receptor type IIB (ActRIIB) signaling induces skeletal muscle hypertrophy and increases muscle mass and strength in wild-type mice. To determine the effect of ActRIIB inhibition-induced hypertrophy on dystrophic muscle, mdx mice were treated with RAP-031, a fusion protein comprised of a form of the ActRIIB extracellular domain fused to a murine Fc. 7-week old mdx mice were treated with vehicle or RAP-031 for 42 weeks. Body composition, grip strength and *extensor digitorum longus* (EDL) contraction force were assessed. RAP-031 treatment increased lean tissue mass compared to the vehicle cohort ($P<0.001$). Grip strength normalized to lean mass was 28.3% higher ($P<0.01$) in RAP-031-treated mice compared to controls. EDL absolute twitch and tetanus contraction forces were increased 30% ($P<0.01$) and 42.5% ($P<0.01$) respectively in the RAP-031 group compared to controls. Forces generated during an eccentric contraction protocol (5 trials) were significantly greater ($P<0.01$) in the RAP-031 treated group compared to controls whereas there was no difference in percent force drop. While the RAP-031 treated muscle lost force at the same rate as untreated muscle, starting with greater absolute force allows the treated muscle to maintain function despite contraction-induced damage. Overall, these data support a therapeutic benefit provided by ActRIIB inhibition to dystrophic muscle.

PSM-TT-04 – Chemotherapy of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is an X-linked muscular abnormality caused by the loss of dystrophin and is one of the most gravely genetic disorders. We have recently found that hematopoietic prostaglandin (PG) D synthase (HPGDS) was induced in grouped necrotic muscle fibers in DMD patients. Cytosolic form of phospholipase A(2) and cyclooxygenase-2, the upstream enzymes of the arachnoid acid cascade, were similarly observed in the HPGDS-positive fibers (Okinaga *et al.,* Acta Neuropathol., 104, 377-384, 2002). We developed novel HPGDS inhibitors based on the X-ray crystallographic analysis of human HPGDS complexed with its prototype inhibitor (Aritake *et al.,* J. Biol. Chem., 281, 15277-15286, 2006). In this study, we developed a novel therapy for DMD by inhibition of HPGDS. HPGDS was localized in the necrotic muscle fibers and accumulated macrophages in mdx mice. Oral administration of HPGDS inhibitors for 5 days prevented the expansion of muscular necrosis in an mdx mouse model, as measured by X-ray computed tomography (CT) imaging enhanced by non-ionic contrast media. The treatment with HPGDS inhibitors also decreased the expression of mRNAs of pro-inflammatory cytokines. These results indicate that PGD2 produced by HPGDS plays important pathological roles on the expansion of muscle damage. HPGDS inhibitor also accelerated the accumulation and activation of macrophages in the necrotic area. These results indicate that PGD2 produced by HPGDS is involved in the expansion of muscle necrosis in DMD and that inhibition of H-PGDS is a novel therapy for DMD.

PSM-TT-05 – Genetic and pharmacological inhibition of cyclophilin D rescues mitochondrial defects and prevents muscle apoptosis in collagen VI myopathic mice

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Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM) are inherited muscle disorders caused by mutations of genes encoding the extracellular matrix protein collagen VI. Mice lacking collagen VI (*Col6a1-/-*) display a myopathic phenotype associated with ultrastructural alterations of organelles, mitochondrial dysfunction with abnormal opening of the permeability transition pore (PTP), and spontaneous apoptosis of myofibers. Treatment with cyclosporin (Cs) A, a drug that desensitizes the PTP by binding to cyclophilin (Cyp) D, was shown to rescue myofiber alterations in *Col6a1-/-* mice and in UCMD patients, suggesting a correlation between PTP opening and pathogenesis of collagen VI myopathies. We found that inactivation of the gene encoding for Cyp-D rescues the disease phenotype of collagen VI deficiency. In the absence of Cyp-D, *Col6a1-/-* mice show negligible myofiber degeneration, rescue from mitochondrial dysfunction and ultrastructural defects, and normalized apoptosis. These findings demonstrate that lack of Cyp-D is equivalent to its inhibition with CsA at curing the mouse dystrophic phenotype and establish a cause-effect relationship between Cyp-D-dependent PTP regulation and pathogenesis of collagen VI myopathies. We investigated the therapeutic effects of the non-immunosuppressive CsA derivative Debio 025. Treatment with Debio 025 prevents mitochondrial dysfunction and normalizes the apoptotic rates and ultrastructural lesions of *Col6a1-/-* mice. Thus, desensitization of the PTP can be achieved by selective inhibition of Cyp-D without inhibition of calcineurin, resulting in an effective therapy of *Col6a1-/-* myopathic mice. These findings validate Cyp-D and the PTP as pharmacological targets for the therapy of collagen VI myopathies.

PSM-TT-06 – Exercised dystrophic mdx mice and specific modulation of disease-sensitive indices by drugs clinically relevant for DMD: effects of a chronic treatment with the ACE inhibitor enalapril

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Angiotensin (Ang)-converting enzyme (ACE) inhibitors have clinical value for treating cardiomyopathy in Duchenne Muscular Dystrophy (DMD) patients. Also, Ang-II antagonist losartan reduces advanced and induced muscle fibrosis in mdx mouse. We verified the involvement of Ang-II in earlier stage of muscle pathology, by treating 4-5 week-old treadmill-exercised mdx mice with 1-5 mg/kg enalapril (6 days/week i.p. for 4-8 weeks). *In vivo*, enalapril counteracted the exercise-induced decrease of fore limb strength in a dose-dependent manner and significantly ameliorated resistance to exercise. *Ex vivo*, the treatment contrasted dose-dependently the exercise-induced reduction of macroscopic chloride conductance, gCl, in EDL muscle. Enalapril might have blunted a direct effect of Ang-II on muscle chloride channel, since Ang-II *in vitro* (10-300 nM) produced a concentration-dependent decrease of gCl in wild-type EDL muscle, through an AT-1 receptor-mediated activation of PKC pathway. Enalapril at 5 mg/kg partially ameliorated mechanical threshold and calcium-dependent contractile parameters of EDL muscle, suggesting positive effects on the altered calcium homeostasis. No effect, at any dose, was observed on plasma creatine kinase and lactate dehydrogenase. A mild improvement of histology profile was observed in both diaphragm and gastrocnemius muscle, along with a significant decrease of NF-kB positive fibres. Dihydroethidium-positive nuclei were also reduced by 80% in tibialis anterior muscle, supporting a drug-induced decrease in superoxide production. Thus, enalapril significantly ameliorated mechanical and inflammation-sensitive parameters in dystrophic muscle, reinforcing the interest of early treatment with ACE-inhibitors in DMD patients.

Preclinical studies in models of NMD – antisense approaches in NMD

PSM-AA-01 – AVI-5038: initial efficacy and safety evaluation in cynomolgus monkeys

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Duchenne Muscular Dystrophy (DMD) has a frequency of approximately 1 in 3,500 male births. Mutations that cause DMD occur spontaneously, and cluster around two 'hot spots' within the large, 2.4 megabase, 79 exon gene. AVI recently conducted a screen of peptide conjugated phosphorodiamidate morpholino (PPMO) compunds to induce skipping of exon 50 of human dystrophin, in order to select an effective candidate to restore the reading frame in DMD patients with deletions in exons 51, 51-53, or 51-55. As a result, AVI is now in development with AVI-5038, our lead exon 50 skipping candidate. The objective of this study was to evaluate the efficacy of AVI-5038 at inducing skipping of dystrophin exon 50, as determined by RT-PCR, when administered via intravenous or subcutaneous injection to cynomolgus monkeys once weekly for 4 weeks. The efficacy endpoint, RT-PCR, was evaluated following a 21-day post-dose period. Initial evaluation of toxicology of AVI-5038, clinical pathology and histological examination of selected tissues was also performed. The results of this study will be discussed.

PSM-AA-02 – Long term systemic antisense-mediated exon skipping in dystrophic mouse models

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Antisense-mediated reading frame restoration is a promising therapeutic approach for Duchenne Muscular Dystrophy (DMD). In this approach, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing of mutated dystrophin transcripts. This is aimed to restore the disrupted open reading frame and allow synthesis of internally deleted, partly functional Becker-like dystrophin proteins. The approach is theoretically applicable to over 70% of all patients. Proof of concept has been achieved in cultured muscle cells from patients, in the mdx mouse and dog models and recently in patients as well. In a first trial in 2006, we showed exon 51 skipping and dystrophin restoration in each patient after local intramuscular AON injections. A subsequent trial where patients are treated systemically is currently ongoing and results are expected soon. Due to AON turnover, repeated treatment is necessary. Therefore, long term safety and efficacy of 2'O-methyl phosphorothioate AON treatment was tested in mouse models with varying levels of severity. We compared intravenous, intraperitoneal and subcutaneous routes of administration. In further studies, weekly subcutaneous injections of 200 mg/kg for up to 6 months were well tolerated, and no toxic effects were observed (based on liver and kidney function parameters). Treatment resulted in significantly improved serum creatine kinase levels and rotarod running times compared to saline treated controls. These results indicated that long term treatment with 2'-O-methyl phosphorothioate AONs is safe and efficient in dystrophic mouse models, which is encouraging for future long term trials in patients.

PSM-AA-03 – Dual exon skipping in myostatin and dystrophin as a potential therapy for Duchenne Muscular Dystrophy

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Myostatin is a member of the transforming growth factor-beta family that inhibits muscle growth. Mutations leading to non functional myostatin have been associated with hypermuscularity and enhanced muscle regeneration. In this study, we aim to knockdown myostatin by means of exon skipping, a technique which has been successfully applied to reframe the genetic defect of dystrophin gene in Duchenne Muscular Dystrophy (DMD) patients. We targeted myostatin exon 2 with antisense oligonucleotides (AON) in human primary myoblasts cell culture. We observed skipping of myostatin exon 2, which disrupted the open reading frame and specifically decreased myostatin mRNA expression level. Furthermore, upon myostatin downregulation, the expression levels of its target genes CDKN1A, PAX7, MYF5 and MYOG were found to be elevated. This suggests that myostatin downregulation leads to enhancement of satellite cells self renewal and differentiation of myoblasts towards myofibers. In addition, we combined two AON targeting dystrophin and myostatin in our *in vitro* culture system and showed that exon skipping in both target genes occurred without apparent interference. Therefore, we propose dual exon skipping in dystrophin and myostatin to simultaneously restore dystrophin synthesis and enhance muscle regeneration as a potential therapy for DMD.

PSM-AA-04 – Guidelines for exon skipping quantification in the DMD gene

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Antisense-mediated exon skipping aiming for reading frame restoration is currently one the most promising therapeutic approaches for Duchenne Muscular Dystrophy (DMD). Several antisense oligonucleotides (AONs) and other molecular approaches have been tested in the last years to induce exon skipping throughout the dystrophin transcript to investigate how to optimize the exon skipping process, especially in cultured muscle cells and the mdx mouse model. Since the major outcome measure of the treatments is the exon skipping levels, here we present the comparison of several different techniques to quantify exon skipping in cells and mouse muscle treated with AONs. We compared densitometry of RT-PCR products on ethidium bromide stained agarose gells, primary and nested RT-PCR followed by lab-chip analysis, real-time PCR (ESRA) and melting curve analysis. The (expensive) Fluidigm digital PCR system allows absolute quantification of skipped versus non-skipped transcripts, and was used as a reference. Preliminary results suggest that quantification through lab-chip of primary PCR products gives similar results as the fluidigm system for muscle tissue. Analysis for cells is ongoing. Hopefully, these results will allow better comparison between different laboratories.

PSM-AA-05 – Applicability of antisense-mediated exon skipping for Duchenne Muscular Dystrophy mutations

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Antisense-mediated exon skipping aiming for reading frame restoration is currently a promising therapeutic application for Duchenne Muscular Dystrophy (DMD). It employs of antisense oligoribonucleotides (AONs) to induce exon skipping during pre-mRNA splicing of mutated dystrophin transcripts. This will restore the disrupted open reading frame and allow synthesis of internally deleted, partly functional dystrophin proteins found in the less severe Becker muscular dystrophy. After proof of concept in patient-derived cell cultures and the mdx mouse model, AONs for exon 51 are currently in early phase clinical trials (van Ommen *et al.* this meeting). This approach is mutation-specific, but as the majority of DMD patients have deletions that cluster in hotspot regions, the skipping of a small number of exons is applicable to relatively large numbers of patients. To assess the actual applicability of the exon skipping approach, we here determined for deletions, duplications and point mutations reported in the Leiden DMD mutation database, which exon(s) should be skipped to restore the open reading frame. In theory, single and double exon skipping would be applicable to 79% of deletions, 91% of small mutations and 73% of duplications, amounting to 83% of all DMD mutations. Exon 51 skipping, which is being tested in clinical trials, would be applicable to the largest group (13%) of patients.

PSM-AA-06 – Multiexon skipping in Duchenne Muscular Dystrophy

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Duchenne musclular 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.

PSM-AA-07 – Chronic long term administration of low and high doses of phosphorodiamidate morpholino oligomer ameliorates the dystrophin phenotype in mdx mice

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The administration of antisense oligonucleotides (AOs) is one of the most promising approaches for the treatment of Duchenne Muscular Dystrophy (DMD). DMD is characterized by premature termination of dystrophin translation and the use of AOs can restore the correct reading frame in the dystrophin transcript. This approach in humans would convert the DMD to the milder Becker Muscular Dystrophy phenotype. Due to the nature of this approach, chronic administration of AOs for all the life of the patient would be necessary. The phosphorodiamidate morpholino oligomer (PMO) has high affinity to the sequence target and resistance to endonucleases which allows a long lasting exon skipping wich reduces the number of administrations. In this study mdx mice were systemically treated with 2 different dosages distributed in 20 injections in a time of 12 months: a high dose to verify the eventuality of toxic effects, and a low dose to analyse the final outcome of a clinically applicable amount of PMO. PMO was injected via tail vein in 6 weeks old animals. Mice were sacrificed 4 and 12 months after the beginning of treatment. Immunostaining, RT-PCR and western blot were used to analyse the dystrophin biodistribution. Morphological outcome was verified by central-nucleated fiber index, presence of fibrosis and infiltrate. The physiological improvement was monitored by creatine kinase assay, *in vivo* force measurement and open-field behavioural activity monitoring test. Biochemical assays were used to test possible toxic effects after PMO administration. Our results support the clinical feasibility of this approach with naked PMO.

PSM-AA-08 – Muscle and heart targeted splice correction for DMD

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Duchenne Muscular Dystrophy (DMD) is caused by mutations in the DMD gene that prevent the production of dystrophin protein. Antisense oligonucleotides (AOs) targeted to trigger excision of an exon bearing a mutant premature stop codon in the DMD transcript have been shown to skip the mutated exon and partially restore functional dystrophin protein in dystrophin-deficient mdx mice. To fully exploit the therapeutic potential of this method requires highly efficient systemic AO delivery to multiple muscle groups, including heart, to modify the disease process and restore function. Here we report the discovery and application of novel peptides which when conjugated to PMO AOs facilitate highly efficient muscle and heart targeting and enhanced systemic splice correction. Data relating to two novel peptide classes will be presented; chimeric peptide conjugates of the form X-TSP-PMO where X is an arginine rich transduction domain and TSP is a tissue targeting peptide domain (the prototype for which is MSP – a muscle specific heptapeptide), and Pip peptides, a novel series of positively charged cell penetrating peptides, the prototype for which is Pip5e-PMO. Data following systemic intravenous administration of both peptide classes will be presented, showing highly efficient systemic dystrophin splice correction including in heart tissue, with further data showing functional and phenotypic correction in mdx mice. We therefore describe two novel peptide classes with potential for highly efficient targeted AO delivery to muscle and heart which offer promise for the delivery of biotherapeutic agents to these target tissues in clinical studies.

PSM-AA-09 – Antisense correction of SMN2 splicing in the CNS of mouse models of spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is characterized by progressive degeneration of spinal-cord motor neurons. It is caused by mutations in the survival-of-motor-neuron (SMN1) gene. The paralogous SMN2 gene, present in all SMA patients, attenuates disease severity, but expresses little full-length SMN protein, due to alternative splicing that results in inefficient inclusion of exon 7. Increasing the extent of SMN2 exon 7 inclusion to express more full-length, functional SMN protein in motor neurons is a promising approach to treat SMA. Previously, we identified a 2'-O-(2-methoxyethyl) (MOE) 18mer antisense oligonucleotide (ASO) that targets an hnRNP A1 bipartite motif in an intron-7 splicing silencer (ISS-N1) and efficiently promotes SMN2 exon 7 inclusion in the liver and kidney of transgenic mice after systemic administration. Because ASOs do not cross the blood-brain barrier, we explored direct delivery to the mouse central nervous system. Using a surgically implanted micro-osmotic pump, the ASO was delivered into cerebrospinal fluid through the right lateral ventricle in adult type-III Smn+/- or Smn-/- SMA mice carrying a human SMN2 transgene. Dose-response studies revealed that intracerebroventricular (ICV) infusion of the ASO increased SMN2 exon 7 inclusion in spinal cord to ~90%, compared to ~10% in control mice. Western blotting and immunohistochemical analysis demonstrated a robust increase of the human transgenic SMN protein levels in spinal-cord motor neurons. We are using this and other ICV delivery methods, in combination with available SMA mouse models, to optimize the efficacy of the ASO, determine phenotypic improvement, and establish a time window for effective treatment.

PSM-AA-10 – Repeat dose mechanistic toxicology evaluation of AVI-4225 PMO in mdx mice

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Mdx mice are dystrophic, which is the result of a spontaneous nonsense point mutation within exon 23 of dystrophin that completely prevents dystrophin expression. The severity of the phenotype is less than that of DMD patients, although there are several important similarities. Both DMD boys and mdx mice typically show signs of vigorous muscle degeneration / regeneration and muscle hypertrophy early in life. In both cases, muscle damage and fibrosis result, however, in the skeletal muscle of mdx mice, the rate of muscle wasting is much slower. As in DMD patients, mdx mice also exhibit reduced cardiac and respiratory capacity, which worsens with age. Systemic administration of the PMO, AVI-4225, has been demonstrated in mdx mice to target the genetic lesion in exon 23, and repair or restore dystrophin expression through exon skipping. In this 12-week study, administering AVI-4225 to mdx mice, we evaluated any possible toxicity due to the mechanism of action of the compound in a dystrophic subject (consequent to the new appearance of truncated but functional dystrophin). In parallel, groups of wild-type C57BL/6NCrl mice were dosed at the highest dose level or vehicle control, to evaluate the effects of AVI-4225 in a healthy, well-characterized C57 mouse similar to the background strain of mdx mice. The results of this study will be discussed.

PSM-AA-11 – Repeat dose toxicology evaluation of AVI-4658 PMO in mdx mice

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The mdx mouse is a strain of C57BL/10 mice that harbors a spontaneous nonsense mutation in exon 23 of dystrophin which completely abrogates dystrophin expression, leading to a dystrophic phenotype. Although the severity of the phenotype is less than that of Duchenne Muscular Dystrophy (DMD) patients, there are several important similarities. DMD boys typically show signs of muscle hypertrophy early in life. Like DMD patients, the mdx mice exhibit muscle degeneration/regeneration, hypertrophy and loss of strength although, in the skeletal muscle, the rate of muscle wasting is much slower. Mdx mice also exhibit reduced cardiac and respiratory capacity, which worsens with age. As AVI-4658 (which targets exon 51) does not address the exon 23 mdx mutation, mdx mice were used to evaluate the potential chemical toxicity of the drug in a dystrophic subject, without the concern of possible confounding mechanism based effects. In parallel, groups of wild-type C57 mice were dosed at the highest dose level or vehicle control, to evaluate the effects of AVI-4658 in a healthy, well-characterized C57 mouse similar to the background strain of mdx mice. It was recognized that the muscle pathology, elevated CK, ALT and AST presented a higher background, from which to detect adverse events. However, the inclusion of an age-matched, vehicle treated control arm in the study allowed for the detection of any significant deviations in these endpoints in the four treatment dose cohorts. Results of this study will be discussed.

PSM-AA-12 – Repeat dose toxicology evaluation of AVI-4658 PMO in cynomolgus monkeys

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Duchenne Muscular Dystrophy (DMD) affects 1 in every 3,500 males worldwide and results from a mutation of the dystrophin gene. AVI BioPharma is currently in clinical development with AVI-4658, a Phosphorodiamidate Morpholino Oligomer (PMO) drug, designed to induce exon 51 skipping and restore dystrophin expression in DMD patients. The objective of this preclinical study was to evaluate the toxicity and toxicokinetic profile of AVI-4658 when administered via intravenous or subcutaneous injection to cynomolgus monkeys once weekly over 12 weeks. The reversibility, progression, or delayed appearance of any observed changes was evaluated following a 28-day post-dose observation period. Guidance from FDA suggested that toxicology studies of antisense compounds should include non-human primate, due to original concerns of complement activation in monkeys by phosphorothioate oligonucleotides following a high-dose, bolus intravenous injection. The primate is also preferred as a large animal species due to the close genetic relationship to humans and the experience of the antisense field in demonstrating the predictability of toxicity effects between primates and humans. The results of this study will be discussed, including the safety and tolerability of AVI-4658, up to and including the maximum feasible dose.

PSM-AA-13 – Exon skipping for non-deletion dystrophin mutations

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Manipulation of dystrophin pre-mRNA splicing to by-pass Duchenne Muscular Dystrophy (DMD)-causing mutations is predicted to reduce the severity of the disease in a proportion of DMD patients. Examination of the dystrophin gene structure in mildly affected Becker MD (BMD) individuals has provided templates for potentially function dystrophins that may be induced by selected exon removal. Patients with deletions or nonsense mutations affecting the dystrophin rod domain are likely to be particularly amenable to exon skipping therapy, and as deletions are clustered in two hotspots, relatively large numbers of patients may be treated using similar strategies. Intramuscular injection of oligomers, designed to exclude dystrophin exon 51, have provided unequivocal proof-of-concept that exon skipping can restore dystrophin in muscles of DMD patients. However, non-deletion mutations are spread across the gene and many of these will require personalised interventions. We are developing exon skipping protocols to restore dystrophin expression in cells from patients with mutations that lie outside the deletion hotspots. Exon duplications present a particular challenge, and a number of different strategies have been applied to (1) restore the reading frame and (2) 'normalize' the size of the transcript. Where multiple exon skipping strategies one mutation are possible, it is imperative that the most functional dystrophin isoform be induced. These studies will contribute to the effort to extend splice manipulation therapy to all potentially amenable dystrophin mutations.

PSM-AA-14 – Induced non-productive splicing to study muscle gene expression

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Endogenous alternative splicing can regulate gene expression through a process called Regulated Unproductive Splicing and Translation by either incorporating an exon carrying a nonsense mutation, or excising an exon to disrupt the reading frame. Antisense oligomer mediated splice manipulation can exclude exons to overcome protein truncating mutations, but can also be used to alter isoform expression, or induce a frame-shift. As a result, the mature gene transcripts cannot be translated into functional products. We show that it is possible to efficiently disrupt the normal dystrophin mRNA reading frame and ablate dystrophin expression in mouse muscle. Total suppression of dystrophin gene expression can be induced by selected exon removal and maintained for several weeks *in vivo*. A severe dystrophic pathology was observed in the diaphragm within 4 weeks of commencing treatment in wild type neonatal mice. This approach to gene down-regulation is very efficient and specific when cell penetrating peptide-conjugated phosphorodiamidate morpholino oligomers are used. Disruption of gene expression through altered splicing patterns could be applied to many different genes, offering the opportunity to induce transient mouse models to study the consequences of gene suppression *in vivo*. In addition, exclusion of selected exon blocks to yield in-frame transcripts can allow mapping of functional protein domains, based upon exon boundaries, and permits physiological evaluation of dystrophin isoforms. This technique provides a cost effective alternative to transgenic mouse models for the study of muscle gene expression.

Preclinical studies in models of NMD – cell and gene therapy approaches

PSM-CGTA-01 – Efficient systemic AAV9-mediated microdystrophin gene transfer in the mdx heart

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Dystrophin plays an important role in muscle contraction linking the intracellular cytoskeleton of striated muscle cells to the extracellular matrix. Mutations in the dystrophin encoding gene can lead to a complete loss of the protein causing Duchenne Muscular Dystrophy (DMD) frequently associated to severe cardiomyopathy. Mutations that permit production of a truncated, less functional protein are usually associated to the milder Becker muscular dystrophy clinical phenotype. While clinical phase-I-studies for therapy of skeletal muscle are performed, trying to convert DMD in the milder Becker form, therapies for cardiac muscles have barely been evolved. The aim of our study is to establish an efficient long term treatment for DMD-associated cardiomyopathy in a mouse model for dystrophin-deficiency (mdx). Mdx mice were treated with a microdystrophin-cDNA (μDys), delivered by adeno-associated-viral vectors (AAV9) specifically into cardiac muscle. μDys, under the control of the CMV or CMV enhanced myosin light chain promoter (CMV-MLC), was inserted into AAV9 and 4×1011 or 1×1012 viral genomes were administerd to mdx mice via tail vein injection. Four weeks after injection, heart and quadriceps femoris muscle (MQF) were dissected and analysed by immunofluorescence for expression of μDys. We found (1) a maximum transduction-efficiency of 60% of all cardiomycytes and (2) no expression of μDys in the MQF. This demonstrates a high specificity and efficiency of the chosen therapy for cardiac muscle. In conclusion, we established an efficient and specific AAV9-mediated gene transfer of microdystrophin-cDNA in mdx hearts, which may represent a promising tool to develop treatment strategies for DMD-associated cardiomyopathy.

PSM-CGTA-02 – Automation of biodistribution study

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GenoSafe proposes methods and develops tests to evaluate innovating biotherapeutics products. As a TREAT-NMD partner, GenoSafe is involved in 'Production, toxicology, safety and delivery of therapeutics' aspect of the program. To evaluate the safety of a gene therapy product, regulatory agencies request to perform a biodistribution study. The purpose is to look for the presence and the persistence of the vector in animals used for preclinical studies. It also checks the absence of transmission in the germinal lineage. A typical biodistribution study implies several dozens animals receiving the therapeutic product and to analyze numerous (from 12 to 20) organs from these animals. This represents a large amount of samples, and then a long work associated to high risk of errors. To reduce this working time, we have considered the automation of different steps of the biodistribution study. We have started to set up and validate the automation of the genomic DNA extraction and normalization, which consists in dilute DNA into given concentrations. For this purpose, we compared new protocols developed with the automate, to the protocol of reference, the manual process. We obtain at least same yields of genomic DNA after extraction but in 2.5 folds less time and we observed a strong repeatability in normalization step. Thus, we realized that more than saving time, the automation of this process leads to increase the repeatability and the reliability. Thus, the automation appears to be indispensable to evaluate the safety and the efficacy of gene therapy product.

PSM-CGTA-03 – Cell therapy for muscular dystrophy: CD34 negative muscle derived cells present high myogenic and no adipogenic potential

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The objective was to identify, within myogenic muscle precursors, a cell population with a high myogenic and low adipogenic potential in the perspective of cell therapy for muscular dystrophy. Myoblast transplantation is based on intramuscular injection of a population of CD56 positive myogenic muscle precursors (MMP). This population contains multipotent stem cells able to differentiate into distinct mesenchymal lineages including adipocytes. Adipocyte accumulation is observed in human muscular dystrophies and stem cells transplanted in muscle environment permissive to fat development, may be committed towards adipogenesis at the expense of myogenesis. After Cytofluorimetric analysis of cell surface markers, distinct MMP populations were sorted using specific magnetic microbead associated antibodies. Their ability to differentiate in adypocytes and myotubes was assessed *in vitro*. Populations of interest underwent *in vivo* studies conducted in immunodeficient Rag2-/- gc-/- mice. The stem cell marker CD34 allowed us to sort two distinct populations: CD34 positive (CD34+) and CD34 negative cells (CD34-). In vitro, the CD34+ cells were myogenic and adipogenic whereas the CD34- cells were only myogenic. Muscle regeneration potential after transplantation in cryo-injuried muscle of immunodeficient Rag2-/- gc-/- mice was the same for CD34+ and CD34- cells. Using clodronate-containing liposomes we could obtain adipose degeneration in cryo-injured tibialis anterior muscle of Rag2/gc-/- mice. After transplantation in these mice model CD34-cells were shown to participate solely to muscle regeneration while CD34+ cells were driven partially to differentiate into adipocytes. In conclusion, muscle derived CD34 negative population might represent a new tool for cell therapy of muscular dystrophy.

PSM-CGTA-04 – Combining stem cells and exon skipping approach to treat muscular dystrophies

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The main goal of this work would be to combine gene modification strategies with cell-mediated therapies. This approach could permit the autologous transplantation of cells, minimizing the risk of implant rejection. Muscular dystrophies are a group of disorders characterized by the primary wasting of skeletal muscle. Mutations in the dystrophin gene cause hereditary muscular diseases as BMD and DMD, the most severe form. The characterization of dystrophin gene and the evidence that different types of adult stem cells are capable of muscle regeneration has lead to the development of potential gene therapy and stem cells treatments for DMD. In some cases, forced exclusion (skipping) of a single or multiple exons can restore the reading frame, giving rise to a shorter, but still functional protein. In this work we collected blood and muscle CD133+ from normal and dystrophic tissues. The cells were transduced with lentiviral vectors constructed to convey specific antisense oligonucleotides able to induce an efficient exon-skipping and to correct the initial frameshift caused by the DMD deletion. The skipped stem cells were injected to verify the dystrophin expression and the capacity of the skipped cells to fuse with regenerating muscle fibers. Autologous engrafting of blood or muscle-derived CD133+ cells, preliminary genetically modified to re-express a functional dystrophin, seems to represent a promising approach for DMD. This approach should offer the chance to distribute the autologous corrected stem cells to the whole body musculature with an intra-arterial injection providing a clinical benefit for the dystrophic patients.

PSM-CGTA-05 – Gene therapy and glucocorticoids for muscular dystrophy and cardiomyopathy of BIO14.6 hamster

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The delta-sarcoglycan deficient BIO14.6 hamster is one of the most studied models for inherited dilated cardiomyopathy and muscular dystrophy. This carries a spontaneous deletion of the delta-sarcoglycan gene promoter and first exon. Its lifespan is shortened to 12-15 months because heart slowly dilates towards failure. We injected the human delta-sarcoglycan cDNA by AAV2/8 by single intraperitoneal injection at two weeks of age. We obtained the body-wide restoration of delta-sarcoglycan expression associated with functional reconstitution of the sarcoglycan complex and with significant lowering of centralized nuclei and fibrosis in skeletal muscle. Motor ability and cardiac functions were rescued. Using serotype 2/8 in combination with serotype 2/1, lifespan was extended up to 22 months with sustained heart function improvement. It is know that corticosteroids have beneficial therapeutic roles in the treatment of Duchenne and Becker muscular dystrophies and sarcoglycanopathies. These drugs allow the maintenance of walking, slowing down the progression of the disease. At present, all patients with a defined diagnosis of muscular dystrophy are corticosteroid-treated. We evaluated the combined effects of gene and glucocorticoid treatments using BIO14.6 hamsters. We treated at the age of 45 days BIO14.6 hamsters using 0.3 mg/kg deflazacort for 3 weeks followed by 3 weeks of interval without drug in both BIO14.6 hamsters receiving AAV and controls. The effects of the interaction were studied by serial echocardiography, behavioral tests and histology.

PSM-CGTA-06 – Gene therapy or antisense oligonucleotide-mediated augmentation of SMN levels for spinal muscular atrophy

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Spinal muscular atrophy (SMA) is caused by a deficiency of functional SMN due to mutations in the survival motor neuron (SMN1) gene. Loss of SMN activity results in motor neuron cell death in the spinal cord. An approach to treating SMA is adeno-associated virus (AAV)-mediated delivery of SMN (AAV2/8-CBA-hSMN1) to the CNS. Administration of the virus into neonatal SMA mice resulted in SMN expression throughout the spinal cord and an increase in the number of motor numbers compared to untreated controls. Treated SMA mice exhibited increased myofiber size, improved motor function and reduced pathology at the pre-synaptic nuclei. Importantly, AAV2/8-CBA-hSMN1 significantly increased the median lifespan of SMA mice to 50 days compared to 15 days for SMA controls. Remarkably, mice treated with a self-complementary AAV vector showed enhanced improvement in median survival to 157 days. Another therapeutic approach is antisense oligonucleotide (ASO)-mediated re-direction of SMN2 splicing to enhance production of functional SMN. Administration of a 2'-O-methoxyethyl (MOE) ASO with a phosophorothioate backbone at birth into the lateral ventricles of SMA mice resulted in an increase in SMN levels in the spinal cord. Concomitant with this increase were improvements in ambulation, motor function and longevity (31.5 days). These data demonstrate that CNS-directed, AAV- or ASO-mediated SMN augmentation is highly efficacious in addressing both the neuronal and muscular pathologies of a mouse model of SMA Type I.

Outcome measure research and evaluation in NMD – biomarkers

PSM-BIO-01 – SMN transcript quantification as a surrogate outcome measure in Spinal Muscular Atrophy (SMA) clinical trials: towards a validated international Standard Operating Procedure (SOP)

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SMA is caused by SMN1 gene mutations. All patients retain at least one copy of a highly homologous gene, SMN2, and higher copy number is generally associated with milder phenotypes. A number of compounds have been shown to increase the amount of full-length SMN mRNA and protein *in vitro* and *in vivo* and some have also been tested in clinical studies. The demonstration that SMN mRNA levels in blood leukocytes correlate with clinical outcomes of pharmacological treatments would indicate that SMN transcript analysis could be adopted as a surrogate outcome measure in SMA clinical trials. At present, the recently developed absolute real time PCR assay (Tiziano *et al.*, in press) is the International Coordination Committee (ICC) gold standard to evaluate whether SMN mRNA represents a valid biomarker for SMA clinical trials. In view of up-coming International double blind placebo-controlled studies, SMN dosage must be reproducible in different laboratories worldwide. To this end, we are validating SMN transcript quantification in two laboratories and developing an International SOP. Preliminary data indicate that RNA quantification of samples extracted in two different laboratories and sent to the collaborating unit was highly reproducible ($P<10e-5$), albeit using different instruments. Also, the data on transcript quantification of a housekeeping gene (GAPDH) in the two labs, used as PCR control, showed relatively modest variability (<20%). A more detailed analysis of SMN transcripts will be presented. The development of this SOP will permit reproducible and comparable SMN transcript analyses in molecular laboratories involved in SMA clinical trials.

PSM-BIO-02 – Biomarkers for Spinal Muscular Atrophy study (BforSMA): design and implementation considerations for an unbiased search for biomarkers of disease severity

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SMA is a common pediatric neuromuscular disorder manifesting across a broad range of severity. Identification of a valid biomarker of disease severity could prove useful to clinical trial design or generate new hypotheses about pathophysiology. Best practices in biomarker discovery were incorporated into the study design: comprehensive clinical data collection, control for major variability factors and rigorous sample collection practices were implemented. In this study, a version of the Hammersmith Functional Motor Scale was chosen as the primary measure of disease severity. Power estimates were based on an average of 100 simulated datasets using data collected in an ongoing natural history study. A sample size of 100 SMA subjects should achieve 83% power for the primary outcome of the Hammersmith scale, assuming a 0.75 correlation between the observed and theoretical outcomes. Analysis of 20 age and gender-matched controls will provide 92% power to detect a univariate biomarker with a mean fold change of 1.5 between SMA and control cases (t-test) when the false discovery rate is controlled at level 0.05. The sample size was biased toward most achievable enrollment numbers: 15 Type I, 45 Type II, and 40 Type III SMA patients and 20 controls. 18 clinical sites were recruited across North America; enrollment and sample collection were completed in 4 months. Sample analysis is in process. This is the first study to identify and evaluate potential biomarkers that may have sufficient sensitivity and specificity to be useful as a pharmacodynamic marker or surrogate endpoint in SMA clinical trials.

PSM-BIO-03 – Utility of SMN transcript levels as a surrogate measure to track drug response in the SMA CARNI-VAL clinical trial

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Spinal muscular atrophy (SMA) results from mutations in the Survival Motor Neuron 1 (SMN1) gene. Valproic acid, thought to target the SMN2 gene, was tested in a SMA CARNI-VAL Phase II multicenter double-blind trial. SMN transcripts were quantified in blood samples to assess their utility as surrogate markers of drug response. SMA 'sitters' ages 2-8 years were randomized to placebo or VPA plus L-carnitine groups. Relative amounts of SMN transcripts for 2 baseline and 3, 6 and 12 month treatment visits were quantified as previously published. The CARNI-VAL group received treatment for 12 months and the placebo group only after the 6 month visit. Drug effects were assessed by linear regression. Mean normalized threshold cycle (Ct) for SMN transcripts was determined for 22 placebo and 18 CARNI-VAL patients. We did not detect any significant change from baseline full-length (fl) or exon7-deleted (del7) SMN values between the 2 treatment groups at 6 ($P=0.620$ (flSMN) and $P=0.620$ (SMNdel7)) or 12 ($P=0.992$ (flSMN) and $P=0.998$ (SMNdel7)) months. Indeed, a mixed effect model analysis indicated that baseline values for both transcripts were highly predictive of one year values ($P<0.0001$). A trend towards decreased SMNdel7 at 6 ($P=0.136$) and 12 ($P=0.166$) months was observed in patients under 3 years; however, this sample was small (n=14). In conclusion, SMN mRNA levels in blood were not affected by the VPA doses used in this study. A more effective treatment is needed before we can adequately assess the value of SMN expression as an appropriate surrogate.

PSM-BIO-04 – Development and validation of an immunoassay for the measurement of survival motor neuron protein

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Genetic defects leading to the reduction of the survival motor neuron protein (SMN) is the causal factor for Spinal Muscular Atrophy (SMA). While there are a number of compounds under evaluation as potential treatments for SMA, there is a critical lack of a biomarker for assessing therapeutic interventions, particularly those targeting upregulation of SMN protein levels. Towards this end we have engaged in developing an immunoassay capable of accurately measuring SMN protein levels in peripheral blood mononuclear cells (PBMC) as a tool for validating SMN protein as a biomarker in SMA. A sandwich ELISA was developed and validated for the measurement of SMN protein in PBMC and HeLa cell lysates. The assay provides a limit of detection (sensitivity) of 0.17 ng/ml for human SMN antigen and 0.74 ng/ml for mouse SMN antigen. Native and recombinant SMN proteins demonstrate parallel dose response curves, allowing accurate determination of the analyte. Initial analysis with the ELISA reveals that PBMCs yield 65 ng of SMN protein per 109 cells in control adult samples. Further work to assess the yield of SMN protein in PBMCs from SMA patient samples and in mouse model tissues is planned.

Outcome measure research and evaluation in NMD – function and strength

OMR-F&S-01 – The use of ulnar length in height calculation for boys with DMD: results from a CINRG natural history study

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Height measurements are essential in monitoring growth and nutrition, as well as determining normal ranges and percent of predicted values for pulmonary function tests (PFTs). In boys with Duchenne Muscular Dystrophy (DMD) for whom respiratory complications are a leading cause of mortality and morbidity, PFTs are particularly important, and help describe the extent of disease. Therefore, PFTs may serve as an excellent surrogate marker and outcome of treatment efficacy. However, it is difficult to obtain accurate standing height measurements in DMD patients due to decreased mobility, lower extremity contractures, poor posture, and muscle weakness. In this study, we examined whether standing height and ulnar length calculated height measures could be used interchangeably to accurately assess height in boys with DMD. We obtained data from the Cooperative International Neuromuscular Research Group (CINRG) Natural History study on DMD (n=347). Ulnar length measurements (using a Rosscraft segmometer) and standing height (using a stadiometer) were obtained in 187 participants at study entry. Standardized measurement techniques were certified through ongoing CINRG reliability training. The height prediction equation was based on ulnar length and age using the linear regression provided by Gauld (2004). Analysis of correlation coefficients between standing height measures and calculated height showed a correlation coefficient of 0.96. We conclude that ulnar length measures used for calculated height maybe used as an alternative for standing height in DMD patients. Further analyses seek to identify factors contributing to inaccuracy in either measurement method as well as steroidal effects on ulnar bone growth.

OMR-F&S-02 – Relationship between different timed tests in Duchenne Muscular Dystrophy: the CINRG experience

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Timed tests of mobility are measures that have been used in Duchenne Muscular Dystrophy (DMD) as a surrogate marker for current status of disease. The tests include the time to walk 10 meters, time to climb four steps, and time to rise from a supine to standing position. The tests can be performed by patients who still have even a small amount of ambulation left. These tests are proven to be highly reliable and easily administered. If these tests correlate well with functional measures and are good predictors to life altering events, such as loss of ambulation, they can serve as excellent surrogate markers in drug development. In a natural history study conducted by following DMD patients (n=347, ages 2-28), we have evaluated >200 patients for at least one year (4 quarterly measurements). While the fact that the disease progresses with age is clearly established, the variability in these measurements within ages, how they relate to each other, and how they change over time is not well-established. Our results show the increasing variability through mid to late teen years. Although the results from the tests are clearly correlated with each other, each timed test explains only approximately one-third of the variability of the other test; thus, these tests may be expected have different predictive ability with regard to life-altering events.

OMR-F&S-03 – Standardization of the six minute walk test (6MWT) in ambulant individuals with spinal muscular atrophy

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The purpose of this project was to develop standardized methodology for performance of the 6MWT in patients with SMA. The 6MWT has been used to assess function and endurance in a number of diseases and recently been chosen as primary outcome in therapeutic trials in several neuromuscular diseases (NMD). As the 6MWT has been accepted by regulatory and funding agencies and has been used in other NMD it has been recommended as a potential primary outcome measure for clinical trials in SMA. American Thoracic Society guidelines for the test have traditionally been used but various modifications have been made to customize the test for NMD. To allow comparison of results of the 6MWT between various protocols we believe a standardized procedure for test performance should be developed and promoted. A committee was established through the International Coordinating Committee (ICC) to standardize the procedures for performance of the 6MWT in SMA. Committee members reviewed the literature, collected 6MWT procedures from multiple protocols, e-mailed, conference called and met in person to come to consensus and recommend a protocol to address the specific needs and features of SMA patients. Procedures will be presented to the ICC for review and acceptance as a standardized 6MWT for potential implementation in clinical trials in SMA. Implementation of standardized 6MWT procedures across multiple trials will allow comparison of data and results between trials. This has relevance for the harmonization of information in the clinical study of ambulant SMA.

OMR-F&S-04 – A CINRG study of the relationship between impairment, activity limitation, participation and quality of life in persons with confirmed dystrophinopathies: methods and baseline characteristics

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Investigators studying therapeutics in B/DMD face increasing pressure from regulatory authorities to develop sensitive, reliable and meaningful outcome measures of strength, function and person-reported health-related quality of life (HRQoL). We enrolled males with confirmed B/DMD from 20 participating centers from 10 countries of the Cooperative International Neuromuscular Research Group (CINRG). Following consent and review of diagnostic testing, participants undergo assessments at baseline, months 3, 6, 9, 12, 18, 24, 36, 48 and 60. DNA is collected for banking. Study teams collect age-appropriate measures of clinical history (medical events, medications, durable medical equipment, supportive services), anthropometrics, goniometry (wrists, elbows, knees, ankles), Medical Research Council manual muscle strength, quantitative knee and elbow flexor/extensor and hand grip strength, timed function (stand from supine, stair climb, 10M walk/run), Brooke and Vignos functional scales, pulmonary function (FVC, FEV1, MIP, MEP) and health-related quality of life (PedsQL, SF-36, POSNA). Three hundred forty seven males with B/DMD aged 2-28 years were enrolled at 20 CINRG centers worldwide between Fall 2005 and Winter 2008. Participants were well-represented from the youngest ages to the mid-teens, with fewer individuals in their late teens to early twenties. At enrollment, 65% were current corticosteroid users, 63% were fully- or partially-ambulatory and 13% required some ventilatory assistance. We will present baseline assessment data relative to age, ambulatory status and corticosteroid use. This study provides investigators with data on age-related patterns of clinical, functional and person-reported outcomes in individuals with B/DMD. This will contribute to optimal design of future clinical trials of emerging therapeutics.

OMR-F&S-05 – Psychometric evaluation of quality of life (QoL) questionnaires in patients with muscle disease and myasthenia gravis (MG)

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There is increased interest in psychometric evaluation and validation of different outcome measures in different diseases. We investigated psychometric properties of quality of life (QoL) measures used in muscle diseases and myasthenia gravis. 303 patients with variety of muscle diseases and 163 patients with myasthenia gravis completed INQoL and SF-36 as part of a US based multi-center quality of life research project. We used Rasch model to compare item fit, separation, difficulty and differential item functioning (DIF) for symptom impact and life domains of INQoL as well as for SF-36 domains. Most domains of both outcome measures showed relatively reliable psychometric properties (SF-36 infit mean square(mnsq) 0.96 -1.00, outfit mnsq 0.92-1.39; INQoL symptom impact infit mnsq 0.98-1.02, outfit msnq 0.90-0.98; INQoL life domains infit mnsq 0.0.76-0.98, outfit mnsq 0.73-0.93). This analysis will allow discussion regarding mis-fitting items and groups that may lead to suggestions to improve these QoL measures for muscle disease.

OMR-F&S-06 – Steroids treatment in DMD patients: effects evaluation with gait analysis and functional scales

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We studied 20 walking DMD patients aged between 7 and 8 years. Patients were divided in two groups (10 patients each): Group A, without treatment and Group B, treated with steroids (Deflazacort 0.9 kg/die continuatively) since at least one year. All patients were evaluated by collecting the HAMA score, the get-up time with Gower's sign, the 10-mt walking time, and through kinematic data of spontaneous gait. HAMA scale evaluation showed a statistically significant difference (P=0.01) between group A (24.8±6.3/40) and B (31.9±4.8/40). In group A, two children were not able to get-up from floor. All group B patients were able to get-up and were faster than those of group A (B mean time 5,5 vs. A mean time 7.1 s – P=0.03). Treated children used a significantly lower time to walk for 10 meters, as fast as possible (5.4 vs. 13.7 s – P<0.01). Height normalised walking speed and cadence were higher in treated patients (88% vs. 75%, P=0.03 and 140 vs. 124, P=0.01 respectively). Untreated patients presented a reduced hip extension band, a stiff extended knee in stance, an equinus foot with reduced dorsiflexion both in stance and in swing. Despite the small number of patients, the load acceptance mechanism appeared, in median, better conserved in the treated group, with the presence of both the ankle plantar flexion in loading response, and the knee flexion in stance. Kinematic data corroborate functional results on the efficacy of steroids in terms of both reduction of muscle strength loss and ambulation prognosis.

OMR-F&S-07 – Comparing child and parent-proxy responses regarding function and assessment of quality of life: report from SMA CARNI-VAL clinical trial

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Currently no treatment for spinal muscular atrophy (SMA) is available. SMA CARNI-VAL, a Phase II multicenter double-blind trial evaluating oral valproic acid (VPA) and L-carnitine (CARNI-VAL) in children with SMA, collected quality of life (QOL) assessments utilizing the parent-proxy (PP) and child self-assessment (SA) instruments to compare parent and child perceptions of QOL and function. The study included 94 children accrued to 2 cohorts: Cohort 1 were SMA 'sitters' ages 2-8 years randomized to placebo or CARNI-VAL; Cohort 2 were SMA 'standers and walkers' age 3-17 years enrolled into open-label CARNI-VAL. Parents completed the PP PedsQL and children >4 years completed the age-appropriate PedsQL at baseline, 6, and 12 months. PP QOL was collected on 92 subjects: age 1.8-16.3 years. SA QOL was collected on 44 subjects: age 4.1-16.3 years. Age and pulmonary function were associated with improved SA emotional, school, and psychosocial functioning at baseline. At baseline, gross motor function was associated with PP and SA physical functioning QOL, and fine motor function was associated with SA psychosocial functioning. Baseline strength, as measured by lower extremity (or knee extension) myometry scores were positively associated with change in PP psychosocial over 1 year, but negatively associated change in SA psychosocial over 1 year. Motor function is associated with better QOL in children with SMA. Patients and parents have divergent views on psychosocial QOL. Children with SMA associate fine motor function with psychosocial QOL; whereas, parents associate physical function with psychosocial QOL.

OMR-F&S-08 – Validity of the motor function measurement scale when routinely used in the follow-up of adult outpatients in a neuromuscular center

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The Motor Function Measure (MFM) is used to assess severity and progression of neuromuscular diseases. Validity was established in patients aged 6-60 years with suspected or confirmed diagnosis of neuromuscular diseases, Duchenne Muscular Dystrophy being the most frequent diagnosis in the population tested. Our aim was to check the validity of the MFM in the follow-up of adult out-patients presenting various type of myopathy. One hundred patients were randomly selected in our Center and submitted to the MFM score evaluation, manual muscular testing (MMT) of lower and upper limb, face and spine, Brooke and Vignos scores. MFM and its three dimensions D1 (standing position and transfers), D2 (axial and proximal limb motor function) and D3 (distal motor function) were compared to the other scores with the Spearman Correlation Coefficient and the Principal Component Analysis. Patients were aged 18-78 years. The most frequent diagnoses were Steinert's Muscular Dystrophy (DM1) and Facio-ScapuloHumeral Dystrophy (FSHD) (30% and 29%). MFM was significantly correlated to all other scores except for Face MMT. Our results confirm the validity of the MFM in adult patients with muscular diseases. However, the MFM global score and its three dimensions D1, D2 and D3 are variously correlated with the facial and axial muscle testing. Therefore, we recommend using separately the three dimensions D1, D2, D3 (rather than the global score) and, if more accuracy is required, the facial and axial muscle testing.

OMR-F&S-09 – Registry of outcome measures: supporting systematic reviews of outcome measures

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The objective was to provide web-based self help tools and guidelines for systematic review (SR) of outcome measures (OMs) for use in clinical trials/studies. Selecting the right OMs for clinical trials/studies is critical to success and is best done by SR of the available OMs so that the most suitable ones can be chosen or an informed decision to create a new one can be made. The Registry of Outcome Measures (ROM) already helps this effort by offering information on an expanding number of potentially suitable OMs. We have introduced additional web based tools to aid SR of OMs. ROM has been expanded to incorporate: (1) a 'Tree of OMs' allows the reviewer(s) to record OMs by category as being considered for a specific study or trial; (2) a search engine that enables investigators to find potential OMs in ROM; (3) a comparison table that displays information about multiple OMs to aid selection of OMs; (4) a document in progress which will evolve to be a Manual of SR for OMs. As these tools are web based they are easily accessible to collaborative groups. They can be open access so that all investigators can see work in progress, avoid duplication of effort, and contribute their views. These tools have already led to the publication of more OM records in ROM and are appreciated by investigators. These new tools available on http://www.ResearchROM.com will play an important part in helping translational research.

OMR-F&S-10 – Development of a functional scale for use in Limb Girdle Muscular Dystrophy 2I

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As part of a wider study to use magnetic resonance imaging and magnetic resonance spectroscopy to assess muscle damage in the limb girdle muscular dystrophy 2I (LGMD 2I) a functional scale was developed that could potentially correlate with imaging techniques and with other measures of strength and motor ability. Twelve ambulant individuals were recruited via Newcastle Muscle Centre, UK (Age range 21-64, male to female ratio 9:3, mean age 46:43, median age, 42:43 respectively) and assessed on two consecutive occasions no more than three weeks apart. The functional assessment consisted of: (a) forced vital capacity in sitting and lying; (b) myometry of the hip flexors, hip abductors, hip adductors, ankle dorsiflexors and knee flexors and extensors; (c) timed tests including: timed up and go, a 10 m walk/run, stair climb and descend and the timed rise from a chair. A quality of movement score from 1 to 6 was also given for these tests. (d) A six minute walk test; (e) an adapted Northstar scale for LGMD 2I. This functional scale was first developed for use in Duchenne Muscular Dystrophy but includes many relevant functional items; (f) activity monitors for 1 week and an activity questionnaire. Two physiotherapists were trained in these measurements prior to the study starting. Test-re-test reliability was good although a training element was shown for some individuals. Preliminary analysis shows that this test is suitable for LGMD 2I patients. Further work needs to be done on assessing change over longer time periods and on its correlation with imaging techniques.

OMR-F&S-11 – Pretreatment data from phase 2b study of Ataluren (PTC124T) in nonsense mutation Duchenne and Becker muscular dystrophy (nmDMD/BMD)

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Ataluren is an orally administered investigational drug designed to treat nonsense-mutation-mediated genetic disorders by enabling the production of full-length, functional proteins. This phase 2b controlled study is assessing 48 weeks of Ataluren treatment in patients with nmDMD/BMD. Screening and baseline evaluations (~6 weeks apart) included 6-minute walk distance (6MWD); StepWatchT activity monitoring (SAM); timed function tests (TFTs) (10-meter walk/run, 4-stair climb, 4-stair descent, stand from supine); grading of how TFTs are performed; and myometry (shoulder abduction, elbow and knee flexion/extension). Pretreatment data are available for 174 patients (median [range] age = 8 [5-20] years, height = 123 [97-174] cm, weight = 27 [15-80] kg, corticosteroid usage = 123/174 [71%], cardiac medication usage = 20/174 [12%]). The median [range] between-test interval was 42 [0-91] days. Test-retest reliability was high: 6MWD (r=0.91), SAM (range r=0.73-0.81), TFTs (range r=0.78-0.90), TFT method grades (range r=0.75-0.83), and myometry (range r=0.78-0.92). 6MWD correlated more strongly with TFTs (range r=-0.79--0.67) and TFT method grades (range r=0.63-0.70) than with SAM (range r=0.48-0.63) or myometry (range r=0.35-0.68). Younger age and corticosteroid usage were predictive for improved test performance. This first registration-directed study in DMD/BMD provides one of the largest and most contemporary experiences in assessing functional ability in boys with this disease. Pretreatment 6MWD and other clinical outcome parameters in this study are reproducible, complementary, and well correlated with factors known to predict disease severity.

OMR-F&S-12 – Can OPC (objective performance criteria) be used to show efficacy or safety in rare neuromuscular diseases clinical trials?

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Could an alternative to double blind randomization in rare disease, like OPC used in FDA approval for some medical devices in the USA, be used as a valid scientific alternative to show safety or efficacy in neuromuscular clinical trials? The OPC (objective performance criteria) concept has allowed the FDA to take a 'least burdensome' approach to the regulatory approval of some medical devices (e.g. prosthetic heart valves, carotid stents) without compromising the scientific integrity required by its mission of public health safety. The OPC is a criteria based on published literature and/or other sources of reliable data. It is a number used as a comparator in single arms trials where randomization is impractical or impossible. An OPC must be established by a multidisciplinary team of physicians in cooperation with statisticians, and should be followed by a detailed analysis in a peer-review journal. An OPC must reflect the current level of care and must be periodically re-evaluated. The OPC use offers several advantages over randomized clinical trials: smaller sample size, standardized comparator for future trials, reduced cost, shortened time to completion, simpler logistic. However the determination of an OPC is no simple task. An approach based on the OPC methodology represents an acceptable and scientifically valid alternative to show the efficacy or safety of new treatments in neuromuscular clinical trials when randomization is problematic.

OMR-F&S-13 – Assessment of ankle flexion/extension strength: from mouse to man

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There are not many means of assessing ankle function This is of particular importance since the tibialis anterior is often chosen as a test muscle in pre-clinical and clinical trials. Moreover this joint is fundamental in many human activities. Since it can be simplified as a two-dimensional rotational joint when performing pure isometric flexion/extension, the movement produces a rotation torque depending on muscle strength itself but also on the point of application of the force vector. In order to assess muscle strength, a torque has then to be measured, not a translational force alone. In our presentation, we will describe several dynamometers that have been specifically designed to measure ankle flexion and extension torques in various animal species (mouse, hamster, rabbit, cat, dog) and in humans. These dynamometers are equipped with adapted and highly precise sensors, analog output for possible visual feedback or acquisition and convenient maintaining settings. In animals, the contraction is produced by stimulating the proper nerve, either in extension or in flexion. Several methodological issues must be considered to debate on how the stimulation, hence the contraction, is specific, how the animals must be positioned and maintained, how the stimulation and rest parameters must be chosen... In humans, we have developed a normative database from 5 to 80 years of age and assess the repeatability and reproducibility of the measure. This presentation will give the opportunity to debate on dynamometry of the ankle in particular but also generally of any joint.

OMR-F&S-14 – Early complications in chronic spinal muscular atrophy

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Spinal muscular atrophy (SMA) begins with severe hypotonia and muscular weakness. Several complications appear at an early phase. In childhood muscle strength is difficult to measure and evaluation of muscle function is more effective. To describe the early complications in children with chronic SMA, their severity and their rehabilitation management. We evaluated the complications appearing in the course of disease in children with type II and III SMA younger than 10 years old including: walk disabilities, joint deformities, scoliosis and respiratory involvement. Severity subtype SMA of these complications was correlated with the Hammersmit Functional Motor Scale (HFMS) and the rehabilitation programme. We collected 27 children with SMA (23 type II and 4 type III). The average of age was 5 years for type II and 4 for type III. There was a majority of boys. All children were treated with physiotherapy and ergotherapy under the coordination of a rehabilitation physician. Eighteen of the type II patients used electrical wheelchairs and 5 used baby-carriage. Eighteen had several scoliosis and wore spinal orthosis. Five of them underwent surgical intervention. Seventeen had pulmonary complications and were under mechanical ventilation. All type III children walked unaided and none of them had joint deformities, scoliosis and pulmonary complications. In total, 22 patients were assessed by HFMS. The score average for type II was 18 and for type III was 39. Children with type II SMA present early complications. The HFMS was useful to demonstrate the severity of their disability.

OMR-F&S-15 – North Star ambulatory assessment, timed items and 6 minute walk test in ambulant boys with Duchenne muscular distrophy

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The aim of our study was to assess a cohort of DMD ambulant boys using the North Star ambulatory assessment (NSAA), a functional scale specifically designed for ambulant DMD boys that also includes timed items, and the 6 minute walk test (6MWT). More specifically, we wished to establish the spectrum of findings for each measure according to age and steroids therapy and their possible correlation. The study is a prospective multicentric study involving 12 leading tertiary neuromuscular centres in Italy and one centre in the UK. 115 ambulant DMD boys were assessed using the NSAA and 6MWT. Their age ranged between 4,10 and 17 years (mean age 8.5±2.5 DS). 91 of them were on steroids, 47 on intermittent and 44 on daily regimen. The scores on the NSAA ranged from 6/34 to 34/34. The distance on the 6MWT ranged from 127.00 to 560.58 metres. Walking 10 meters was between 3 and 22 sec. The time to raise from floor ranged from 1 to 59 sec. Some patients were unable to raise. As expected the results changed with age and were overall better in children treated with steroids. NSAA had an overall good correlation with 6MWT and 10 meters and less with Gowers. The results suggest that the different outcome measures provide different aspects of function and that should be used in combination to obtain a better estimate of the overall function.

OMR-F&S-16 – Criterion validity of MDQoL-60

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We have developed a muscular dystrophy-specific QOL evaluation scale (MDQoL-60) to evaluate the effects of treatment. MDQoL-60 facilitates QOL evaluation in 11 domains in patients with muscular dystrophy. The criterion validity of this scale was evaluated using the established generic QOL measure SF-36 or WHOQOL-26 as a criterion. QOL measurement using each generic QOL measure in patients with muscular dystrophy showed a marked floor effect in physical function using SF-36 as a criterion but no such effect using WHOQOL-26. In SF-36, the score is adjusted so that 50 becomes the mean. Patients with muscular dystrophy generally show a low score. Therefore, SF-36 has many problems when the effects of intervention such as treatment are evaluated in patients with muscular dystrophy. In WHOQOL-26, the mean value is almost in the middle of measurement values. However, WHOQOL-26 contains only a few QOL items regarding ADL or motor function. Evaluation of correlations between subitems showed that the generic QOL measures can be used instead of MDQoL-60 for items regarding the psychological aspect and social roles such as psychological stability, expectations, relationships, or activities, but not for items regarding the physical aspect such as ADL and respiratory and circulatory functions. In addition, sex-associated problems in patients with muscular dystrophy are not adequately reflected by the generic QOL measures. To evaluate the effects of intervention such as treatment for muscular dystrophy, the use of a disease-specific scale is desirable.

OMR-F&S-17 – Reproducibility of muscle strength measurement by hand-held dynamometer ISOFORCE

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We evaluated an effective method of muscle strength measurement using a hand-held dynamometer and intra- and inter-examiner reproducibilities. In the manual muscle strength test, muscles at 4 levels are selected, and, considering the examinee's fatigue, it is desirable to limit measurement to the neck (flexion), 2 sites in the upper limbs, 2 sites in the lower limbs, and grip. Sites showing high reproducibility in a pretest should be selected. The proximal segment is firmly fixed, and measurement is performed in the middle of the range of motion. The hand-held dynamometer is fixed at the most distal site of the distal segment, allowing isometric contraction. Muscle strength measurement is performed 3 times in succession, and the maximum or mean value is adopted. The mean intra-examiner variation should be considered to be about 10%, and the mean inter-examination variation to be about 25% (See the paper by Kawai). Effective methods for muscle strength measurement with high reproducibility in the bedside sitting, supine, prone, and lateral recumbent positions were summarized.

OMR-F&S-18 – Validation of the ABILHAND questionnaire to measure manual ability in children and adults with neuromuscular disorders

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Neuromuscular disorders (NMDs) can lead to specific manual disabilities due to hand muscle weakness, atrophy and myotonia. The aim of this study was to adapt and validate the ABILHAND questionnaire in children and adults with NMDs using the Rasch model. This questionnaire contained specific manual activities for children and for adults. One hundred and twenty-four adult patients and the parents of 124 paediatric patients were asked to provide their perceived difficulty in performing each manual activity on a three-level scale: impossible (0), difficult (1) or easy (2). Items were selected from well-established psychometric criteria (ordered categories, equal item discrimination, adequate fit to the Rasch model, lack of redundancy) using the Rasch Unidimensional Measurement Models (RUMM2020©) computer programme. The 22 selected items contain 4 children specific items, 4 adult specific items and 14 items commonly applicable to both children and adults. They define a unidimensional and linear measure of manual ability and demonstrate continuous progression in their difficulty. The item hierarchy of difficulty was invariant across six patient-related factors. The scale exhibited good precision (r=0.95) and the 22 items were well targeted to the patients' locations. The ABILHAND measures were strongly related to the ACTIVLIM measures (r=0.76), and poorly related to grip strength. Moreover, the resulting scale can be used for adults and children, allowing manual ability to be assessed from childhood to adulthood.

OMR-F&S-19 – Assessment of changes in motor function in patients with Duchenne Muscular Dystrophy using the motor function measure (MFM)

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To assess changes in motor function in patients with Duchenne Muscular Dystrophy using the Motor Function Measure (MFM). Three studies were performed. Two studies included only physiotherapy-treated patients, with 13 patients (males mean age 11 years 7 months, SD 1 year 10 months, range 8-14) in the 3-month study and 41 patients (males mean age 14 years 1 month, SD 5 years 5 months, range 6-32) in the 1-year study. A third study compared 12 patients treated with steroids with 12 age- and motor-function-matched untreated patients (males mean age of treated patients 10 years 2 months, SD 2 years 2 months range 6-14) over a 12-months period. Over 3 months, the MFM D1 subscore (standing and transfers) decreased significantly (-4.7%; P <0.01). Over 1 year, all MFM subscores decreased significantly: -4.9% for D1 (P<0.01); -7.7% for D2 (axial and proximal motor capacity; P<0.01); -4.3% for D3 (distal motor capacity; P=0.03); and -5.8% for the total score (P<0.01). A threshold value for loss of ambulation and a predictive value 1 year before loss were estimated (total score 70% and D1 subscore 40%). Compared with the controls, patients treated with steroids had more stable total scores (-0.59 vs. -5.87; P=0.02) and D2 subscores (0.98 vs. -8.50; P<0.01). These results support the use of the MFM in everyday patient management to prepare for loss of ambulation and in clinical trials to follow up patients receiving various treatments.

OMR-F&S-20 – Description of motor function impairment in SMA patients with Motor Function Measure (MFM)

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Spinal muscular atrophy (SMA) is a genetic disease with a high morbidity rate in childhood. Some treatments may be of benefit and clinical trials conducted to allow safety and efficacy using valid and sensitive outcomes measures. Our objective was to describe motor function impairment in SMA patients with the Motor Function Measure (MFM) a valid tool designed for all neuromuscular diseases and to estimate annual rate of change in MFM scores according to subtypes. Results of patients age ≥6 years, assessed at least once with the MFM and up to 5 years, were collected. Distributions of the MFM scores were analysed within subtypes, relation with age was studied and slopes of change for patients were estimated. Data was collected from 95 patients with SMA (9% type I, 39% type II, 51% type III), age 6 to 59 years. Fourty patients were assessed at least twice (followup ranging from 1 month to 5.5 years). The cooperation of the patients was rated good in 90% of type II and 92% of type III. Patients with short followup (less than 12 months) demonstrated little variation in scores. Responsiveness for those with longer duration of observation, showed slow deterioration as expected (-0.8 pts/y for type II and -0.6 pts/y for type III) with varibility in both subtypes, especially in type III. In conclusion, the present results are promising regarding the use of the MFM in clinical trials with SMA patients, as to detect differences in disease progression in relation to a specific treatment.

OMR-F&S-21 – Validity of the shuttle walk test in patients with polyneuropathies

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Patients with polyneuropathies frequently experience walking disability. However, there is no golden standard for assessing walking ability, and the frequently used 10 Meter Walk Test (10 MWT) shows ceiling effects. The incremental Shuttle Walk Test (SWT) is a reliable, valid, and responsive test in various patient populations with cardiac and lung diseases and could be of interest for patients with polyneuropathies. The aim of this cross-sectional study, involving 88 patients with chronic idiopathic axonal polyneuropathy and multifocal motor neuropathy, was to assess ecologic and concurrent validity of the Shuttle Walk Test. Outcome on the SWT was related to the 10 MWT scores and the Short Form Health Survey-36 (SF-36). Patients preferred the SWT to the 10 MWT with mean scores of 7.6 (SD 1.3) and 6.9 (SD 1.3), respectively (P<0.05) (10-point Likert scale). Spearman correlation between the SWT scores and the 10MWT scores was r= -0.87 (P<0.01). Regression models showed that, when adjusted for age, body length, and walking aids, the SWT scores explained 67% of the variance in the 10MWT scores. Correlation scores between the SF-36 (domain physical functioning) on the one hand, and the 10MWT and SWT on the other, were found to be r=-057 and r=0.60, respectively (P<0.05). The Shuttle Walk Test may be helpful to assess walking ability, and tailor neurological and rehabilitation interventions. Reliability and responsiveness however have to be assessed.

OMR-F&S-22 – Comparison of quantitative muscle testing with hand-held myometry in spinal muscular atrophy

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The purpose of the study was to compare two methods of strength testing in children with spinal muscular atrophy (SMA). Strength has been the most used primary outcome for SMA. Many studies have utilized Quantitative Muscle Testing (QMT), a computer based method for maximum voluntary contraction (MVC) while others measured MVC using hand-held myometer (HHM). In order to find a method that could be used in an international clinical trial, we compared QMT and HHM on elbow flexors. Five evaluators convened at one site (Philadelphia) to examine 10 patients with SMA type II/III. Each evaluator tested 4 patients and each patient was seen by 2 evaluators using QMT and CITEC myometers. Reliability was assessed using the highest rating for QMT and HHM separately and calculating intraclass correlation coefficients (ICC). Validity was assessed by correlating QMT and HHM with age, independent ranking for severity of disease by one author (RSF) and wheelchair use. Both HHM and QMT were found to have high and significant ICCs (ICCs>0.98). Bradley-Blackwood tests were non-significant; measurements had similar means and variances. Bland-Altman plots showed HHM measurements were slightly but not significantly biased compared to QMT. Validity was established by demonstrating Spearmen correlations for age with QMT or HHM were all non-significant (*P*>0.42), QMT was significantly related to wheelchair use (*P*=0.02) and ranking of disease severity (*P*=0.04). Both QMT and HHM strength testing were reliable in SMA type II/III. The QMT showed a slight advantage for inter-rater reliability and significantly higher validity than HHM.

OMR-F&S-23 – 6-minute walk test in Duchenne Muscular Dystrophy: longitudinal observations

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Walking abnormalities are prominent in Duchenne/Becker muscular dystrophy (DMD/BMD). Prior to a Phase 2b 48-week registration trial of ataluren (PTC124T) in DMD/BMD, a pilot observational study established the feasibility of a 6-minute walk test (6MWT) in boys with DMD and documented ambulation differences relative to healthy controls. This long-term follow-up provides data on 6-minute walk distance (6MWD) (DMD n=12; healthy n=9) and stride length (DMD n=9; healthy n=7) for observational study participants who underwent repeat testing ~1 year after initial testing. Median [range] age at repeat testing was similar at 8.5 [5-12] years for boys with DMD and 8 [5-12] years for healthy controls. After ~1 year, respective height and weight increased from median of 129 cm to 134 cm and 35 kg to 43 kg for boys with DMD and from 136 cm to 143 cm and 35 kg to 42 kg for healthy boys. 6MWD decreased in 9/12 boys with DMD (median [range] from 350 [252-481] m to 304 [150-530] m) and increased in 7/8 healthy boys (median [range] from 616 [525-687] m to 620 [590-724] m). Stride length decreased in 7/10 boys with DMD and was maintained or increased in 7/7 healthy boys. Most boys with DMD experience declines in ambulation over 1 year with changes that significantly diverge from those in healthy control boys. Stabilization or improvement in 6MWD with 48 weeks of ataluren treatment in the ongoing Phase 2b registration trial may represent a clinically meaningful benefit to boys with DMD/BMD.

Outcome measure research and evaluation in NMD – imaging

OMR-IMA-01 – Improved diagnosis and treatment of weakness in periodic paralysis

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Mutations in Cav1.1 or Nav1.4 channels cause hypokalemic periodic paralysis (HypoPP), a dominantly inherited muscle disorder characterized by weakness aggravated by cooling, glucose/insulin or exercise. Special *in vivo* 23Na magnetic resonance imaging (MRI), fat-suppressed 1H-MRI, and force assessment were used at rest, after provocation, and during treatment to determine intramuscular Na^+ and water concentration, and muscle strength in HypoPP patients. Membrane potentials and twitch force were measured on HypoPP and normal fibre strips. Of the 36 patients, 25 presented with continuous muscle weakness of varying degrees, up to wheelchair-dependence. The weakness was associated with intracellular Na^+ overload and oedema. Weakness, intracellular Na^+ overload and oedema were increased by cooling and glucose/insulin; and almost completely normalized by 4 weeks of treatment with the carbonic anhydrase inhibitor acetazolamide or the new aldosterone antagonist eplerenone (the 2 formerly wheelchair-bound patients are now ambulatory). *In vitro*, the continuous weakness correlated to membrane depolarization. Acetazolamide repolarized the membrane and restored force. Membrane depolarization associated with intracellular Na^+ and water overload causes muscle weakness. Acetazolamide has direct and beneficial effects on the muscle and can markedly improve continuous weakness. 23Na-MRI is a helpful clinical tool to visualize intracellular Na^+ overload for diagnosis and treatment monitoring. Fat-suppressed 1H-MRI (STIR) can be used instead because of the relatively high correlation of oedema to Na^+.

OMR-IMA-02 – Test/retest and machine/machine reliability of dual energy X-ray absorptiometry (DEXA) measurements in patients with DM-1 and DM-2

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DEXA measurements are frequently used in longitudinal natural history studies and multi-center therapeutic trials as an outcome measure in patients with DM. There are no data available in the literature documenting the test-retest reliability OR the machine/machine reliability of these measurements. We performed repeated DEXA measurements on two separate occasions within 1-36 hours on 15 patients with DM participating in clinical trials at our site. The measurements were made with instrumentation and software from the Lunar corporation, Madison WI. We also made repeated measurements on another 6 patients within a 24 hour period on 2 separate machines, one from the Lunar corporation and the other from the Hologic corporation. We found the following results for test/retest reliability: The Intra class correlations (ICC) for the test/retest measurements were as follows: DEXA total 0.99, DEXA Bone Mineral Content (BMC) 0.99, DEXA Fat 0.99, and DEXA Lean Body Mass (LBM) 0.99 machine/machine correlation. The Pearson correlations for the machine/machine measurements were as follows: DEXA total 0.99, DEXA Bone Mineral Content (BMC) 0.96, DEXA Fat 0.99, and DEXA Lean Body Mass (LBM) 0.93. All of the above correlations had a *P*-value of <0.0001. All the ICC's were greater than 0.90 which denotes excellent test/retest reliability. The excellent machine/machine correlation documents the reliability of measurements taken across several sites in a clinical trial and also the reliability of longitudinal measurements that may have been made over an extended period of time during which hardware and software changes may have occurred.

OMR-IMA-03 – Test/retest reliability of regional lean body mass (LBM) measurements using dual energy X-ray absorptiometry (DEXA) in patients with DM-1

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The objective of this study was to document the test-retest reliability of Regional Lean Body Mass (LBM) measurements using Dual Energy X-ray Absorptiometry (DEXA) in patients with DM-1. Total Lean Body Mass measurements using DEXA have been used in longitudinal natural history studies and therapeutic trials in patients with DM-1. Recently, correlations between Regional LBM and quantitative strength measurements have been documented by McDonald *et al*. The regional measurements are made by technicians using software provided with the DEXA instrumentation. The test/retest reliability of these regional measurements is not known. Regional LBM measurements were performed twice over a 1-24 hour period on DEXA scans of 10 patients with DM-1 using the Hologic post processing software. The whole body scans were divided into 5 body segments – trunk, right and left arm and right and left leg – using well defined anatomical landmarks and standard procedure as reported in previous studies. The Pearson correlations between the 2 regional measurements for each of the areas were as follows: trunk 0.998, right arm 0.995, left arm 0.984, right leg 0.993, left leg 0.996, the *P*-value for each of the correlations was <0.0001. In conclusion, all the correlations were greater than 0.90 which denotes excellent test/retest reliability. The excellent correlation documents the reliability of regional measurements derived from scans that may be made over an extended period of time as part of natural history studies and/or therapeutic trials.

OMR-IMA-04 – Muscle function and metabolism as outcome measures in neuromuscular disorders by multi-parametric NMR imaging and spectroscopy

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Beyond conventional imaging (MRI) of muscle trophicity and composition, Nuclear Magnetic Resonance (NMR) offers multiple imaging (NMRI) and spectroscopic (NMRS) modalities to non-invasively and dynamically investigate muscle function, applicable to both humans and animal models of myopathy. Multi-parametric functional (mpf) NMR simultaneously measures: muscle perfusion, by arterial spin labelled (ASL) NMRI; BOLD, an index of capillary blood oxygen by NMRI; myocellular oxygenation, by proton NMRS of deoxy-myoglobin; oxidative mitochondrial capacity and myocellular pH, by phosphorus NMRS of phosphocreatine (PCr) and inorganic phosphate. As examples: in calf muscle of glycogen storage disorder type III patients, mpf-NMR following aerobic exercise, revealed altered perfusion, whilst impaired oxidative mitochondrial capacity correlated with retarded oxygen uptake and reduced and delayed maximum capillary oxygen level. These combined results suggested that, in addition to inadequate substrate supply to the mitochondria, oxygen supply was unequal to consumption and also limited metabolic efficacy post exercise. In a hypertrophic murine model, exercising myostatin deficient mice (mstn-/-), developed the same force normalised for increased muscle mass as wild-type controls (WT). However, hyperaemic perfusion profiles were prolonged in mstn-/-, and oxidative mitochondrial capacity was reduced compared to WT, despite identical levels of phosphocreatine depletion, myocellular pH, and maximal perfusion at the end of exercise. Here mpf-NMR substantiated a non-pathologic, functional shift towards more glycolytic metabolism in these mstn-/- mice, corroborating a shift in muscle fibre typology. Investigating interplay of oxygen supply, uptake and mitochondrial consumption is thus possible in clinical and pre-clinical contexts, furthering possibilities for understanding and evaluation of myopathies.

OMR-IMA-05 – The measurement of muscle volume in the patients with muscular dystrophy, using muscle CT

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The muscle volume in patients with muscle diseases is an index of the progression of disease and of the efficacy of therapy. This study showed the method for the measurement of muscle volume using CT in patients' thigh muscle which mainly contributed to the daily function. We used the following methods: (1) The standard CT value of muscle and fat were calculated from CT value of iliopsoas muscles and subcutaneous fat in 114 non-neuromuscular disease patients aged between 20 to 30 years old. (2) Helical CT scanners were used to investigate the muscle volume in 16 patients with clinically diagnosed myopathies aged between 26 to 53 years old.CT imaging was performed between the great trochanter and the patella, with an 1-cm slice thickness. Using MATLAB®, CT images were analyzed applying estimated linear-function, which fitted muscle and fat standard CT value, and muscle density map was obtained. Vessels and skin tissues were erased manually from the density map, and accumulation of outcomes resulted in muscle volume of thigh. Standard CT values of muscle and fat were determined 56.3±5.65 (SD) and -98.3±11.4 (SD), respectively. The muscle volume of thigh on one side was calculated as between 450 and 4,300 cm^3 by CT, and these volumes were well correlated to the muscle strength of the legs. In conclusion, the measurement of muscle volume based on CT, which was validated by the muscle strength, was developed. The muscle was not anatomically distinguished from the other tissue, but calculated from the CT values.

OMR-IMA-06 – Estimation of skeletal muscle mass in patients with neuromuscular diseases using dual-energy X-ray absorptiometry

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Dual-energy X-ray absorptiometry (DXA) can determine fat mass (FM), fat-free mass (FFM), and bone mineral content for both the whole body and specific regions. In a healthy person, skeletal muscle mass (SMM) will be proportional to appendicular FFM; in neuromuscular disease (NMD), proliferation of interstitial adipose tissue (IAT) associated with muscle fiber loss should be taken into account, since fat-free content of IAT will also increase. The present study estimated the SMM using appropriate functions of DXA estimates. FM and FFM were measured with a Hologic QDR-4500w in 73 NMD patients. Regional analysis demonstrated that the ratios of FM to FFM were in the range from 2:8 to 7:3 in the mid-thigh region, and about 7:3 in the subcutaneous region. These findings indicate that IAT is radiologically similar to subcutaneous adipose tissue (SAT), since in the advanced stage of NMD almost all muscle fibers are replaced by IAT. The SMM can then be calculated using following equation: SMM = FFM–3/7×FM. To validate this equation, mid-thigh CT slices were obtained from 15 patients using a Toshiba Asteion TSX-021A/3J. From the area and mean CT value of each muscle, a corrected muscle area (MAc) was calculated. Regression analysis demonstrated that a correlation between MAc and DXA-derived SMM was 0.97. The intercept of the regression line was about zero. In conclusion, the composition of IAT is similar to that of SAT. The estimation function we proposed is expected to be a useful outcome measure in a future clinical trial.

OMR-IMA-07 – Quantitative assessment of skeletal muscle by NMR imaging: pitfalls and solutions

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Muscle imaging, and in particular NMR imaging, will play an increasingly important role, together with muscle force and movement measurements, for the evaluation of therapeutic interventions in patients with neuro-muscular disorders. To fulfil this mission, NMR imaging must be able de detect early changes high sensitivity, precision and reproducibility. Qualitative evaluation by visual inspection will not achieve this goal and one needs to turn to truly quantitative imaging protocols. We shall review the different approaches currently available to perform such quantitative investigations of skeletal muscle by NMR imaging. Muscle trophicity can be very precisely measured, particularly using 3D anatomical protocols. Voxel by voxel analysis of the NMR signal, has a potential to identify three main components: muscle tissue itself, fatty replacement and fibrosis. Newer approaches, from T1-rho or ultra-short TE sequences to Gd-molecules with high affinity for collagen, aim at a more direct evaluation of the fibrotic component. Sarcolemma integrity can be assessed by time-course studies of muscle imaging signal enhancement after injection of Gd contrast agents, possibly coupled to albumin. There are two main obstacles to the development of the muscle quantitative imaging concept: the lack of automatic procedures, particularly for data analysis, and the hardware imperfections, mainly inhomogeneities of B0 and B1 transmit and receive and gradient non-linearities. We shall provide an update on the solutions being elaborated to speed up and automatize data processing and to compensate for the hardware limitations, a prerequisite for a widespread use of quantitative NMR imaging as non-invasive outcome measure.

OMR-IMA-08 – Quantative analysis of muscle wastings in various muscle disease by computed tomography

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The purpose of our study is to establish the method to measure the muscle volume of patients with myopathies using CT scan. (1) CT measurements of the thigh were performed in 16 patients of myopathies and 4 healthy volunteers. Twentyeight to 30 scans with 10 mm intervals were acquired from trochanter major to pattela. DICOM CT data were transferred to workstations for histogram analysis. Areas of pure muscle were determined in the interval from 50 to 65 HU, and areas of adipose tissue were determined in the interval from -170 to -60 HU. The pixel with the interval from -60 to 50 HU was considered to show the mixture of muscle and adipose tissue. Estimated muscle area was obtained from pure muscle areas plus muscle part of mixture areas. Volume of muscle was calculated from corresponding cross-sectional areas in all scans and distances between the scans. (2) We calculated muscle volume index (MVI) by using the histogram-based prosedure. We applied this method to 44 CT scans retrospectively selected of patients with DMD. Results showed that the muscle volumes of thigh were correlated with the disability stage. In DMD, MVI decreased with disease progression. In conclusion, the method we propose is useful to evaluate muscle volumes.

OMR-IMA-09 – MR Imaging and spectroscopy of muscle and brain in DMD

B.H.A. Wokke, C.S.M. Straathof, J. van den Bergen, I.B. Ginjaar, A. Aartsma-Rus, H.A.M. Middelkoop, J.G.M. Hendriksen, M.A. van Buchem, A.G. Webb, G-J. van Ommen, J.J.G.M. Verschuuren and H.E. Kan
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Duchenne Muscular Dystrophy is caused by an X-linked mutation of the dystrophin gene. Due to absence of dystrophin production patients have progressive muscle weakness and a reduced life expectancy. Recently the first successful restoration of *in vivo* dystrophin expression in human skeletal muscle was established. To evaluate treatment effects the histopathological status of the muscle needs to be characterized. About one third of the DMD boys shows cognitive impairment. This may be caused by the lack of dystrophin, but the mechanism remains to be clarified. We plan imaging studies with magnetic resonance (MR) in muscle and in brain. We will include DMD patients with and without steroid treatment to control for steroid treatment related effects, like behavioral problems, structural changes in brain, and anti-inflammatory effects in muscle. The focus in the muscle MRI study is finding a non-invasive tool to be used in therapeutic trials. We propose to study the leg muscles in DMD patients and compare the results with those in age matched healthy controls. High field strength MR imaging (3 Tesla) will provide quantitative parameters of tissue pathology. Results will be correlated to muscle strength. For the brain we propose to study DMD boys and compare the results to those in healthy controls using 3 and 7 Tesla MR, and correlate the results to cognitive and behavioral functioning. The primary focus will be on regions that normally have high dystrophin expression like cerebellum and hippocampus.

Poster abstracts – session 2

Wednesday 18th November from 12:30 - 14:00

Registries

Reg-01 – DuchenneConnect registry: expanding services to meet evolving needs

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DuchenneConnect is a web-based registry developed to link patients, providers, and researchers in the Duchenne/Becker muscular dystrophy (DBMD) community. The program provides resources for genetic testing, genetic counseling, improved care/management, and clinical trials, and aims to better characterize the DBMD community. As a member of the TREAT-NMD global registry, we provide patient recruitment tools for clinical trials and research studies. Registered participants complete a self-report survey regarding their medical history and diagnosis, and submit questions to program staff. Submission of over 1000 questions from participants identified topic areas shared among families. Questions focused on understanding and knowledge of clinical trials were frequently encountered. To date 1,500 individuals have registered on DuchenneConnect, of which 1,131 have completed the profile. Families contacted DuchenneConnect with questions focused on clinical trials most frequently regarding the purpose of clinical trials, inclusion/exclusion criteria, announcements for current trials, and whether a given mutation-specific investigational therapy could benefit their child. To address these questions, DuchenneConnect developed a portal that provides a repository of information for families to understand the fundamental concepts of trial participation, locate available opportunities, and discuss questions. The DBMD community seeks opportunities to participate in clinical trials and research studies. DuchenneConnect developed a portal (URL forthcoming) to provide educational information in support of these needs. By integrating education with the registry functions, the community can become more knowledgeable and further prepared for future opportunities, in a forum that protects privacy and provides support.

Reg-02 – Neuromuscular REACH: a centralized American registry system to collect physician-entered data from MDA clinics

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Neuromuscular REACH (Research through Enhanced Technology Architecture for Comprehensive Healthcare) is a national registry and bioinformatics architecture that is under development by the muscular dystrophy association. REACH will (1) directly address the fragmentation currently hindering basic and translational research in this area; (2) utilize economies of scale by building a single interface that will house datasets for multiple diseases; and (3) leverage MDA's existing network of 220 clinics to decrease the costs for populating and maintaining neuromuscular disease registries. This bioinformatics architecture will support, in the near and long term, efficient management of disease-specific registries, conduct of natural history studies, development of clinically meaningful outcome measures for clinical trials and genotype-phenotype correlations, coordination of biological sample tracking, and integration with electronic medical records. Major goals for the project are: to design, develop and implement a nationwide system to collect curated core data elements for a broad range of neuromuscular disorders; to initiate integration of REACH with existing national and international registries such as TREAT-NMD; to develop REACH reporting modules; and to initiate integration of REACH with electronic health records (EHR). The project plan includes a web-based interface through which clinic teams can enter and access registry data for the different diseases in MDA's program. Implementation of the registry will occur in phases, with the 40 diseases covered by MDA's services program prioritized into tiers 1, 2 and 3. Tier one diseases will be those for which core data elements have been adopted by TREAT-NMD.

Reg-03 – TREAT-NMD patient registries for DMD/BMD and SMA disorders: the Bulgarian experience

V. Guergueltcheva, A. Todorova, V. Mihaylova, J. Genova, T. Tchamova, T. Todorov, B. Georgieva, A. Jordanova, I. Kremensky, V. Mitev and I. Tournev
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Our collaboration with TREAT-NMD on developing patient registries for DMD/BMD and SMA disorders started in autumn 2007. The registries in Bulgaria are managed in collaboration between Alexandrovska Hospital and Molecular Medicine Center in Sofia Medical University, the Bulgarian NMD society and in the frame of funded by the Bulgarian Ministry of Education and Science project. Information on the registries and our activities with TREAT-NMD could be obtained on the website of the Bulgarian NMD Society (http://www.nmd-bg.com). At present 57 DMD, 23 BMD and 34 SMA (22 SMA type III, 11 SMA type II and 1 SMA type I) patients are registered. The MLPA analysis/direct sequencing of the DMD gene in 51 DMD/BMD patients managed to clarify the disease-causing mutations in all of them. In total 42 deletions (82%), 6 duplications (12%) and 3 point mutations (6%) were detected in our patients' group. All deletion/duplication borders were precisely determined. In addition, four families with no index patient available were also analyzed by MLPA and the disease-causing deletions were detected in all of them by direct analysis of patients' mothers and sisters. All SMA patients are genetically verified. By collaborating on a national and supranational level we expect to be part of the efforts for finding cure for these disorders and to promote better care for the patients.

Reg-04 – TREAT-NMD patient registries for spinal muscular atrophy: natural history and clinical care in Germany and the United Kingdom

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TREAT-NMD patient registries are an important infrastructure of the network to test trial feasibility in the planning phase and help to efficiently recruit patients. The German and UK SMA registries are based on an online self-report questionnaire harmonized for all TREAT-NMD SMA registries. Data analysis of 500 patients shows a high rate of similarity between both countries. 9%/13% of the registered patients in the German and UK registry have SMA Type 1, 27%/23% have SMA Type 2 and 53% of SMA patients in both registries have SMA Type 3. 94% of the German and 97% of the UK patients have a homozygous deletion of the SMN1 gene; the others show a deletion compound heterozygous to a point mutation. Some difference between Germany and the UK in clinical care of registered SMA patients can be documented. Whereas in the UK, 52% of SMA type 1 patients and 11% of SMA type 2 patients use a gastral tube for feeding, only 22% of the German SMA type 1 patients and 2% of SMA type 2 patients receive this treatment. More patients with SMA type 1 receive scoliosis surgery in Germany than in the UK (17%/8%); however, scoliosis surgery is in the UK more common than in Germany among SMA type 2 and 3 patients. Further analysis is needed to identify differences in natural history and clinical care at different stages of each subtype of SMA.

Reg-05 – CMDIR, congenital muscular dystrophy international registry

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The CMD international registry is a patient self report registry that will be launched August 14th, 2009. The CMDIR will register patients globally with a diagnosis of congenital muscular dystrophy and/or a mutation in one of the genes that lead to either a CMD or LGMD phenotype. The genes targeted by this registry include: col6alpha1, col6 alpha2, col6 alpha3, LAMA2, LARGE, fukutin, POMT1, POMT2, POMGnT1, SEPN1, integrin alpha 9 and integrin alpha 7. Patients with confirmed mutations in FKRP and lamin A/C will be directed to existing registries. The goal of the CMDIR is to facilitate clinical trial readiness in the CMDs by collecting contact information on children and adults with CMD. The CMDIR will identify 3 levels of disease confirmation: both genetic mutations confirmed, disease confirmed through muscle immunohistochemistry, history and clinical exam and undiagnosed. Emory Genetics will provide counseling services to registrants, directing them to both local resources and diagnostic labs. An approach that involves referral to a CMD expert physician will be emphasized to promote targeted genetic testing. All confirmed genetic mutations will have deidentified information sent to the TREAT-NMD global database. In order to ensure global participation, the CMDIR will be translated into 6 languages, rolled out over August and September. The languages include: French, German, Spanish, Italian, Japanese and Portuguese. The poster will review enrollment and de-identified responses to current questions. The poster's aim is to facilitate awareness of this resource for physicians and people with CMD.

Reg-06 – The Dutch dystrophinopathy database

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The development of clinical trials for Duchenne Muscular Dystrophy (DMD) patients requires solid data on epidemiology and natural history of both DMD and Becker muscular dystrophy (BMD). The densely populated Netherlands with 16 million inhabitants forms a good candidate for studies into these fields. Estimates predict the existence of about 500 DMD and 250 BMD patients in the Netherlands. DMD and BMD patients received written information about the registry through patient organizations (Vereniging Spierziekten Nederland and Duchenne Parent Project), physicians, a genetic database and a website (http://www.lumc.nl/duchenne). They filled out a questionnaire about their disease course and gave consent to contact their physicians. The database includes all items required for registration in the TREAT-NMD database. Up to date, information was gathered about 310 Duchenne and 100 Becker patients, including data from 60 deceased DMD and 6 deceased BMD males. The DMD information shows a relatively uniform disease course: diagnoses at an average age of 4 years (range 0-10), loss of ambulance at 10 (range 5-15) and scoliose surgery at the age of 15 (range 11-18). Age at start of mechanical ventilation is less uniform, with ages ranging from 14 to 28 years. The disease course of BMD is more diverse with, for example, age at diagnoses ranging from 4 to 65 years. After one year our registry covers about 50% of the estimated dystrophinopathy population in the Netherlands. It gives important information about the natural course of these diseases, relevant to the development of future treatments.

Reg-07 – Construction of a database for a nationwide Italian collaborative network of mitochondrial diseases

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A flurry of epidemiological studies in recent years has confirmed the notion that mitochondrial disorders (MD) are one of the most common genetic disorders (with an overall estimate prevalence 1:5,000) and a major burden for society. In contrast to the extraordinary progress in our understanding of the biochemical and molecular bases of the MD, we are still extremely limited in our ability to treat these conditions. For rare diseases, small patient populations represent the major impediment to progress in research and care. This limitation is most effectively overcome by a patient register in combination with a biomaterial bank. The principal goal of this network is to develop a web-based register of patients with MD to better understand the phenotypes and the natural history of these diseases. In particular, this goal should be reached by: (1) establishing an Italian network of clinical centers – mainly neurologists and neuropaediatricians – with expertise on MD; (2) by setting up a web-based database, which will be harmonized with other registries for rare diseases and by interfacing with European databases (i.e. Treat-NMD); (3) by planning longitudinal studies. We believe these fundamental steps are necessary to better understand the natural history of the MD and, finally, to improve the management of these disorders.

Reg-08 – The FKRP global registry

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TREAT-NMD, a network of excellence for rare inherited neuromuscular diseases funded by the European Commission, together with patient organisations and clinicians from around the world has launched a global registry for patients with mutations in the fukutin-related protein gene (FKRP). This is the first international collection of data from both patients and professionals involved in the care of individuals with both limb girdle muscular dystrophy (LGMD2I) and the more severe FKRP-associated congenital muscular dystrophy syndromes (MDC1C, Muscle Eye Brain disease, Walker Warburg syndrome). The FKRP registry aims to collect data on a longitudinal basis and is divided into a patient report part including a consent form and a second part, which is completed by the professional involved. The patient part is available in a range of common languages and consists of easy drop down menus that can be completed online. They include sections describing both current and previous best motor function achieved, with optional 'pain' and 'quality of life' questionnaires. The professionals are required to confirm the genetic diagnosis and record respiratory and cardiac function. They are also asked to assess the patient's motor function, including manual muscle testing on selected muscle groups and a 6 minute walk test. The global registry for patients with FKRP mutations will be an important resource for future clinical trials as it will provide valuable natural history data, information about disease prevalence and genotype-phenotype correlations and important aspects about outcome measures. Trial readiness is essential in this evolving era of potential therapy.

Reg-09 – Current status of DMD/BMD patient registry in Japan

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The development of orphan medicines presents many challenges. Clinical trials with new therapeutic strategies are now being planned for Duchenne and Becker muscular dystrophy (DMD/BMD), however since adequate numbers of patients are needed to achieve significant results for clinical trials, patient registries are an important infrastructure all over the world, especially in the case of rare diseases such as DMD/BMD. We developed a registry of Japanese DMD/BMD patients on the national basis. The main purpose of this registry is the effective recruitment of eligible patients for a clinical trial and may provide timely information to individual patients. The registry data gives us more detail of natural history, epidemiology and clinical care. All required items for The TREAT-NMD global patient registries are included in our Japanese version. It would be possible to match our date to global registry database. Particularly with regard to orphan diseases, it is appropriate to include Japanese patients in Multi-Regional clinical Trial (MRCT), because such a large-scale clinical trial is very difficult to be conducted only within Japan. MRCT will accelerate drug development for neuromuscular diseases in Japan. We hope that in the near future, this registry will gain trial readiness in Japan and accelerate more effective harmonization with other countries to fight orphan diseases.

Reg-10 – Achievements and challenges towards patient registry for Duchenne Muscular Dystrophy in India

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Patient registries represent powerful tools for the collection of observational, molecular and epidemiological data and are becoming a widely accepted tool in both private and public health care settings. Scientific advances on orphan diseases like Duchenne Muscular Dystrophy have erased boundaries between countries, turning out to be global initiatives. With a population more than a billion, the expected cases of DMD in India are high. Moreover, there are no programmes/strategies to understand the number and take necessary steps for identification, prevention and management. Our efforts over the past 3 years have been towards molecular diagnosis, genetic counselling and outreach measures for Duchenne/Becker muscular dystrophy. Amongst the 700 patients analysed for DMD mutations, complete mandatory information, as specified by the Global registry initiatives such as Treat-NMD, are available for 650 cases. Despite diverse set of health care practices in India, our holistic efforts on care and support have been fruitful through active communication with the clinicians, request forms capturing complete clinical data, services on molecular diagnostics, carrier analysis and genetic counseling. We brought together laboratories across the country during the UPPMD conference in India and discussed the need for adopting global standards of diagnostics for DMD. The challenge lies in updating clinicians on the efforts towards creating a system for the fulfillment of a future national registry. The efforts on creation of patient registries will pave way to understand progression, enable dissemination of updates on standards of care, facilitate networking of clinicians and researchers and accelerate clinical trials efficiently.

Clinical trial design

CTD-01 – Moving forward with TACT

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The TREAT-NMD Advisory Committee for Therapeutics (TACT) was officially launched on the 22nd May 2009 (http://www.treat-nmd.eu/research/TACT). The TACT Chair, Cristina Csimma (Virdante Pharmaceuticals, Cambridge, USA) will lead this exciting initiative with the support of the TREAT-NMD secretariat and a multi-disciplinary team of experts, nominated by the neuromuscular community. The overall aim of the TACT is to provide guidance and advice to the neuromuscular community on the prioritisation of likely candidates for entry into clinical trials in inherited neuromuscular diseases. The remit of the TACT will be to evaluate the therapeutic development potential of drugs based on the available preclinical data support and taking into account all key aspects of drug development. This should assist TREAT-NMD in prioritising clinical trials to be run via the network; provide the background for preparing funding applications and investigational drug applications; and provide an objective and well informed appraisal to be shared with the wider neuromuscular community. To support these goals, the TACT is comprised of 43 members, each with specific expertise in one or more of the areas: drug discovery, chemistry, preclinical pharmacology, toxicology, regulatory, biostatistics, clinical care and research, ethics, patient organisation, drug development, and funding of clinical trials. We hope this important initiative would be endorsed by the major funders and will assist the community as a whole. Starting with the drug candidate proposals already submitted by the neuromuscular community, TACT is formulating a list of potential drug candidates and two review meetings have been scheduled for 2010 (February and June).

CTD-02 – Developing and delivering antisense strategies for Duchenne Muscular Dystrophy

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The MDEX consortium, (http://www.mdex.org.uk) is a multidisciplinary group of scientists and clinicians involved in experimental therapies for Duchenne Muscular Dystrophy (DMD). Recent key achievements are summarised below. Selection of AOs to induce skipping: collaborative *in vivo* studies identified the morpholino (PMO) as the backbone of choice; studies on cultured human cells and in the humanised DMD mouse selected the lead sequences to skip exon 53 and 51 (AVI-4658, AVI-Biopharma, currently in clinical trial). Selection of muscle for IM AO injection: MRI and histological studies in non-ambulant DMD boys identified the *extensor digitorum brevis* muscle (EDB) as an optimal target. Correlating age and revertant fibres: as an increase in revertant fibres with age could confound the interpretation of dystrophin restoration therapies, we performed a study which indicates that revertants do not increase with age in DMD. Quantification of dystrophin restoration: a method to evaluate relative levels of sarcolemma-associated proteins using digitally captured images of immunolabelled muscle sections was developed. Efficacy of repeated administration of low dose of PMO in mdx mice: we studied the efficacy of repeated low dose PMO administration in inducing dystrophin expression and restored mechanical properties in mdx mice. Improving muscle uptake of AOs: cell penetrating peptides conjugated with PMOs gave widespread systemic correction of dystrophin expression in mdx mice; ultrasound in combination with contrast enhancing microbubbles increased cardiac gene delivery. Clinical trials: we completed an intramuscular study assessing safety and efficacy of administering a single dose of PMO AVI-4658 into EDB muscles of 7 DMD boys; we are currently involved in a repeated intravenous administration, dose escalation study to assess safety and efficacy of AVI-4658 in a group of 16 DMD boys.

CTD-03 – MELTIMI: a Phase 2 double-blind, randomized, placebo-controlled, dose-finding study with idebenone (Catena®) for the treatment of MELAS

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Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS) is a progressive and devastating multi-system disorder. The 'classical' MELAS syndrome, which often starts in childhood or young adulthood, is characterized by seizures, progressive neurological and muscular impairment, loss of cognitive function, hearing loss, myopathy, peripheral neuropathy and superimposed episodes of acute neurological worsening. Both the relentless, gradual impairment and the acute episodes of neurological deterioration associated with MELAS have been attributed to impaired function of the mitochondrial respiratory chain. Idebenone (Catena®), a modulator of mitochondrial energy production and ROS scavenger, represents a potentially effective treatment for MELAS. We present details on the design of the MELTIMI study in which we evaluate 21 patients with the A3243G mitochondrial DNA mutation and MELAS (defined by a history of either seizures or stroke). Patients will receive Catena® (900 mg/day or 2,250 mg/day) or matching placebo for one month. The primary outcome measure is cerebral lactate levels measured by Magnetic Resonance Spectroscopy (MRS), a biomarker associated with disease worsening. Secondary endpoints include effects on fatigue and quality of life, as well as information on safety and tolerability in MELAS patients. This study will help the investigators to determine whether to proceed with longer-term efficacy studies.

CTD-04 – Lessons from clinical trials in spinal muscular atrophy: struggles and successes

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We hypothesize that a review of how effectively subjects were recruited and retained in clinical trials in spinal muscular atrophy (SMA) will be informative in the design of more efficient clinical trials in the future. We identified all prospective natural history, intervention and exploratory clinical trials in SMA since discovery of the SMN gene in 1995. An investigator from each study group provided enrolment data and comment on the challenges, successes and shortcomings of their study. This retrospective review analyzed study design, recruitment, subject retention, test item completion and whether sufficient subjects completed the study to enable informative a priori data analysis. Studies in SMA type I have had difficulty with clinical sub-classification, slow and often incomplete recruitment, high rate of missed study visits and drop-out rate, and limited subjects who completed all study visits and testing. Studies in SMA type II and III have been more successful in these areas, but with greater difficulty when dealing with type II subjects who are young, particularly weak and have pulmonary issues. Type III studies are challenging in terms of the differing ambulatory status and extreme variation in degree of weakness. Clinical trials in SMA types I-II have been confounded by issues related to dealing with frail, young subjects. A trial design which limits the number of study visits and avoids extensive travel to the study site may prove beneficial to reaching the study's objectives. Type III studies need to capture a wide range of ambulation skills.

CTD-05 – Outcomes in glycogen storage disease type II to evaluate enzyme replacement therapy: a new approach in translational research

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Glycogen storage disease type II (GSDII) is an autosomal recessive lysosomal disorder due to mutations in the gene encoding alpha-glucosidase (GAA). The disease is clinically divided in: a severe infantile, a juvenile and an adult onset form. Enzyme replacement therapy (ERT) has provided encouraging results in the infantile form. It is not yet known if ERT is effective in late-onset GSDII. Cases are diagnosed by biochemical assay, molecular characterization of GAA gene. We have diagnosed over 40 patients affected with GSDII with juvenile, late-onset and adult form (Nascimbeni *et al.*, Neurology, 2009). Natural history of patients with the heterogeneous late-onset GSDII is needed to evaluate ERT efficacy. A significant decline was observed over the years, before ERT, in skeletal and respiratory muscle function. We evaluate several outcomes such as muscle strength by MRC, timed and graded functional tests (Gait, Stairs, Gowers, Chair score), the six Minute Walk Test, forced vital capacity on spirometry, and QoL by SF36. We examined in 11 patients the effect of ERT: we found evidence of partial improvement in our cases during a prolonged observation from 3 to 24 months. The use of different clinical parameters seems crucial to determine the efficacy of ERT, since not all late-onset patients respond similarly. We investigate correlation with mutations and autophagy in their biopsy. The low prevalence and the clinical heterogeneity of GSDII requires multicenter studies: our protocol is valid for long-lasting studies to confirm the presence of clinical results.

CTD-06 – Preliminary results from a first-in-human study with ACA-031, a novel agent to increase skeletal muscle, in healthy volunteers

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Activin receptor type IIB (ActRIIB) binds ligands of the TGF-β superfamily, including myostatin (GDF-8) and several other negative regulators of muscle mass. ACE-031, a fusion protein derived from a form of the extracellular domain of ActRIIB linked to a human IgG Fc, binds to and prevents signaling of these negative regulators of muscle mass. In preclinical studies in disease models, including the mdx model mouse model of Duchenne Muscular Dystrophy, treatment with RAP-031, a murine analog of ACE-031, resulted in significant dose-dependant increases in lean skeletal muscle mass and strength, as well as a decrease in adiposity. In this first-in-human, Phase 1 study of ACE-031, the primary outcome was to evaluate the safety and tolerability of ACE-031 in healthy postmenopausal women. Secondary outcomes included pharmacokinetic analyses, pharmacodynamic effects on lean body mass (DXA) and muscle volume (MRI), quantitative muscle testing, and biomarkers of muscle growth and differentiation. In total, 48 subjects were randomized to one of 6 cohorts of 8 subjects, each to receive either a single subcutaneous dose of ACE-031 or placebo (6 active: 2 placebo) at dose levels ranging from 0.02 to 3 mg/kg and followed for 2 months. Subjects were requested to maintain their baseline physical activity for the duration of the study. A safety review was conducted following each cohort prior to dose escalation. To date, all 48 subjects have been treated with ACE-031 or placebo and completed the follow-up phase. Analyses of the data are ongoing and preliminary results will be presented at the meeting.

CTD-07 – Preparation for a phase 1/2 clinical trial of myoblast transplantation to DMD patients

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A previous phase 1 clinical trial, completed by some of us, has shown that the transplantation of normal allogeneic myoblasts to tacrolimus immunosupressed DMD patients leads one month later to the expression of dystrophin in 8/9 participants, with up to 29% of dystrophin positive muscle fibers in the cm^3 of muscle that was transplanted with cells. We also observed 34% dystrophin positive muscle fibers in a 26 years old DMD patient that received a compassionate treatment. We recently observed in the muscle biopsies of these patients and of animal models that the transplanted myoblasts not only fused with host muscle fibers but also formed satellite cells. The satellite cells were able to participate in repair of the muscle fibers that were damaged 1 month after transplantation. Given these encouraging results, we are currently trying to organize a phase 1/2 clinical trial to verify whether myoblast transplantation could improve the strength of the muscles injected with these precursor cells. The planned multi-centre clinical trial would involve randomized transplantation of myoblasts into the Extensor carpi radialis muscle with placebo saline injection into contralateral muscle. A double-blind approach would be taken for strength evaluation using standardized myometry as well as dystrophin expression in muscle biopsy as one of the muscles would be injected with myoblasts and the contralateral muscle would be injected with saline. All participants will be monitored for adverse events of the procedure and immunosuppressive therapy. We are currently planning for a 6 months follow-up of the patients under tacrolimus immunosuppression.

CTD-08 – AVI-4658 Phase 2 clinical safety and efficacy in DMD patients

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Duchenne Muscular Dystrophy (DMD) affects 1 in every 3,500 live male births worldwide and results from a mutation of the dystrophin gene. The mutation manifests as a disrupted reading frame that prevents production of dystrophin, the protein that enables proper muscle function. AVI BioPharma is currently in clinical development with AVI-4658, a Phosphorodiamidate Morpholino Oligomer (PMO) drug, designed to induce exon 51 skipping and restore dystrophin expression in DMD patients. While AVI-4658 is designed to specifically restore dystrophin expression in patients with deletions in exons 50, 52, 52-63, 45-50, 48-50 or 49-50 (13% of all DMD patients), the exon skipping approach should be applicable to up to 83% of all patients. Here, we present the trial design and initial data from a Phase 2, open-label, multicenter, dose-ranging study in DMD patients, to assess the safety, tolerability, pharmacokinetics and efficacy of 12 weekly intravenous administrations of AVI-4658. The study objective is to permit selection of a well tolerated dose to take forward into further placebo controlled clinical trial.

CTD-09 – Current progress with the systemic administration trial of AVI-4658, a novel phosphorodiamidate morpholino oligomer (PMO) skipping exon 51 in Duchenne Muscular Dystrophy (DMD)

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AVI BioPharma in collaboration with the MDEX consortium have identified a PMO to skip dystrophin exon 51 in DMD patients, restore the reading frame and enable dystrophin expression, proven by our recent single IM dose study. Here, we test 6 PMO doses to select an effective, tolerated dose for subsequent registration. 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 (6 minute walk), skeletal muscle, pulmonary and cardiac function is being assessed. Safety assessment include adverse events, physical examinations and laboratory tests – including hematology, coagulation studies, chemistry and anti-dystrophin antibodies. A DSMB guides dose escalation decisions (across the 6 doses 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/kg). Cohorts 1, 2, 3 and 4 have completed 12 weeks dosing. Cohort 5 is proceeding with dosing, and cohort 6 is due to start in October. No SAEs or severe drug related AEs reported so far. To date, maximum single dose is 300 mg and cumulative PMO dose is 3,000 mg. Current safety data will be presented at the meeting. Study drug well tolerated to date. Dosing and follow up continue on schedule. This preliminary data bodes well for safe long term administration in Duchenne patients. Preliminary, interim efficacy results of cohorts 1-4 are due in December 2009, and the data from the remaining cohorts in the first quarter of 2010.

CTD-10 Bayesian diagnosis of Amyotrophic Lateral Sclerosis (ALS) to improve clinical trial eligibility

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Amyotrophic Lateral Sclerosis (ALS) is unique among the neuromuscular diseases (NMD) in that it's diagnosis is clinical, and cannot be made with genetic tests, biopsies, or imaging. Current ALS diagnostic criteria reflect what 'the experts' believe ALS is, not necessarily what ALS really is. Consequently, some 10 to 20% of ALS patients do not meet current diagnostic criteria. Bayesian diagnosis ignores expert opinion in favor of data; in this case we used a dataset of over 1000 patients, half with ALS, and half with conditions from which ALS must be distinguished. Using a logistic regression model, 'Bayes Factors' (BF or odds ratios), were determined for the key clinical signs that distinguish ALS from other NMD; tongue atrophy, tongue fasciculations, deep tendon reflexes, Babinski sign, and muscle tone. The BF of a clinical sign is the odds ratio that a patient with that sign has ALS. The BF's provide a relative weighting for each clinical sign, and can be combined when more than one sign is present, to provide a final 'Odds of ALS' for that patient. When applied to a new dataset of patients with progressive weakness and motor disability suggestive of ALS, the Bayesian method shows that even people who, by current criteria, do not have ALS or who have 'suspected or possible ' ALS, have extremely high 'odds of ALS'. As new drugs become available for ALS trials, this Bayesian diagnosis will allow the inclusion of more subjects, especially those early in the course of the disease.

Standards of care and diagnosis in NMD

SoC-01 – The development of standards of care and patient recruitment for pregnant women with NMDs

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Women with neuromuscular disorders are often advised against having own children because obstetric risks and an influence on muscle function cannot be predicted. However, motherhood is an essential perspective in life for many women and increasingly more patients wish to have children. Since 1990 we have been collecting data pro- and retrospectively in women who had a defined muscle disease and at least one completed pregnancy. We asked for obstetric and neurological histories and the personal attitude expressed towards gestation and raising children. Patients were mostly recruited in Germany but also in Sydney/Australia, Helsinki/Finland, Pembrokeshire/UK and a few through self support groups in New Zealand and Italy. By June 2009, we had data sets of 185 participants belonging to different disorders (congenital myopathies n=12, spinal muscular atrophies n=25, muscular dystrophies n=33, Charcot-Marie-Tooth disease n=31, myotonic dystrophies n=74, others n=10). For many conditions, e.g. metabolic myopathies and myotonias, there is still a lack of information. As the national patient numbers are too small to conduct systematic studies, we attempt to initiate a prospective recruitment of patients through the networks of TREAT-NMD, MD-NET and other organisations. Given a confirmed pregnancy after the first trimester, first contact should be established between the patient and the study group in Aachen. After delivery, questionnaires will be sent (mostly via e-mail) to the participant which should be completed by the patient and her referring clinicians. With joint efforts we hope to enhance our knowledge in the important issue of motherhood in neuromuscular diseases.

SoC-02 – Identifying mitochondrial oxidative phosphorylation disorders: a diagnostic flowchart combining clinical, biochemical and histological tests used in the Ghent university hospital

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It is estimated that deficiencies in mitochondrial oxidative phosphorylation (OXPHOS) are present in 1 in 5,000 births. Some patients present with a specific clinical phenotype, but most have non-specific clinical characteristics. Also, due to the large number of proteins encoded by either the nuclear and the mitochondrial genome, identifying the underlying gene defect is a difficult and time consuming task. After almost two decades of experience, we have come to develop a step-by-step diagnostic approach, coordinating several medical sub-disciplines. It is a cascade of laboratory tests that is started when enough clinical arguments have been collected to suspect a defect in OXPHOS. Skeletal muscle is most often retrieved, or a skin biopsy can be taken to start up a fibroblast cell culture. OXPHOS enzyme activities are evaluated using spectrophotometrical assays, and histological signs of mitochondrial disease are investigated. If the results provide evidence for defects in OXPHOS, a second line of highly specialized tests is started, which includes blue native polyacrylamide gel electrophoresis, western blotting and immunohistochemical staining. Based on the data acquired, gene analysis is performed. Using the diagnostic flowchart described here, we are able to identify the underlying gene defect in approximately 40% of patients with OXPHOS deficiency. Our laboratory continues effort to improve the diagnosis of these debilitating diseases by looking for novel diagnostic techniques.

SoC-03 – Molecular characterization of COL6 genes in a cohort of 73 patients by extensive sequencing and aCGH highlights UCMD/BM genetic heterogeneity

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We have characterized by full sequencing and aCGH the three COL6 genes in a cohort of 73 patients clinically and/or immunohistochemically diagnosed as having a collagen VI-related phenotype (36 UCMD, 35 BM, 1 case of Myosclerosis myopathy and 1 patient with LGMD presentation). We identified mutations in 55 patients (75.3%) with a detection rate highly similar for UCMD (75%) and BM patients (74.3%). We identified de novo heterozygous mutations in 74% of UCMD patients, whereas only 7 patients (26%) carried an homozygous or compound heterozygous genotype for recessive mutations. Among BM cases, we found heterozygous mutations in 23 cases (88.5%), whereas in 3 patients (11.5%) we detected compound heterozygous recessive COL6A2 mutations. By array-CGH we identified a deep intronic mutations abolishing COL6A2 gene transcription. In 18 patients (9 UCMD and 9 BM cases), no pathogenic mutations were identified neither by sequencing nor by COL6 genes CGH analysis exploring large genomic rearrangements. This result supports genetic heterogeneity, with still unknown genes responsible for UCMD-BM phenocopies. Among cases negative for COL6 mutations, we identified three autosomal dominant large BM families, suitable for gene mapping by SNPs haplotyping and linkage analysis. Mitochondrial membrane potential was investigated in muscle cells from a BM patient belonging to a COL6 mutations negative family, and a latent mitochondrial dysfunction was observed. This results supports the occurrence of UCMD/BM patients without COL6 genes mutations but sharing with COL6 mutated patients a common pathogenic background and consequently potentially eligible for mitochondrial-related therapies.

SoC-04 – Muscular dystrophies: diagnostic approaches in Romania

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Muscular dystrophies are a group of inherited disorders of skeletal muscle characterized by progressive muscle weakness and wasting. Since the discovery of the dystrophin, protein involved in DMD/BMD several other dysfunctions of muscular proteins have been described be involved in different types of muscular dystrophy. In the last few years, our lab established an efficient way in the diagnosis of Romanian patients with muscular dystrophy by introducing of cellular and molecular techniques to provide exact diagnosis. After a clinical diagnosis of muscular dystrophy, confirmation of the DMD/BMD diagnosis for male patients is usually made by genetic testing, using MLPA technique, that enables the screen the whole dystrophin gene and to analysis the deletions/duplications. If no deletions/duplications are detected, a muscle biopsy is taken for the analysis of muscle proteins in an attempt to establish a precise diagnosis. Immunofluorescence and immunoblotting are the two methods used in order to evaluate the modifications of protein expression as a diagnostic tool. We use antibodies for: dystrophin, utrophin, sarcoglycans α, β and γ, calpain-3, caveolin-3, dysferlin, merosin and nNOS. The combination of these techniques allowed us to establishment of a high diagnostic accuracy of these diseases for Romanian, to identify and evaluate the expression protein deficiency primary, type of mutations importance for the prognosis of the disease. During the last 4 years, on a lot of 80 patients with muscular dystrophy, our laboratory establish the DMD/BMD diagnosis for 55 patients, LGMD 2A for 10 patients, LGMD 1C for 1 patient and LGMD 2C for 1 patient.

SoC-05 – TREAT-NMD strategies for standards of care generation and dissemination for NMD

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Harmonisation of standards of care for NMD is crucial to allow patients access to the best possible diagnosis and management of their disorder, as well as to develop a logical baseline for design of clinical trials and the development of novel therapies. The harmonisation of care standards is a priority for patient organisations and clinicians and needs to be taken forward internationally to carry maximum weight and allow full consensus. For this reason, TREAT-NMD has been actively engaging on the generation and dissemination of standards of care in a number of NMD. A collaboration with the ICC group for SMA led to the generation of care standards in this condition, which are now available in eight languages via the TREAT-NMD website. Collaboration with the CDC initiative for the generation of care standards in DMD has led to the generation of two papers on diagnosis and management in this condition, which are shortly to be published in Lancet Neurology. Working with patient organisations, a 'family-friendly' version of this, ready for translation into different languages has been generated. Interim care guidelines for DMD are already available in five languages. TREAT-NMD is currently working with groups developing standards of care for congenital muscular dystrophies and congenital myopathies, and this model can be extended to other disease groups in the future. Generation of care standards is only one part of this process however, with dissemination and implementation being ultimately even more important. TREAT-NMD can disseminate care guidelines via its communication infrastructure, via the patient and care and trial site registries and through teaching courses. A supplementary EU project, CARE-NMD intends to provide funding to establish models of dissemination and implementation in Eastern Europe and large scale collaborative trials performed via the network will also provide the opportunity to disseminate care standards further.

SoC-06 – Evaluation of awareness level and practices of standards of care in Duchenne Muscular Dystrophy amongst parents of DMD children in India

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Duchenne Muscular Dystrophy is an X-linked lethal pediatric neuromuscular disorder, with no curative treatment till date. The disorder is progressive requiring multidisciplinary medical care. The current study is an attempt to understand the awareness level of standards of care and practices amongst parents of DMD children. The questionnaire-based study is anchored on the interim recommendations from the Centre for Disease Control, USA and Treat-NMD on Standards of care for DMD affected children. The survey includes families with children suspected to have DMD and have had a confirmed molecular diagnosis at our facility. The questionnaire captures the current awareness and experiences of the family under the various domains of diagnosis and management as per the recommendations. It also gathers information on location, infrastructure, schooling and support groups available to the participants in order to understand the needs of the family. Parental Stress Index is another aspect that assesses the stress levels of the parents. This study enlightens us on the current scenario and the further needs on DMD care. It also aids in understanding the measures needed to minimize the variations in diagnostics and management. In the absence of considerable awareness, steps to better public know-how and diagnostic services are important consequences this study will pave way for. The current study, being the first of its kind in India, brings to light the pressing needs and concerns of the DMD community and hopes to serve as a base for future effective care and therapy.

SoC-07 – Carrier analysis in mothers of DMD children using MLPA

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Duchenne and Becker Muscular dystrophies are X-linked recessive allelic disorders caused by mutations in the DMD gene, affecting only males and transmitted by females, who have a 50% risk of an affected son or a carrier daughter. The incidence has been estimated as 1 in 3,500 male births, but not all the mothers of affected infants are carriers; a substantial proportion of cases (approximately 1/3) represent new mutations. Hence, investigation of the carrier state and genetic counseling are vitally important for the families concerned. In this study we have used Multiplex Ligation-dependent Probe Amplification (MLPA) technique in carrier analysis of mothers of children affected by D/BMD. Forty families where the proband mutation was identified were taken in the study. Family history of DMD was seen in 7 families and the rest 33 cases were sporadic. MLPA analysis results were validated by cross checking some samples with STR marker analysis and quantitative fluorescence multiplex PCR analysis. Of the 40 mothers tested, 17 (42.5%) were ascertained as carriers and the rest 23 (57.5%) were caused due to *de novo* mutations. All samples from families showing positive family history tested positive for carrier status. However, among the sporadic cases, 10 (30.3%) were carriers and 23 (69.7%) were caused to *de novo* mutations. This is the first study from India using MLPA. More than 2/3rd of the sporadic cases are caused due to *de novo* mutations. The lower number of carrier mothers suggests a higher frequency of new mutations in Indian DMD and BMD patients.

SoC-08 – The importance of a correct and comprehensive management in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy, recessive X-linked hereditary disease (Xp21) with a prevalence of 1/3,500 newborn male and due to a mutation of the DMD gene – which encodes the protein called distrofina, is considered the most common and severe neuromuscular disease in humans. This paper presents the diagnostic and treatment strategy in case of two boys of similar ages, affected by DMD. Although clinical diagnosis was the same in both cases, different therapeutic approaches resulted in a different pattern of the disease for both patients. Performed molecular diagnosis revealed the exact type of mutation. To complete the medical act, with a adequate genetic counseling we determined the patients mother`s genotypes. Treatment with corticosteroids resulted in TP patient preservation of motor function, respiratory and heart. Establishing with certainty the gene mutation has been instrumental in managing this disease, the impact is considerable for patients and their families because it: (a) offered the patients a chance to be included in a clinical trial; (b) allowed a relevant genetic counselling. It is imperative that the family doctor explains this pathology in which there need for rapid drug therapy and physiotherapy, and the importance of establishing a molecular diagnosis.

High throughput strategies in NMD

HTS-01 – Drug discovery for Duchenne Muscular Dystrophy via utrophin promoter activation screening

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Duchenne Muscular Dystrophy (DMD) is a fatal X-linked muscle wasting disease, caused by mutations in the gene for dystrophin. Utrophin is an autosomal homologue of dystrophin, which can functionally compensate for its absence in mdx mice (a model for DMD). Pharmacological upregulation of utrophin in muscle is therefore a potential therapy for DMD. We developed a high-throughput, cell-based assay for utrophin promoter activation, using the muscle cell line C2C12, stably transfected with a 1.3 kb human utrophin promoter region linked to a luciferase reporter. The development of a new drug is a lengthy process, but could be expedited by screening compounds already FDA-approved for other applications. We therefore used our utrophin promoter activation assay to screen the Prestwick chemical library, which contains 90% FDA-approved drugs and 10% natural compounds. Initial screening yielded 19 hits out of a total of 1,120 compounds (1.7%), using a cut-off of 20% activation above controls. In a second round of experiments, 13 out of these 19 compounds showed dose-dependent activation of the utrophin promoter. Based on magnitude of upregulation and lack of cellular toxicity, four compounds were selected for further validation. A TaqMan qPCR assay showed that all four compounds upregulate endogenous utrophin RNA in normal C2C12 muscle cells. The compound producing the greatest increase in utrophin RNA was selected for *in vivo* studies to determine its effectiveness in the mdx mouse.

HTS-02 – Discordant prednisone vs. deflazacort effects in mouse mdx and human DMD miniature BioArtificial muscle (mBAMS)

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A tissue-based approach to *in vitro* drug screening allows the determination of the cumulative positive and negative effects of a compound at the tissue rather than the cellular or subcellular level. Conditionally immortalized control, mouse mdx and human DMD myoblasts were tissue engineered into three-dimensional muscle constructs with characteristics of functional tissue, i.e. parallel arrays of striated myofibers. These miniature BioArtificial Muscles (mBAMs) were grown attached to two flexible microposts (µposts) acting as artificial tendons in 96 microwell plates, and when electrically stimulated, generated active tetanic forces measured with an automated µpost motion tracking system (Vandenburgh *et al.*, Muscle and Nerve, 2008; FASEB J., 2009). Tissue engineering and drug screening were semi-automated with a customized Beckman Coulter Biomek Liquid Handler. Over a range of concentrations, both corticosteroids could increase maximum tetanic forces by over 50%. However, in human DMD mBAMs, deflazacort exerted favorable max tetanic effect at 100-fold less concentration compared to prednisone. Human DMD mBAMs appear more sensitive to deflazacort than mdx mBAMs in these conditions, suggesting these two corticosteroids are indeed not nearly dose-equivalent for enhancing muscle function. Moreover, this new high content drug screening technology may be a useful tool for the identification of new compounds or combinations for better treatments in human muscular dystrophies. [Supported by the NIH (NIA, NINDS, NIAMS), the NSF, AFM and the Hood, Sharp, Ryan's Quest, Charley's Fund, Gals for Cal, CureDuchenne, Quest, Zach Heger, Ryan's Hope, Harvey Family and Jett Foundations].

HTS-03 – Identifying new treatments for neuromuscular disorders

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Identifying treatments for progressive, severely disabling neuromuscular disorders that lead to premature death is a major effort at PTC Therapeutics. Our current efforts are focused on Duchenne Muscular Dystrophy (DMD), spinal muscular atrophy (SMA) and myotonic dystrophy (DM). In DMD, which affects children and is characterized by progressive muscle wasting, we are working on three key therapeutic targets (myostatin, IGF1 and utrophin) that may compensate for the loss of dystrophin protein. Research is ongoing to identify molecules that modulate expression of each target. Spinal muscular atrophy, the most common form of infant mortality, is caused by the loss of the survival motor neuron (SMN1) gene. SMN1 is a protein essential to motor neuron survival. Increasing SMN2 protein levels may compensate for the loss of SMN1. We are investigating three therapeutic approaches for SMA: increasing SMN2 protein production, suppressing alternative splicing of SMN2 RNA, and enabling readthrough of the aberrant stop codon in SMN2 mRNA. Research is ongoing to identify and evaluate molecules active in these drug discovery programs. Myotonic dystrophy type 1 (DM1) affects adults and leads to progressive disability and premature death. Symptoms include muscle wasting and weakness in the lower legs, hands, neck, and face. Muscleblind (MBNL1) protein plays an essential role in the maturation of muscles and eyes. Research has shown that sequestering MBNL1 to CUG repeats in the DMPK1 3'UTR plays a role in DM1. Research is ongoing to identify compounds that increase MBNL1 protein levels. An overview of these drug discovery programs will be presented.

Other – networking

Ot-Net-01 – Engaging the regulators to facilitate antisense oligonucleotide therapies for Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is one of the most common fatal genetic disorders to affect children around the world and is caused by mutations in the dystrophin gene. Two proofs of concept studies using intramuscular administration of exon 51 antisense oligonucleotides or oligomers (AONs) have recently been completed successfully in the Netherlands and UK; two follow up studies with the same AONs are assessing the safety and efficacy of repeated administration of AONs in DMD boys. If these studies prove that this approach is safe and effective, the repeated systemic administration of AONs could prove to be one of the first effective tools, restoring dystrophin expression, to slow down the disease progression. Skipping of exon 51 by systemic AON administration would be applicable to approximately 13% of patients with DMD. Skipping of other exons could be beneficial for DMD boys carrying different deletions or nonsense mutations. In order to discuss development of future AON targets and the unique regulatory issues posed by this approach, TREAT-NMD and EMEA organised an international meeting, on the 25th September 2009, to discuss the development of future antisense oligonucleotide therapies for DMD. TREAT-NMD aims to address the bottlenecks which currently hinder the development of promising treatments in DMD. The TREAT-NMD/ EMEA meeting focused specifically on the development of AON therapies for DMD, and key points for discussion included: regulatory and toxicology issues, outcome measures and ethical aspects. The meeting was attended by approximately 100 clinicians, scientists, industry representatives, patient representatives, funding organisations and regulatory representatives. The results of the meeting and recommendations from the regulators for moving forward with AON clinical trials are presented in this poster.

Ot-Net-02 – Cochrane reviews: the best evidence for treating neuromuscular diseases?

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With emerging new therapies for muscle disease, such as corticosteroids and drugs that modify RNA processing currently in trials for Duchenne Muscular Dystrophy, it is important that the most up to date systematic reviews of the literature are readily available to patients, clinicians and healthcare commissioners. The Cochrane Neuromuscular Disease Group is one of 51 editorial groups contributing to the Cochrane Library. A Cochrane systematic review investigates a defined research question. The first stage is creation of a protocol which outlines the search strategy, type of study to be included, type of participants and methodological quality of trials to be evaluated. This process seeks to minimize bias and is peer reviewed prior to the initiation of the systematic review. Only randomized or quasi-randomized studies are included for meta-analysis. All completed reviews are peer reviewed once more prior to electronic publication and are then open to electronic criticism. All published reviews are updated regularly to ensure that available evidence is recent and unbiased. The processes involved in writing a systematic review will be illustrated by reference to reviews of treatment for muscular dystrophy, metabolic myopathy and spinal muscular atrophy. A detailed summary of the reviews relevant to TREAT-NMD will be presented. We will seek volunteers for topics which still need review authors.

Ot-Net-03 – The EuroBioBank network: a vital link in translational research on neuromuscular disorders

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EuroBioBank, the first operating European network of DNA, cell and tissue banks for rare diseases was established in 2001 to facilitate access to quality human biomaterials for researchers worldwide. Composed of 13 biobanks from 7 EU countries and coordinated by EURORDIS (European Organisation for Rare Diseases), this network was selected to be the biobanking service structure within the TREAT-NMD network of excellence (WP04.1 'Develop and manage supranational biobanks'), as it is the only network of its kind in Europe and also as most of its banks primarily deal with neuromuscular biomaterials. A main objective of the EuroBioBank network is to continue improving the availability, exchange and use of human biomaterials for translational research on neuromuscular disorders. A central tool to reach this objective is the EuroBioBank website http://www.eurobiobank. org, which provides services for TREAT-NMD researchers, such as the online catalogue of samples, Standard Operating Procedures (SOPs) and ethical documents. Implementation of quality control for biomaterials from NMD patients and additional evaluation of the SOPs are also part of this project. Over 400,000 samples are available across the EuroBioBank network. Last year, 3,330 neuromuscular samples were collected, 2,969 neuromuscular samples distributed to researchers, and 16 peer-reviewed studies were published acknowledging EuroBioBank. By facilitating access to high quality DNA, cell and tissue samples for the worldwide research community, while working closely with Research Ethics Committees (RECs) and maintaining ethical standards along donors'consent, the EuroBioBank network thus plays a major role in the acceleration of cutting edge treatments for neuromuscular diseases.

Ot-Net-04 – The GENAME project: an example of Spanish collaborative translational research in SMA

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A strong research infrastructure may improve and accelerate basic science to the clinical level. Translational research in neuromuscular disorders has proven to be a powerful process that drives the clinical research machinery. With the main financial support from Genoma España (a Spanish government agency) and FUNDAME (Spanish SMA foundation) we have established in 2007 a collaborative project involving a wide spectrum of groups from basic neurobiology to clinical care pointing to a concerted action to generate SMA translational research in Spain. The main goal is to provide the groundwork for identification of promising targets that will allow the treatment of the disease. This project establishes collaboration with 16 Spanish Centres and investigators. We aim to perform a multidisciplinary approach in four main areas: clinical, genetic-proteomics, neurobiology and therapeutics of the disease. Clinical aspects include the creation of a national registry of patients, biobanking, natural history, early intervention and the validation of outcome measures. The first accomplished objective was the genetic characterisation of all available Spanish SMA patients (Alias *et al.*, 2009). In addition, genetic influences that affect SMN protein abundance or its role in the motor neuron are approached by genomic, transcriptomic, metabolic and proteomic studies. A thorough neurobiology approach of the disease studying the available animal models is also ongoing. Finally, novel experimental therapies are under development. The sponsorship groups include parent and family associations, national and foreign research foundations, collaborative laboratories and companies. This ample variety of funding sources reflects the significant interest generated by the project.

Ot-Net-05 – MDA Venture Philanthropy: a new approach to bridging the valley of death for neuromuscular diseases

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MDA Venture Philanthropy, Inc., or 'MVP', is a nonprofit subsidiary of the Muscular Dystrophy Association, developed to fund the commercialization of new treatments for neuromuscular diseases. MVP will: make strategic investments in therapeutics with promising paths to market; leverage MDA's investment to date of over $1 billion in science, manpower and infrastructure; conduct professional due diligence in evaluating investment opportunities; work with donors to target major gifts to specific disease areas and engage donors directly; seek to grow its assets through a return on investment strategy; measure its impact in objective and definable terms. MVP's evaluation processes relies upon members of its business and scientific development advisory committees, as well as a wide range of external reviewers. The process has been designed to be complete, including a signed contract where applicable, within 13 weeks of receipt of an inquiry and is relatively unique in its format among non-profits. MVP will fund projects ranging from high throughput screens to phase III-enabling phase II studies, with a focus in Duchenne Muscular Dystrophy, spinal muscular atrophy and amyotrophic lateral sclerosis. Since its launch in early 2009, MVP has received letters of intent from 26 biotechnology companies and reviewed eight full data packages. Two projects have passed scientific diligence and are in the final stages of financial diligence. MVP has also completed detailed analyses of four potential therapeutic pathways and indexed these targets against the Pharmaprojects database to identify relevant existing drugs.

Ot-Net-06 – How to explain Duchenne research to Duchenne families

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I am a German biochemist, 79 years old and now 'retired' from running a private newborn CK screening program for the early detection of Duchenne and Becker muscular dystrophy since 1977. Writing reports and recording interviews for the Duchenne boys and young men, their families and care givers in Germany and elsewhere about scientific research for finding a therapy for this disease was part of the screening program and I am still writing them, first in English, then in German, and Ricardo Rojas, a young man with Becker dystrophy in Mexico, translates them into Spanish. There are also many other translations, even into Japanese and Chinese. As soon as they are ready, I am sending them to more than 1000 addresses on my e-mailing list, and the latest ones can be seen on my internet pages http://www.duchenne-information.eu. I am writing these reports in a way that will let the readers, who mostly have not studied modern biochemistry and genetics, understand what scientists and clinicians in many laboratories of the world are doing for them and their children. To make certain that the information does not contain mistakes, the researchers get an opportunity to check and correct my summaries about their work before they are published. Examples of the reports and interviews, which are now written on behalf of TREAT-NMD, Parent Project Muscular Dystrophy, and ActionDuchenne, will be shown in the poster.

Ot-Net-07 – ALADIN, All Against Duchenne in the Netherlands

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The new perspectives for treatment of patients with a dystrophinopathy and the planning of clinical trials demand robust outcome measures and knowledge of the natural history of the disease. Ideally, clinical care for patients with DMD or BMD would be uniform throughout a country, according to internationally established standards of care. A long follow-up with multiple standardized measurements would clearly help to decide which measurements are most reliable and clinically relevant to follow disease progression. The goal of ALADIN is to improve life-long clinical care and facilitate research on dystrophinopathies. Representatives from all seven Dutch neuromuscular centers and the university hospital of Leuven (Belgium), several larger hospitals, rehabilitation clinics and of the two Dutch patient organizations started to meet twice yearly. Meetings are free for anyone interested in the field of dystrophinopathies. A first newsletter was produced to inform all patients and physicians known to be involved in the care of DMD or BMD patients. Four meetings have taken place. The location of the meeting rotated among the participants. So far 58 physicians, basic researchers, physical therapists, representatives of patient organizations were regular visitors of the ALADIN meetings. The topic of the presentations covered a wide range of topics, from basic laboratory experiments to standards of care, and included regular updates on the progress of the TREAT-NMD activities. In conclusion, we have created a low profile platform that successfully facilitates the exchange of knowledge, research plans and clinical trials within the Netherlands and Belgium.

Ot-Net-08 – MDA clinical research network

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In August 2008, through a competitive application process, MDA awarded clinical research network grants to ten sites, five focused on DMD and five focused on ALS. The goals of the network are to promote and accelerate clinical research investigations and clinical trials for neuromuscular diseases and to make this process more collaborative and efficient. In 2009, the network centers plan to initiate five clinic studies: (1) Treatment of dystrophin deficient cardiomyopathy: the study, led by Dr. Jerry Mendell, will (a) define the natural history of cardiomyopathy and (b) compare the effects of various treatments on the progression of cardiomyopathy. (2) Clinical outcome validation in non-ambulatory and young boys/men with DMD: led by Drs. Anne Connolly and Julaine Florence, this project will develop standardized outcome measures for boys under age five and non-ambulatory boys. (3) Clinically meaningful changes scale: led by Dr. Hiroshi Mitsumoto, the ALS network will develop and test a clinically meaningful changes scale which will ultimately be used to assess to what effect drugs or other therapies have on individuals with ALS. (4) Trial of high fat/high calorie diet versus optimal calorie replacement in ALS: led by Drs. Merit Cudkowicz and Anne-Marie Wills, the network will test recent studies indicating a ketogenic-high calorie diet may slow neuronal death. (5) A multicenter prospective study of hyperlipidemia in ALS: this study, led by investigators Dr. Stan Appel and Dr. Ericka Simpson will examine 500 individuals with ALS to determine if hyperlipidemia is neuroprotective and predicts progression and survival.

Ot-Net-09 – TREAT-NMD and the ICC: a fruitful collaboration to foster clinical trials and research in SMA

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TREAT-NMD partners and the US colleagues working under the auspices of the International Coordinating Committee (ICC) group joined their efforts to reach consensus on clinical trials' organization in SMA. A few milestones have been reached already: (1) a consensus statement on standards of care for SMA generated by an ICC working group was disseminated internationally via ICC and TREAT-NMD; (2) a general consensus on outcome measures (OMs) for evaluating patients with SMA was developed. The consensus-building process went through the analysis of several factors, including available data and literature on natural history, validation processes, use of the scale worldwide (info collected by ICC). A workshop hosted by EMEA (London, October 2008) involving healthcare professionals, scientists, patients, industry and EMEA Committees' representatives was a key milestone for developing consensus on OMs and endpoints, setting a collaborative agenda for future trials in SMA and starting a dialogue on regulatory issues. Participation by the ICC ensured global representation and that the outcomes will also be shared with the FDA. In April 2009, a meeting in Rome built on the opportunity provided by SMA Europe to further validate (and crossvalidate with QoL questionnaires) the existing OMs. Agreement was reached on next steps for the motor function scales and PedsQL's neuromuscular modules for SMA typeII. A protocol for European studies and plans for training sessions should be available soon. New conjunct initiatives under development concern validation of genetic biomarkers, identification of topics for training and education and of potential for multinational clinical trials for SMA.

Ot-Net-10 – TREAT-NMD clinical trials coordination centre: efficiency of networking

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The Clinical Trials Coordination Centre (CTCC) is part of the network of excellence TREAT-NMD. It is attached to the Clinical Trials Center (ZKS Freiburg) and the Department of Neuropediatrics of the University Medical Center Freiburg. The CTCC coordinates the clinical research and enables through methodical training, outcome research and performance of randomized controlled studies the promotion of evidence production after the criteria of Evidence based Medicine. It has implemented the Care and Trial Sites Registry (CTSR, http://www.treat-nmd.eu/trialsites) which shows now (07/2009) 166 registered sites in 42 countries worldwide. Out of those countries 14 are non-European including 52 registered centres with 21 centres from North America. The overall amount of reported treated patients is 13,935, 6,945 with Duchenne/Becker muscular dystrophy, 3,058 with spinal muscle atrophy and 3,932 with other rare phenotypes. According to a recent survey, the most important factors deciding on the success of a trial are good organisation through the responsible Contract Research Organisation and well-trained investigators (Hark *et al.*, Applied Clinical Trials, 2009). 67% of the registered centres reported that there are familiar with ICH-GCP. As a network of centres caring for patients with neuromuscular disorders the CTSR will be used for dissemination and implementation of the latest evidence based treatment recommendation. The CTCC will use the CTSR to analyse in some exemplary countries how well the standards of care are implemented. In conclusion, the CTSR will not only facilitate multi-centre clinical trials but also help to improve clinical care for patients with neuromuscular disorders.

Other – general

Ot-Gen-01 – Can physical activity or iinflamation influence the outcome of LGMD2B?

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LGMD2B is a disto-proximal myopathy due to the lack of disferlin protein which is involved in the repair of sarcolemma. Abnormalities in phosphatidylcholine environment have been found also in sarcolemma in DMD and LGMD patients. Dysferlinopathy may present as disto-proximal myopathy, myalgia/hyperCkemia; its progression is highly variable in the same family, implying epigenetic or environmental factors. We evaluated whether strenuous exercise might influence the clinical progression of the disease as well the role of several serum citokynes. We have followed 20 adult patients with molecularly proven LGMD2B. We conducted a retrospective study of natural history, including muscle MRI and outcomes using the modified Gardner-Medwin-Walton (GMW) scale. Our investigation evaluated the role of sport activity performed before the onset of muscle weakness in the progression of the disease. We collected patients' sera to study the expression of cytokines. Patients were divided into two groups: cases with past history of sport activity (at least 3 hours/week for 2 years) and cases without physical activity. Sportive patients presented a significant more rapid progression than non-sportive patients. The time elapsing from onset to loss of Gowers (grade 4 of the GMW scale) was significantly less in sportive patients (5.75 years) than in non-sportive patients (10 years). We also observed that patients with inflammatory component in muscle might had sudden deterioration; we postulated a defect of chemio-actractant as a possible modulator of disease course. In conclusion, we suggest that both intense physical activity and muscle inflammation could be adverse factors in disease course.

Ot-Gen-02 – The genetic skeletal muscle channelopathies: genotype-phenotype correlation and longitudinal studies

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The skeletal muscle channelopathies are rare Mendelian disorders caused by mutations in genes that encode ion channel subunits. As members of the CINCH group (Consortium for the Clinical Investigation of Neurological Channelopathies), we at the MRC centre for neuromuscular diseases are involved in the first ever large scale multi-centre natural history trial of the non-dystrophic myotonias (NDM) and Andersen-Tawil syndrome (ATS) The non-dystrophic myotonias encompass a spectrum of heterogeneous disorders ranging from myotonia congenita (MC), to paramyotonia congenita (PMC) through to the potassium aggravated myotonias with muscle stiffness being the cardinal symptom. ATS is characterized phenotypically by the triad of periodic paralysis, cardiac dysrhythmias and dysmorphic facies. Mutations in the potassium channel gene, KCNJ2 underlie the disorder. The aims of the trials are as follows: to characterize the phenotypic spectrum associated with specific genetic defects; to collate information on symptom progression; to evaluate investigations used in the diagnosis and to assess endpoints for future treatment trials. We present the results of all patients who have been genotyped together with preliminary neurophysiology data.

Ot-Gen-03 – Divergent effects of pathogenic mutations in primary desminopathy on oligomer assembly and multimerization of desmin revealed by single particle spectroscopy

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Mutations in the intermediate filament protein desmin cause a distinct class of myofibrillar myopathies, which are characterized by deposition of aggregated desmin. To assess the effect of different disease-associated mutations at the molecular level, we applied confocal single particle fluorescence spectroscopy. We studied (1) the *de novo* aggregation properties of desmin *in vitro* and (2) the aggregation state of desmin in homogenates of transfected cells rendering purification unnecessary. We detected divergent assembly patterns for three different desmin missense mutations. Whereas R350P-desmin showed a strong inhibition of assembly formation that was associated with a reduced level of tetramers and an increase in dimers in native cell extracts, E413K-desmin formed hyper-stable tetramers. Moreover, we found subtle effects on assembly for R454W-desmin at dimer and tetramer level by single particle spectroscopy not detectable by classical fluorescence microscopy. Furthermore, by co-transfection and mixing studies of wt-desmin-GFP in combination with variable amounts of R350P-desmin, we showed that the mutant protein efficiently interacts with wt-protein resulting in a dominant-negative effect on desmin assembly. Our results provide a molecular basis for a detailed functional classification of mutations in the desmin gene and may help to explain the variable spectrum of phenotypic manifestations. In addition, our findings may also have implications regarding diagnostic and therapeutic strategies for primary desminopathies, based on the different molecular events that disrupt physiological filament formation.

Ot-Gen-04 – Severe nemaline myopathy associated with consecutive mutations E74D and H75Y on a single ACTA1 allele

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Nemaline myopathy is among the most common congenital myopathies. We describe for the first time a novel double *de novo* mutation in two adjacent codons resulting in two amino acid changes E74D and H75Y in the ACTA1 gene. The hypotonic male infant was the first son of healthy unrelated parents with no family history of neuromuscular disorders. Pregnancy was complicated: decreased fetal movements were noted on the 25th week of gestation, premature labour pains were present from the 29th week onwards and because of breech presentation a Caesarian section was carried out in the 39th week. The patient presented with multiple congenital fractures and joint contractures. He was dependent on ventilatory support until his death at 2 months. Muscle biopsy revealed severely atrophic and rounded muscle fibers with considerable variation in diameter and pronounced disorganization of the myofibers. Electron microscopy indicated a distinct disturbance of the myofibrillar architecture and nemaline rods. In view of previously described cases carrying different single missense mutations of the amino acid residues E74 or H75, we suggest that the particular genotype E74D/H75Y is compatible with the severity of the patient's phenotype. The possibility of germ cell mosaicism should be taken into account in genetic counseling.

Ot-Gen-05 – Talin 1 and 2 are required for myoblast fusion, sarcomere assembly, and the maintenance of myotendinous junctions

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Integrins are essential for development and maintenance of skeletal muscle. Mutations in α7-integrin cause congenital myopathy in humans, and integrin ablation causes muscular dystrophy and defects in myofibre development in mice. Talin 1 and talin 2 mediate a connection between integrins, actin and signaling proteins, and in muscle, they concentrate at the myotendinous junction (MTJ). We have used genetically modified mice to identify the specific functions of the talin genes in muscle development. Ablation of either talin 1 or talin 2 leads to a myopathy characterized by the detachment of myofilaments from the sarcolemma at the MTJ. Defects are more pronounced in talin 2-null mice, which present with centrally nucleated fibers, and appear not to be caused by an increase in sarcolemmal damage as observed for example in mdx mice. Interestingly, the phenotype of talin2-null mice resembles that of α7-integrin-null mice, which also present centrally nucleated fibres with only a moderate increase in serum creatine kinase. Ablation of both talin isoforms causes severe developmental defects, with impaired myoblast fusion and myofibrillogenesis. Together, the data reveal an essential function for talin 1 and talin 2 in muscle development and integrity. The similarity of the phenotype of talin 2- and α7-integrin-null mice suggests that mutations in talin 2 could lead to a congenital myopathy similar to that caused by α7-integrin mutations, and suggests that this gene should be considered as a candidate in patients without an identified genetic defect.

Ot-Gen-06 – Limb-girdle CMS with tubular aggregates - phenotypic clues for the entity

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Congenital myasthenic syndromes (CMS) are a clinically and genetically heterogeneous group of inherited muscle disorders caused by genetic defects that affect transmission at the neuromuscular junction (NMJ). To date recessive and less frequently dominant mutations in eleven genes were identified as responsible for CMS. In the large Munich cohort of over 750 CMS families from all over the world about 50% have been genetically diagnosed in regard to the known genes. Limb-girdle myasthenia (LGM) is a known entity in the literature and recently DOK7 mutations have been identified as causing the disorder in some of the patients. Of the remaining, at least nine families have been published with LGM phenotype and characteristic tubular aggregates on muscle biopsy. We have characterized seven families with LGM in our cohort with known genes excluded by direct sequencing or linkage analysis. All of them show autosomal recessive inheritance pattern. The onset of the disease is between 2 and 12 years of age. All patients show proximal limb-girdle weakness and no ptosis, ophthalmoparesis, facial, bulbar or respiratory weakness. Creatin kinase levels are normal or slightly elevated. There is a clear decrement upon repetitive nerve stimulation. Muscle biopsies disclose tubular aggregates arising from the sarcoplasmic reticulum. The patients usually respond well to cholinesterase inhibitors. Genome wide scan is in progress in these families. Optimal treatment, genetic counselling and elucidation of the pathomechanisms at the NMJ in CMS rely on the identification of novel genes involved in the disorder.

Ot-Gen-07 – Normal phenotype in a person with spinal muscular atrophy genotype

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SMA is an autosomal-recessive disease caused by homozygotic deletion of exons 7, or 7 and 8 of the telometric copy of the SMN1 gene and a few other proteins within the SMA region on chromosome 5q13. We present the family where parents have the heterozygous deletion of exon 7 of the SMN1 gene and their children are homozygous persons with the same mutation. The boy (proband) developed clinical picture of SMA3 and his sister has normal phenotype. The family has been diagnosed through complete set of clinical investigations including the molecular-genetic methods (PCR, SSCP). Parents had the heterozygous deletion of exone 7 of the SMN1 gene and both their children harboured the homozygous deletion of the same exon. The normal phenotype of the sib with homozygotic deletion of exon 7 of the SMN1 gene could be hypothesised as the result of gonadal mosaicism in one of the parents, gene conversion events that took place in the healthy sib, but not in the affected one, the presence of some modifying genes outside the 5q region that play a role in determining whether or not a patient developes SMA, or some up till now unknown reason. The better understanding of this rare event could help in further treatment of SMA sufferers.

Ot-Gen-08 – Development and characterisation of an *in vitro* model of sporadic inclusion body myositis (IBM)

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IBM is the commonest acquired myopathy among those aged over 50 years and has no effective treatment. The failure of immunosuppressants suggests that non-inflammatory disease mechanisms, including ER stress and mitochondrial dysfunction, may be important. Here we established an *in vitro* model to quantitatively characterise the role of these disease-relevant mechanisms in IBM. Primary rat skeletal muscle cell cultures were exposed to IBM-relevant conditions. Cells were either transfected with β-Amyloid Precursor Protein (β-APP) or a control Empty Vector (EV), or treated with inflammatory mediators IL1-β (5-20 ng/ml), TNFα (5-15 ng/ml) or lipopolysaccharide (LPS, 20-60 ng/ml) for 24-72 hours. The myotube fusion index was reduced by 22% following transfection with β-APP versus EV ($P<0.05$). Cell viability, estimated by an MTT assay, was 16% and 26% lower following transfection with β-APP, versus EV, at 48 and 96 hours, respectively ($P<0.05$). There was dose-dependent reduction of cell viability following exposure to IL1-β, TNFα and LPS. Using fluorescent confocal microscopy, the change in cytosolic calcium was determined following treatment with Thapsigargin, an inhibitor of SR-ATPase, by measuring Fluo4-AM fluorescence, β-APP transfected cells demonstrated a 28% smaller Thapsigargin response than controls ($P<0.01$). IL1-β/LPS treatment caused a dose-dependent reduction in the thapsigargin response, up to 21% at 24 hours. These results indicate a disturbance in calcium homeostasis and vulnerability to ER stress occurs in IBM. Transfection with β-APP or exposure to IL1-β/LPS also induced an increase in heat shock protein expression. We are currently testing whether pharmacological manipulation of this chaperone system is cytoprotective in this model.

Ot-Gen-09 – A new ACTA1 mutation in two unrelated sporadic cases of neonatal form of CFTD: insights on the pathological mechanisms.

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Congenital Fibre Type Disproportion (CFTD) is defined by isolated uniform atrophy of type I fibres, at least 12% smaller than type II fibres in absence of structural changes. CFTD is also clinically and genetically heterogeneous. Recessive, dominant and X-linked inheritance patterns have been described. So far mutations in three genes have been shown to cause CFTD, ACTA1 in three sporadic cases with lethal neonatal presentation, SEPN1 in one recessive family and TPM3 in a panel of patients with dominant transmission and variable clinical course. We report herein the identification of a new ACTA1 mutation in two unrelated sporadic cases of neonatal form of CFTD. Both patients presented with a major axial and peripheral hypotonia, a myopathic facies and a high arched palate during the first year of life. They presented delayed motor milestones, scoliosis and swallowing troubles. They encountered respiratory complications requiring nocturnal mechanical ventilatory assistance. Autonomous walking was acquired between the age of three and four. Both patients indicated real although slow improvements. Muscle biopsy showed in both patients a typical pattern of congenital myopathy with uniform atrophy of type I fibres, that were significantly smaller than type II fibres without structural changes. A c.764A>G change leading to the p.Glu251Gly substitution in the mature form of ACTA1 protein was identified in both children. The good correlation between clinical presentation and histological data may suggest that the pathogenic mechanism leading to fibre type disproportion could be specific of this new ACTA1 mutation identified in both children.

Ot-Gen-10 – Clinical and molecular characterization of patients with defective α-dystroglycan glycosylation

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As clinical trials are becoming a reality in an increasing number of neuromuscular conditions, identification of the molecular defect is required for accurate registry set up. Abnormal α-dystroglycan glycosylation is responsible for several conditions distributed in a continuum of clinical phenotypes; from severe congenital muscular dystrophies (CMDs) with or without brain and eye abnormalities, to mild limb girdle muscular dystrophies (LGMDs). We searched for mutations in the 6 genes known to be involved in α-dystroglycan glycosylation, in 61 patients with reduced α-dystroglycan. We detected 10 patients with FKRP mutations, 4 with POMGnT1 mutations, 5 with POMT1 mutations, 2 with POMT2 mutations and 1 with fukutin mutations. Among the FKRP-mutated patients, 5 had LGMD, either severe (3) or mild (2); 5 patients had severe CMD pure phenotype, 4 without brain involvement; 1 with mental retardation, epilepsy, cortical dysplasia and cerebellar cysts. POMGnT1-mutated patients showed moderate to severe myopathy, mental impairment, and, on MRI, white matter alterations with polymicrogyria, hypoplasia of the corpus callosum or cerebellar cysts. Two POMT1-mutated patients had mild weakness and no central nervous system alterations, two had moderate myopathy, cerebellar hypoplasia and periventricular white matter changes, one had severe muscle weakness and ocular involvement. The POMT2-mutated patients had moderate mental impairment, axial and limbs weakness and minor abnormalities on MRI. The fukutin-mutated patient had hypotonia since birth, mild myopathy, but no brain involvement. Our study confirms that mutations in α-dystroglycan-related genes are associated with a wide clinical heterogeneity and that precise diagnosis in α-dystroglycanopathies remains a challenge.

Ot-Gen-11 – Muscular dystrophy in Kosovo

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If we were to compare the current data about the condition of the persons with muscular dystrophy in Kosovo with those of the last 20 years, we would notice that their economical, social, and health condition is very poor – all this resulting as a consequence of the post-war state. In the future we will continue to advice, suggest and give help to persons with muscular dystrophy in Kosovo without hesitation. While in Kosovo there are many places for employment of people with muscular dystrophy, in our society there exists a prejudice that they cannot do these kinds of jobs. The Association of Persons with Muscular Dystrophy in Kosovo residents of Prishtina, 'Ardian Krasnici' street, No. 6/22, needs financial help to begin a large campaign in order to change the current opinion of Kosovo about these people and together surpass this kind of discrimination. This by means of: (a) informing the public by media about those kinds of workplaces where people with muscular dystrophy can be employed without the risk of showing inefficiency in their job; (b) contact with written and electronic media, arranging conferences for press, seminars and tribunes, with the purpose of persuading journalists to write more on the need of eliminating prejudices in the Kosovo opinion resulting with a greater need for integration of persons with a limited abilities. This awareness of opinion can be achieved only through a massive long campaign; (c) making medial pressure to local administrative institutions and international mechanisms so they seriously consider the problem and foresee obligated quote for employment of persons with muscular dystrophy into their legislation and mechanisms in Kosovo. In its daily activities the Association is facing a great hindrance in the process of registering the new members. In Kosovo society there exists an immense prejudice about people with muscular dystrophy, remarkably in rural communities, regarding their abilities, incorporation, devotion and diseases in society. Their families refuse to accept that they have a person with muscular dystrophy or with limited abilities inside their families, so they hide and separate them. Therefore, our Association thinks that the best way to fight this negative state is to contact directly with families where persons with muscular dystrophy live, and publish their true living life. In addition, we think to achieve this by an automobile which will tour through rural communities (faraway countries). Inside of our Association board there are 4 people employed: one Albanian, which is also the president of Association, one Serbian local president of Graçanica, one Muslim, the manager of the office and one Ashkali office assistant. Volunteers include one neurologist, one internist, two pediatricians and one cardiologist. All of them are contributing a lot in our activities in the Muscular Dystrophy Association of Kosovo.

Ot-Gen-12 – TREAT-NMD training centre: applying the ENMC expertise

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The European Neuromuscular Centre (ENMC) is a European consortium funded and steered by an international group of neuromuscular patient organisations. The ENMC was founded in the late 80's with the aim to increase co-operation and interaction between international researchers and clinicians working in the area of neuromuscular diseases. The main route of enhancing communication is through funding and organising workshops for scientists and clinicians to meet and discuss current topics concerning diagnosis, care and the development of treatments for people with neuromuscular diseases. ENMC is a partner of the TREAT-NMD network. ENMC's role within the TREAT-NMD network is in line with its objective to stimulate co-operation and interaction between scientists and clinical researchers. It is responsible for the organisation of training activities like workshops and conferences for the neuromuscular community, with the aim to: (a) disseminate the achievements of the TREAT-NMD network (dissemination and implementation of Standards of Care, Outcome Measures, Patient Registries); (b) involve Central Eastern Europe (CEE) within the network and increase the level of education with CEE countries; (c) establish a uniform level of training for specialists working in the area of neuromuscular diseases by developing and implementing a Neuromyology Curriculum that is accepted at national level as well as by the learned societies. Part of the training focuses on Eastern Europe, whereby training activities have been organised on Standards of Care and Preparation for clinical trials in Hungary and Serbia. Further plans are in place to train in Romania and Macedonia on various topics related to neuromuscular diseases and the organisation of a summer school in Russia is also currently under way. A strategic plan has been developed, with systems in place for linkage with learned societies, scientific leadership, training need and trainer identification and a plan for integrate these aspects.

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