

Final programme

Workshop on overcoming bottlenecks in the development of therapeutics for rare neuromuscular disorders

President of the Workshop :
Ketty SCHWARTZ (President of AFM Scientific Advisory Board)

Génocentre - Evry - France

15-16-17 January 2007

Workshop on overcoming bottlenecks in the development of therapeutics for rare neuromuscular disorders



Format:

The program will be varied, with plenary sessions and interactive round tables. The aim of this meeting is to address some of the identified bottlenecks on the roadmap to the treatment of rare neuromuscular diseases and to drive us to the initiation of potential new projects. These could be funded by AFM and/or incorporated into TREAT-NMD.

No poster, no students

Only key speakers, experts and invited participants

:: **Monday, 15 January 2007**

8:00 - 9:00: Registration



9:00 - 9:30: Opening

Ketty SCHWARTZ President of AFM Scientific Advisory Board,
Serge BRAUN Scientific Director: aims and wishes



9:30 - 10:00: Special lecture:

Kate BUSHBY, United Kingdom
Bottlenecks in the development of treatments for NMD

Day 1 Patient registries, methodology for clinical trials



10:00 - 12:30: Session 1 / Patient registries

Key Speakers

⇒ Kevin FLANIGAN, USA

Review of US registries

⇒ Hanns LOCHMUELLER, Germany

Treat NMD approach of patient registries

Following the two lectures, short 5 min. presentations will be given by the following invited participants:
2-3 slides to describe their own experience and bottlenecks in patient registries. Interactive round table to follow.

⇒ Olivier POCH, France, Genoret database

⇒ Gregory GIMENEZ, France, Myobase : multi organism database

⇒ Louis VIOLLET, France, Neuromuscular database - Steinert database

⇒ Christophe BEROUD, France, Locus specific database

⇒ Tarek SHARSHAR, France, Myasthenia Gravis database

⇒ Anne D'ANDON, France, Pascal LAFORET, France, Pompe disease registry

⇒ Gisèle BONNE, France, Laminopathies and UMD-LMNA database

⇒ Pat FURLONG, USA, PPMD experience



11:45 - 12:30: Round table

Hanns LOCHMUELLER, Chairman



12:30 - 2:00: Lunch

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- **2:00 - 5:00: Session 2 / Methodology for clinical trials**
 - Key speakers
 - ⇒ Sharon HESTERLEE, USA
 - Translational research in clinical trials
 - ⇒ Thomas MEIER, Switzerland
 - Clinical trials for rare neuromuscular diseases – challenges & solutions
 - ⇒ Claudia HIRAWAT, USA
 - Clinical development of PTC124
 - ⇒ Jean-Louis ABITBOL, France
 - TRO19622 in SMA : clinical Plan
 - ⇒ Channa DEBRUYNE, United Kingdom
 - Clinical development of orphan drugs
- **3:30 - 5:00: Round table**
 - Sharon HESTERLEE, Chairman
 - Thomas VOIT, Chairman
- **5:00 - 5:30: Tea - Coffee break**
- **5:30 - 6:00: The experience of the clinical development of an approved orphan drug**
 - ⇒ Hamadi ZOUABI, France
 - How to get a marketed drug registered in orphan disease ?
 - ⇒ Petra KAUFMANN, USA
 - SMA Clinical trial methodology
- **6:30 - 7:00: Conclusion**
 - Hanns LOCHMÜLLER, Chairman
 - Thomas VOIT, Chairman
- **7:30: Dinner**

:: **Tuesday, 16 January 2007**

Day 2 Immunological issues, inflammation

- **9:00 - 1:00: Session 3 / Inflammation: mechanisms of inflammation whether genetically or non-genetic (i.e. infection) - induced**
Serge HERSON, Chairman
 - ⇒ Olivier BENVENISTE / Olivier BOYER, France
Clinical and pathophysiological features of muscle inflammation
 - ⇒ Robert T. LESHNER, USA
Translational programs in DC: the CINR clinical trial and murine pre-clinical groups

- **10:30 - 11:00: Tea - Coffee break**
 - ⇒ Miranda GROUNDS, Australia
Anti-TNF- therapy (Remicade, Enbrel) protects dystrophic skeletal muscle from necrosis
 - ⇒ Gordon S. LYNCH, Australia
Growth factors, growth promoting agents and anti-inflammatory approaches for improving dystrophic muscle pathology and function in mdx mice

- **1:00 - 2:30: Lunch**

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- **2:30 - 4:30: Session 4 / Immunology: how to circumvent immune response to vectors and transgenes?**
Key speakers
 - ⇒ Maria Grazia RONCAROLO, Italy
Modulation of anti-transgene specific immune response
 - ⇒ Ignacio ANEGON, France
Immunointervention in a model of AAV-mediated gene transfer into the skeletal muscle of non-human primates
 - ⇒ Shin'ichi TAKEDA, Japan
An adeno-associated virus-mediated gene transfer into canine X-linked muscular dystrophy in Japan (CXMDJ)
- **4:30 - 5:00: Tea - Coffee break**
- **5:00 - 6:30: Round table on inflammation and immunity**
David KLATZMANN, Chairman
Maria Grazia RONCAROLO, Chairman
- **7:00: Dinner**

:: Wednesday, 17 January 2007

Day 3 / Functional evaluations, imaging, delivery methods



8:30 - 10:45: Session 5 / Functional evaluation - Standardization

Key Speakers

⇒ Francesco MUNTONI, United Kingdom

General aspects of Functional evaluation in NMD patients

⇒ Gideon DREYFUSS, USA

Molecular characterization of the the SMN complex, development of assays for drug discovery and biomarkers for SMA

⇒ Carole BÉRARD, France

Evaluation of the motor function in NMD with the motor function measure. The case of the DMD

⇒ Jean Yves HOGREL, France

Assessment of muscle strength in NMD patients

⇒ Christian SCHWAKE, Germany

Evaluation of ventilatory function in Neuromuscular Disorders

⇒ Richard S. FINKEL, USA

The CHOP INTEND: a motor scale for infants with neuromuscular disease



10:45 - 11:00: Tea - Coffee break



11:00 - 12:30: Session 6 / Imaging

Key speakers

⇒ Volker STRAUB, United Kingdom

Contrast agent-enhanced MRI of skeletal muscle damage in animal models of muscular dystrophy

⇒ Marc TADIÉ / Gaelle PARADOT, France

Fiber tracking

⇒ Pierre CARLIER, France

Quantitative NMR investigations of skeletal muscle

⇒ Daniel STOCKHOLM, France

In Vivo Fluorescence Imaging of skeletal muscle : Future improvements through imaging of genetically engineered mice



12:30 - 1:00: Round table

Jean-Marie GILLIS, Chairman

François LETERRIER, Chairman



1:00 - 2:30: Lunch

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2:30 - 4:00: Session 7 / Delivery methods / Biodistribution

Key Speakers

⇒ Stéphane BLOT, France / Chris WOODDELL, USA

Hydrodynamic methods: from regional to systemic delivery in large animals

⇒ Daniel SCHERMAN, France

The potential of electroporation

⇒ Didier CAIZERGUES, France

Regulatory and toxicology issues of gene and cell therapies

⇒ Seng H. CHENG, USA

AAV-Mediated Delivery of IGF-1 to the CNS by Deep Cerebellar Nuclei Injection Improves Motor Function and Prolongs Survival in Symptomatic ALS Mice

⇒ Giulio COSSU, Italia

Cell therapy of Muscular Dystrophy: from pre-clinical animal models to clinical experimentation



4:00 - 4:30: Tea - Coffee break



4:30 - 6:00: Round table (Delivery methods / Biodistribution)

Pierre LEHN, Chairman



6:00 - 6:30: Conclusions

Michel FARDEAU, France

Ketty SCHWARTZ, France



8:00: Gala Dinner

Abstracts

Workshop on overcoming bottlenecks in the development of therapeutics for rare neuromuscular disorders

Monday, 15 January 2007

9:30 - 10:00: Special lecture:

Bottlenecks in the development of treatments for NMD

Kate Bushby

Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne NE1 3BZ. UK.

Since the cloning of the dystrophin gene 20 years ago, and the subsequent identification of the gene defects underlying the majority of the inherited neuromuscular disorders, hope of treatments which address the pathophysiology of these often devastating diseases has been high. There are many promising therapeutic strategies which are in preclinical or early clinical development, but no novel therapeutics are in clinical practice. The urgency to develop translational research in NMD has exposed the requirement for specific actions to address the areas where promising work is being held up by research bottlenecks as well as the need for a concerted effort to be more proactive in developing a clinical trials culture in the field. Tangible progress has to be made in many areas, from the standardisation of assessment of animal models, via problems in delivering promising therapeutics to muscle and modulating any possible immune response, to standardised assessment of patients and indeed their better identification through registries and databases.

This meeting, and similar workshops and symposia elsewhere in the world show the commitment of the field to move forward on these issues in a positive and collaborative way, as does the EU investment in the network of excellence TREAT-NMD. We hope that these efforts will enhance the development of a collaborative culture which has at its core a commitment to addressing the bottlenecks which threaten progress in this area and thereby advance the delivery of cutting edge treatments into the clinic.

Patient registries, methodology for clinical trials

10:00 - 12:30: Session 1 / Patient registries

Review of US Registries

Kevin M. Flanigan

University of Utah, Salt Lake City, Utah; and the Institut de Myologie, Paris

Duchenne Muscular Dystrophy presents a model of the opportunities and the challenges facing translational researchers. Modern tools for molecular diagnosis have resulted in improved genotypic characterization of patients. At the same time, potential therapies directed at specific classes of mutations are reaching the clinical trial phase. Nevertheless, the identification of large cohorts of clinically and genetically defined patients for trials is a challenge due to a variety of factors.

Examples of approaches to registries from the United States include the NIH-funded United Dystrophinopathy Project (UDP), the CDC-funded MD STARnet project, and the NIH-funded National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients. The UDP consists of

(1) a consortium of seven academic muscular dystrophy research and treatment centers who participate in a longitudinal, natural history and genotype/phenotype correlation study, and (2) a patient self-report registry, describing reported mutations and current ambulatory status. The UDP has enrolled over 700 patients, who have consented to notification of trials for which they might be candidates. In contrast, MD STARnet uses a surveillance approach in five states, tracking patients from a variety of clinical and laboratory records. This population-based approach should provide improved estimates of disease prevalence, and descriptions of access to care in different populations. Additional CDC-funded projects address family needs, and quality-of-life measures in the dystrophinopathy population. The DM/FHSD registry uses extraction of clinic records provided by self-selected patients. Each of these approaches has strengths and weaknesses which may guide the development of international patient registries.

TREAT-NMD approach of patient registries

Hanns Lochmüller

The development of therapeutics requires pre-clinical and clinical studies. Planning and running of clinical trials rely on the establishment of well-defined patients' cohorts. Several, national registries have been built in Europe, but they are fragmented and not harmonized. Innovative therapies to patients suffering from rare, neuromuscular disorders may be highly specific. In some areas, for example current approaches to DMD, the specific mutation will determine applicability or not of a particular therapeutic technique. For this reason, it is necessary that there is a European resource to identify these patients with respect to their genetic defect (gene, mutation) and clinical status. This shall be achieved by TREAT-NMD databases for DMD, SMA and various muscular dystrophies.

The main objectives are:

Establish standard procedures of data collection for the targeted disorders (SMA, DMD, muscular dystrophies) towards cutting-edge therapies using Universal Mutation Databases (UMDs)

Disseminate information on UMDs and offer specialised trainings for the personnel involved;

involve patient groups

Identify, localize and register patients with the targeted disorders using UMDs for the potential inclusion in therapeutic trials and other research applications

In the first phase of the project, the database content for each gene/disease to be included in the specific UMD will be discussed with experts. Legal, ethical and technical aspects shall be assessed prior of implementation. Patient groups will be informed and involved, and curators for data collection be trained. In the main phase of the project, curators will identify, localize and register patients with the targeted disorders using UMDs. For DMD and SMA, "national" curators will be located in Newcastle (UK, NL, Scandinavia), Montpellier (France, Spain, Belgium), Ferrara (Italy, Portugal), Munich (Germany, Austria) and Budapest (Eastern-European countries). For other genes causing various very rare forms of congenital or limb girdle muscular only 1 pan-European curator each will be assigned over the course of the network as priorities evolve.

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Genoret Database

Olivier POCH

The *Genoret Database* (HYPERLINK <http://www-genoret.u-strasbg.fr/genoret> <http://www-genoret.u-strasbg.fr/genoret>) is a specific component of EVI-GENORET <HYPERLINK "<http://en.wikipedia.org/wiki/EVI-GENORET>"<http://en.wikipedia.org/wiki/EVI-GENORET>>, the European Vision Institute functional GENomics of the RETina Integrated Project.

The aim of the Genoret Database is to centralise phenotypic, genomic and proteomic data concerning retinal diseases as well as a minimal data-type set concerning patients. This should allow implementation of standards (Clinical Report Forms, Standardized Operation Procedures (SOPs), Standardized Experimental Procedures) and permit the establishment of common information networking systems.

MyoBase: a multi organism database focusing on muscle development and muscular diseases.

David Salgado*, **Gregory Gimenez*** and Christophe Marcelle

Developmental Biology Institute of Marseille Luminy (IBDML). CNRS UMR 6216. Université de la Méditerranée. Campus de Luminy, case 907. 13288 Marseille Cedex 09. France

Muscle development and function are regulated by complex and poorly characterized networks of genes whose dysfunctions can cause severe muscular diseases in Human or in animals. To understand the mechanisms of normal and aberrant muscle development, function and repair in different organisms, researchers have generated large sets of highly diverse data. However, they are stored and scattered in several databases. Such spreading makes the comparison and the analysis across species difficult and time-consuming.

MyoBase is an informatics system where all information related to muscle will be available in one place and where data from various sources will be correlated to allow an user to easily retrieve muscle-relevant information across species.

MyoBase will integrate information from Human, Mouse, Chicken, Zebrafish, Ciona, Fly and Worm; the data originate from public databases as well as Muscular Dystrophy Associations... The centralisation and the organisation of such diverse data will facilitate the interspecies comparisons and may highlight unknown relationships.

MyoBase is currently in a developmental phase but it already contains the following information:

Genomic data from Zebrafish, Mouse, Fruitfly and Human together with a genome visualisation tool.

Gene expression patterns from Zebrafish and Mouse.

Orthologous relationships, calculated by three distinct methods, with an alignment or a tree visualisation tool.

Literature information on genes (Abstracts from Pubmed publications)

Gene ontology annotations.

Pathways and molecular function annotations for each gene

Protein domain information.

Regulation data with putative transcription factor binding sites.

In the coming months, we will integrate other types of gene expression data (e.g. microarray) and the in-

formation on the remaining species. We will link muscular diseases to the genes that are involved in them. MyoBase will centralize all relevant data for the study of muscles; we hope that it will become an invaluable tool for the “Muscle community” to orientate researchers during their analyses.

* these authors contributed equally to this work

« Network on Neuromuscular Disorders »

A computerized interactive database for the french Neuromuscular Reference Centers

Christophe BEROUD¹, Louis VIOLLET²

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Comité de Pilotage du Projet « Network on Neuromuscular disorders » Centre de Référence Neuromusculaire GNMH. Hôpital Necker Enfants Malades, 149 rue de Sèvres 75015 Paris, France

Neuromuscular disorders are characterized by the dysfunction of the motoneuron-nerve-muscle system. More than 200 known diseases belong to this group, affecting muscle and/or the peripheral nervous tractus and/or the neuromuscular junction, with an adult or childhood onset. Because of the large diversity of the phenotypes and the lack of exhaustive epidemiological data, the real incidence of neuromuscular disorders is not well established. Diagnosis and management of patients is usually made in one of the Neuromuscular Reference Centers dispatched in the whole territory. Nine Neuromuscular Reference Centers have been recently created since 2005 in France. The aim of the project is to create a logistical network for research in the field of neuromuscular disorders . All Reference Centers are participating in this project and are all in the process of organizing themselves at the present time. A pilot-committee, composed by 2 representants of each Reference Centers has been created for the development of this project

The objectives of the project are :

1-To set up a unique clinical data base using a secured web server for the everyday clinical management of the patients throughout France. This « core » database will contain administrative, genetic, medical and social data for each new patient, obtained after informed consent. Data will be entered by the clinician himself during the medical visit and will be updated at each re-visit. Data will be protected according to the legal rules (CNIL), and data exchange between each Reference Center will be possible under pre-established rules. Administrative and social items were defined according to the INVS (Institut National de Veille Sanitaire) instructions. Data extraction will be made after decision of the pilot committee upon request.

2-To set up satellite data bases, « disease-specific ». Because of the large heterogeneity of neuromuscular disorders, many items are disease-specific and could not be collected in the « core data base ». For each disease or group of diseases (ex : myotonic dystrophies, dystrophinopathies, spinal muscular atrophies, etc), specific databases will be developed, containing informations like the accurate features of the phenotype, the histological, biochemical and genetical findings, the description of the natural history of the disease, etc. These informations will allow to initiate disease specific clinical research projects, like genetic/phenotypic correlations. Of course, all these « disease-specific data bases » will be connected to the « core database » allowing the exchange of data.

This project of neuromuscular databases will allow an homogeneization of the clinical data collection and

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of the clinical management of patients throughout France. In addition, this database will be a powerful tool for clinical research project and for multicentric therapeutic trials on neuromuscular disorders.

Locus specific databases

Christophe Bérout 1, 2, 3, Dalil Hamroun^{1, 3}, Véronique Humbertclaude^{1, 3}, Sylvie Tuffery-Giraud^{2, 3}, Gwenaëlle Collod-Bérout³, Mireille Claustres^{1, 2, 3}

1 : CHU Montpellier, Laboratoire de Génétique Moléculaire, hôpital Arnaud de Villeneuve, Montpellier, F-34000, France;

2 : Université MONTPELLIER¹, UFR de Médecine, Montpellier, F-34000, France;

3 : Inserm, U827, Montpellier, F-34000, France.

A Locus Specific DataBase (LSDB) is designed to collect mutations and associated phenotypic data from a single gene. This information is collected by experts (geneticians curators and clinician curators) currently making LSDB the databases containing the most reliable information in the field of mutations. Since the creation of the HUGO-MDI initiative we have been involved in software design and LSDB creation. We thus have developed the UMD[®] generic software to build LSDB. This product includes many sophisticated analysis tools that make it a unique system recommended by the HUGO and HGVS. The UMD[®] software is widely used (more than 650 users from 50 countries). Fruitful collaborations with experts in the field of neuromuscular diseases and supports from the AFM led to the creation of the UMD-LMNA, UMD-EMD, UMD-SGCA, UMD-SGCG, UMD-FKRP, UMD-CAPN3, UMD-ZMPS-TE24, UMD-DYSF, UMD-DMD and the UMD-LAMA2. Recently the UMD tool has been chosen by the "Translational Research in Europe - Assessment and Treatment of Neuromuscular Diseases" (TREAT-NMD) network of excellence to build the UMD-DMD and UMD-SMA European databases. Our experience in this field allowed us to identify few bottlenecks: a problematical access to phenotypic information, difficulties to find permanent funding and surprisingly a lack of recognition of the new professions born from this new field of the genetics: clinician curators and geneticians curators.

Myasthenia Gravis database

Tarek SHARSHAR

The Association Française contre les Myopathies (AFM) is currently financing a national register of patients with myasthenia gravis. The main objective of this register is to record the relevant clinical and biological data in order to improve caring of the patients but also to foster research by identifying predictive factors of outcome and response to medical or surgical (thymectomy) treatment. For instance this register has been useful for developing a therapeutic trial on intravenous Immunoglobulin sparing effect of steroids. Informed consent from the patient is required. Since 2003, about 600 patients have been included in 12 different centers. However, most of patients have been included in three teaching hospitals. A partnership is near to be settled down between the AFM and the AP-HP in order to help physicians recruiting the patients. Computerization of the register is also on the way.

The French Pompe Registry (Dr Pascal Laforêt and French Pompe disease Group)

A French registry has been recently developed with a financial support of AFM in order to collect prospective clinical data from patients affected with late-onset Pompe disease. The aims of this registry are the following: 1) To collect epidemiological data; 2) To improve the knowledge and to precise the natural history of late-onset Pompe disease with regular standardized evaluations; 3) To evaluate the benefit and safety of treatments, in particular for enzyme replacement therapy (Myozyme) which are currently assessed. This registry has the advantage to be independent from industry, and has been financed by AFG (Association Francophone des Glycogénoses). The data base is harbored in a secure web site: [HYPERLINK "https://eventa.kikamedical.com/maltase/"](https://eventa.kikamedical.com/maltase/) <https://eventa.kikamedical.com/maltase/> (Kika Medical society), and each French neuromuscular center will have access to it. Epidemiological and clinical data from 50 adult patients (approximately half of the estimated French Late-Onset Pompe patients) have already been collected in this database. The first results are currently analyzed thanks to a tight collaboration with the neuromuscular centers and specialized consultations of Angers, Bordeaux, Brest, Garches, Lille, Marseille, Nice, Nîmes, Paris, Rennes, and Saint-Etienne.

Laminopathies and UMD –LMNA database

Rabah Ben Yaou¹, Khadija Chikaoui¹, Dalil Hamroun², Christophe Bérourd², France Leturcq³, **Gisèle Bonne**¹ and the French & European networks on EDMD and other nuclear envelopathies.

1. Inserm U582, Institut de Myologie, GH Pitié-Salpêtrière, Paris, France

2. CNRS UPR 1142 IGH/IURC, Montpellier, France

3. AP-HP, laboratoire de biochimie génétique, Hôpital Cochin, Paris, France

Laminopathies are a group of disorders (up to ten different) due to mutations in the LMNA gene encoding A-type lamins (lamins A and C), proteins of the nuclear envelope. Up to now, 264 mutations of the lamin A/C gene in 1209 individuals have been identified. Faced with this very wide diversity, we have established an UMD-LMNA database which brings together all the clinical and genetic data concerning the mutations described by our networks as well as those reported in the literature ([HYPERLINK "http://www.umd.be"](http://www.umd.be) <http://www.umd.be:2000>). This mutation database is now a useful tool for analysing phenotype/genotype relation in this complex group of disorders.

2:00 - 5:00: Session 2 / Methodology for clinical trials

Clinical trials for rare neuromuscular diseases – challenges & solutions

Thomas Meier Ph.D. (CSO)

Santhera Pharmaceuticals, Switzerland, ([HYPERLINK "http://www.santhera.com"](http://www.santhera.com) www.santhera.com)

Santhera Pharmaceuticals (SWX: SANN) is a Swiss specialty pharmaceutical company focusing on the discovery, development and marketing of small molecule pharmaceutical products for the treatment of severe neuromuscular diseases. Santhera's vision is to become a leading specialty pharmaceutical company offering therapies for a number of indications in this area of high unmet medical need which includes many orphan indications with no current therapy.

Santhera currently has four clinical-stage development programs both in the US and Europe, three of which are investigating its lead compound, SNT-MC17/idebenone, in the treatment of Friedreich's Ataxia

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(FRDA), Duchenne Muscular Dystrophy (DMD) and Leber's Hereditary Optic Neuropathy (LHON). The fourth clinical program is investigating JP-1730/fipamezole for the treatment of Dyskinesia in Parkinson's Disease (DPD) in cooperation with Juvantia.

"Bottlenecks" in the planning and conduct of clinical trials with rare NMDs include but are not limited to uncertainties on assumptions of actual patient numbers, difficulties to enroll pediatric patients into placebo-controlled trials, the validation of endpoints and the paucity of appropriate data on disease progression. Strategies to overcome such bottlenecks and hurdles will be illustrated based on Santhera's experience from its ongoing Phase II and Phase III programs in NMDs in Europe and the US.

TRO19622 in SMA: Clinical Plan

J-L Abitbol, T. Bordet, R.M. Pruss. Trophos, Parc Scientifique de Luminy, 13288 Marseille, France

A 45,000 compound library was screened for the ability of small molecular weight compounds (<500) to rescue purified rat motoneurons from death due to trophic factor deprivation. This phenotypic screening with primary neuron survival as an endpoint aims at avoiding the pitfalls of target-based drug discovery in Motor Neuron Diseases.

TRO19622 was selected for its ability to rescue nearly 100% of rat motoneurons deprived of trophic factors. It also provides significant protection to primary striatal, cortical, hippocampal and cerebellar granule neurons in a number of models of neuronal cell death with an EC₅₀ of ~1-5 μ M. In vivo, TRO19622 rescues facial motoneurons from axotomy-induced death in neonatal rats (30 mg/kg/day, po), accelerates nerve regeneration following nerve crush in adult mice (3-30 mg/kg/day, sc), increases survival in a mouse model of SMA (NSE-Cre;F7/F7 – Judith Melki) (30 mg/kg/day, sc) and increases survival, maintains weight and motor function in SOD1G93A mice (3 and 30 mg/kg/day). TRO19622 binds to the cholesterol site on PBR and to VDAC. The molecule is orally bioavailable, crosses the blood brain barrier and has a large safety margin.

Phase 1 single and multiple dose tolerance studies, drug-drug interactions with riluzole, phase 1b in ALS are completed and achieved target exposure for efficacy with good safety. Phase 2/3 trials in ALS will commence in 2007.

Due to the excellent safety of TRO19622 in adults, a pharmacokinetic study in 6-25 year old patients with SMA type 1b to 3 followed by a 3-month Phase 1b safety study at the target therapeutic dose will start in 2007 in preparation for a phase 2 efficacy and safety study.

Issues regarding the SMA clinical plan, need and difficulties of obtaining PK data in children, main efficacy assessment criteria and sample size calculation, duration of trial, investigative sites for phase 2 will be addressed.

TRO19622 development in SMA and ALS is funded by Association Française contre les Myopathies.

5:30 - 6:00: Session 2 / The experience of the clinical development of an approved orphan drug

How to get a marketed drug registered in orphan disease?

Hamadi Zouabi MD

Pierre Fabre Médicament
Boulogne, France

High dose systemic chemotherapy or chemoradiotherapy followed by allogeneic or autologous haematopoietic stem cell transplantation (HSCT) can be very effective for the treatment of malignant and non-malignant haematological diseases or malignant solid tumours. As the number of HSCT in Europe is no more than 5/10 000 persons in the community, the development of products in this indication can be considered for Orphan drugs.

Busulfan (Bu) is an alkylating agent with myeloablative properties which is part of key conditioning regimens before HSCT. The use of oral Bu, administered at high doses (i.e. 1 mg/kg x 16 doses over 4 days) for the preparative treatment before HSCT in the paediatric population is current practice. However Bu is registered for HSCT in only 3 European countries (Austria, France, and Spain).

Until recently, only an oral form of Bu was available, requiring that the patient swallows a large number of capsules. The main problem with the optimal utilisation of busulfan is the bioavailability of the oral form: its pharmacokinetic (PK) variability between patients is high and Bu concentration has been correlated with graft rejection and disease relapse, as well as with the severity of conditioning regimen toxicities.

Consequently, it should be expected from an I.V. form of Bu (Busilvex®), which contains the active substance of Bu, to reduce these issues. Moreover, it should be taken into account that the use of Busilvex® in children would be particularly interesting because of the toxicity associated with total body irradiation, the difficulties related to oral medications and the favourable outcome of transplant in this group. As the number of HSCT in Europe is in accordance with the definition of prevalence of the condition (HSCT) Busilvex® was designated an orphan medicinal product on December 2000.

In agreement with the EMEA/CPMP a full dossier concerning the use of Busilvex® in adult patients was submitted and a marketing authorisation was granted in the EU on July 2003.

In order to translate in children the advantages offered by Busilvex® to adults, Pierre Fabre Médicament designed and implemented (December 2001) a clinical trial in this population. A variation application for Busilvex® in paediatrics was submitted on December 2004.

The EMEA experts concluded that the rationale of orphan development program performed by Pierre Fabre Médicament for Busilvex® represents a clinically relevant step forward in the conditioning treatment of children.

SMA CLINICAL TRIAL METHODOLOGY

Petra Kaufmann, M.D., M.Sc.

Dept. of Neurology, Columbia University, New York, NY, USA

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these drugs are now candidates for SMA clinical trials based on (1) the availability of a suitable formulation, (2) preclinical safety data including testing in juvenile animals, (3) human trial safety data, (4) bioavailability and CNS penetrance, and (5) strength of the in vitro efficacy data supporting use in SMA. Because the number of potential SMA clinical trial participants is limited and because resources are finite, the current situation represents a “bottleneck” in the evaluation of new SMA treatments at a time when several experimental compounds await clinical investigation

The following approaches may help to overcome the “bottleneck”:

Patient education on clinical trials and collaboration between investigators and patient groups can help to increase the proportion of SMA patients enrolled in clinical trials.

Collaboration between investigator groups can expedite recruitment.

Creating a data repository with placebo group data elements common across trials can improve the design of future trials.

Optimizing the dose regimen for the SMA population through Phase I studies can increase the likelihood for success in subsequent efficacy trials.

Efficient Phase II clinical trial designs can play a strategically important role in SMA drug development.

These innovative trial designs include futility trials and drug selection designs.

In summary, given that there are a number of potential treatments for SMA based on in vitro studies, and given the relative rarity of the disease and thus the limited patient pool, trial efficiency, timely recruitment and investigator collaboration are important in SMA clinical trials.

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Immunological issues, inflammation

9:00 - 1:00: Session 3 / Inflammation: mechanisms of inflammation whether genetically or non-genetic (i.e. infection) - induced

Clinical and pathophysiological features of muscle inflammation

Olivier Benveniste : Service de Médecine Interne 1, Centre de référence des pathologies neuromusculaires Paris Est, Hôpital Pitié-Salpêtrière, Paris, France

Olivier Boyer : Laboratoire d'Immunologie Inserm U. 519, Faculté de Médecine et de Pharmacie, Rouen, France.

Scope: actuality on new classifications of myositis linked with their different physiopathogenesis and introduction to the different sessions of the day.

Current situation and salient points: Many situations may induce inflammation in muscle. This is e.g. a common feature of certain dystrophies such as dysferlinopathies and it should also be a problem during gene therapy procedure because of the immune reaction against new proteins and/or vectors. Nevertheless, the main cause remains idiopathic inflammatory myopathies. The classification of myositis improves recently, taking into account clinical (such as isolated involvement of muscle or not, association with cancer...), immunological (presence or not of auto-antibodies) and pathological criteria. This new classification has the ability to isolate different clinical and physiopathological entities, with finally, also different

prognosis factors. Quoting the most frequent, these myopathies consist of dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM), but also, overlap myositis (defined, among others, by the presence of auto-antibodies), myositis associated to cancers etc... Among these myopathies, they may be distinguished either by their histological features which also reflect their different underlying pathogeneses. The mechanism of DM is complement-mediated microangiopathy, the inflammatory infiltrate is secondary to ischaemic damage. In PM the muscle fibres are damaged by cytotoxic CD8 T lymphocytes. IBM may be a degenerative disease with accumulation of a variety of proteins within the fibres. The inflammatory infiltrate, which is similar to that seen in PM, may be a reaction to accumulated proteins. Perspectives: these diseases with so different physiopathogenesis and prognosis should be treated by specific approaches. That is the reason why 1) we developed animal models and 2) we started specific clinical trials for respectively inclusion body myositis and overlap myositis.

Clinical and Preclinical Trials in Muscular Dystrophy

The Center for Genetic Medicine of the Children's National Medical Center (CNMC)

Robert T .Leshner MD

Washington, DC

CNMC is the coordinating site of the Cooperative International Neuromuscular Research Group (CINRG). The CINRG network consists of 22 academic centers dedicated to performing clinical trials in Duchenne Muscular Dystrophy (DMD) and other neuromuscular diseases. The Center for Genetic Medicine has been designated one of 6 Wellstone Centers established by the MD Care Act of 2001 and fosters collaboration between basic scientists and clinicians with the goal of rapidly translating potential therapies from the bench to human clinical studies. Clinical trials requiring recruitment of large numbers of patients are facilitated through the CINRG network. Skilled clinical evaluators at each of the CINRG sites are given repeated, rigorous training to ensure that outcome measures of strength and function are reliable and reproducible among sites. Clinical trials have addressed potential “downstream targets” of the muscle pathophysiology of DMD. The CINRG network is poised for anticipated clinical trials of gene therapy and gene rescue. Lessons learned from our initial trials will be discussed. We welcome potential collaboration with other neuromuscular investigators.

The CNMC mouse core conducts preclinical drug trials in murine models of muscular dystrophy. Colonies of mice including, mdx, mdx/nude, “limb girdle dystrophy” models, Emery-Dreifuss and inflammatory myopathy animals are maintained in a pathogen free environment, the laboratory is a comprehensive drug testing facility capable of comprehensive clinical, functional and behavioral data collection. Muscle tissue analysis of biochemical, histological and immunological parameters are performed in the Hoffman laboratory. Sophisticated cardiac imaging with ultra high frequency echocardiography supplements skeletal and cardiac muscle morphologic assessment. The laboratory is currently conducting trials of 8 drugs with support to launch several additional trials this year.

Anti-TNF α therapy (Remicade, Enbrel) protects dystrophic skeletal muscle from necrosis

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Dystrophic myofibres of Duchenne Muscular Dystrophy (DMD) boys have defective dystroglycan complexes and are susceptible to sarcolemma damage. Little is known about the balance between the repair of minor damage and the alternative fate of myofibre necrosis. We propose that inflammatory cytokines and cells can increase initial sarcolemmal damage and exacerbate necrosis of dystrophic myofibres. Using the mdx mouse model of DMD we have shown reduced necrosis of dystrophic muscles *in vivo* using highly specific drugs to silence TNF α ; specifically using TNF α antibodies (human Remicade/Infliximab and mouse-specific cV1q) or soluble TNF α receptors (Entercept/Enbrel). Both Remicade and Enbrel are in wide clinical use to treat inflammatory diseases such as rheumatoid arthritis and Crohn's disease. New data using cV1q in mdx mice will be presented. We have shown similar protection using a range of other anti-inflammatory interventions including the depletion of neutrophils, the blocking of mast cell degranulation (Cromolyn), the blocking of complement C5a, and exposure to the corticosteroid prednisolone. The protective effect of these treatments on myofibre necrosis was demonstrated using both adult mdx mice where the relatively low level of muscle damage is increased in response to voluntary exercise, and young mdx mice at the time of acute onset of necrosis. These data support an important role for inflammation in exacerbation of muscular dystrophy and suggest new drug interventions to reduce the clinical severity of DMD. Additional studies show that over-expression of Class 1:Ea IGF-1 specifically within dystrophic myofibres in mdx/mIGF-1 mice reduces necrosis. It appears that TNF α can block IGF-1 signaling. Understanding the interacting signaling pathways of TNF α and IGF-1 is a central focus of our ongoing research.

(For publications see [HYPERLINK "http://school.anhb.uwa.edu.au/personalpages/grounds/"](http://school.anhb.uwa.edu.au/personalpages/grounds/) \n <http://school.anhb.uwa.edu.au/personalpages/grounds/>)

Growth factors, growth promoting agents and anti-inflammatory approaches for improving dystrophic muscle pathology and function in mdx mice

Gordon S. Lynch, Jonathan D. Schertzer, Stefan M. Gehrig, Chris van der Poel & James G. Ryall.
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Although considerable progress has been achieved with gene therapies for rare neuromuscular disorders, there remains a profound need for alternative therapeutic strategies that can improve muscle mass and strength, ameliorate the dystrophic pathology, and enhance patient quality of life. Successful pharmacological approaches may enable patients to survive and thus take advantage of gene therapies when they eventually become available.

Whether a therapy is genetic or pharmacologic, the aim is to restore, maintain, or improve muscle size and strength. From a physiologist's perspective, slowing the loss of muscle tissue will preserve muscle function. Restoring or increasing muscle fibre size and/or number will optimise the potential for improving muscle function. Preventing fibrotic infiltration could increase the potential for successful fiber regeneration and the restoration of function. Exogenous administration of growth factors and growth promoting agents, such as insulin-like-growth factor-I (IGF-I), interleukin-15 (IL-15) and beta-adrenoceptor agonists (e.g. formoterol), have demonstrated therapeutic potential for improving dystrophic muscle function in mdx mice.¹⁻³ However, the optimal doses and timing of the administration of these (or similar)

agents alone or in combination with anti-inflammatory agents have yet to be perfected.

The ultimate efficacy of any novel single or combination treatment strategy must be determined from careful pre-clinical examination of a number of complementary physiological, biochemical, and histological parameters. Physiological assessments are important if one is to gauge the true impact of a therapy on the activities required by the patient for daily living. Understandably, this is not a simple matter, since accurate measurements of force and power output from the limb or diaphragm muscles of dystrophic mice requires considerable experience and expertise. Similarly, accurate assessment of contraction-mediated damage to dystrophic skeletal muscles, particularly muscle fibre susceptibility before and after treatment, provides important information about the efficacy of interventions that could ultimately protect muscles from injury and ameliorate the dystrophic pathology. Including careful analyses of such functional parameters should help to overcome current bottlenecks in the development of effective therapeutic strategies for skeletal muscle diseases.

Gregorevic P, Plant DR, Leeding KS, Bach LA, Lynch GS. Improved contractile function of the mdx dystrophic mouse diaphragm muscle following IGF-I administration. *Am J Pathol* 2002;161:2263-2272.

Harcourt LJ, Holmes AG, Gregorevic P, Schertzer JD, Stupka N, Plant DR, Lynch GS. Interleukin-15 administration improves diaphragm muscle pathology and function in dystrophic mdx mice. *Am J Pathol* 2005;166:1131-1141.

Harcourt LJ, Schertzer JD, Ryall JG, Lynch GS. Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. *Neuromuscul Disord* 2006 (in press: Nov 27).

2:30 - 4:30: Session 4 / Immunology: how to circumvent immune response to vectors and transgenes?

Modulation of anti-transgene specific immune response

Andrea Annoni, Manuela Battaglia, Brian Brown, Ehud Hauben, Angelo Lombardo, Luigi Naldini, **Maria Grazia Roncarolo**.

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) Via Olgettina, 58- I- 20132 Milano

Successful gene therapy is often limited by the immune response to the transgene products. Induction of transgene-specific tolerance is crucial for the success of gene therapy trials in immunocompetent patients. Tolerance can be mediated by two major mechanisms: central tolerance with clonal deletion of Ag-specific T cells, and peripheral tolerance mediated by regulatory T cells (Tregs). Peripheral tolerance can be achieved through expansion of natural CD4+CD25+FoxP3+ Tregs, induction of adaptive Ag-specific type 1 T regulatory (Tr1) cells, or manipulation of antigen presenting cells (APC), including dendritic cells (DC), to render them tolerogenic. The ability of Tregs to induce and maintain tolerance to alloantigens, allergens, food antigens, or pathogens has been demonstrated in several pre-clinical models. However, it remains to be determined whether Tregs can inhibit anti-transgene immune responses.

Due to their ability to transduce non-dividing cells and stably integrate, lentiviral vectors (LV) are a candidate system for therapeutic gene transfer in a number of genetic diseases. However, the use of LV may be hampered by their ability to trigger innate and adaptive immune responses.

Indeed, we recently demonstrated that LV can activate a subset of plasmacytoid DC that in turn can activate other subset of DC and trigger an adaptive immune response. Furthermore, we developed a model in

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which LV-GFP injection in C57BL/6 mice induces antigen-specific cellular and humoral immune responses. GFP specific cytotoxic CD8+ T-cells and antibodies generated in these mice lead to clearance of transgene-expressing cells. Co-injection of LV-GFP and CD4+CD25+ Tregs cells from wt or from GFP-transgenic (tg) mice did not prevent the immune response to the transgene. Conversely, transfer of GFP Tg APC, but not of wt APC, reduced GFP-clearance and down-modulated the expansion of GFP-specific CD8+ T cells. Therefore, anti-transgene immune responses can be modulated by high and persistent expression of the transgene in the APC. Alternatively, a complete de-targeting of APC using lentiviral vector, comprising target sequences for micro-RNA specifically expressed in hematopoietic cell lineage, results in persistent transgene expression. Further studies are ongoing to dissect and modulate the transgene-specific immune responses in these models.

An adeno-associated virus-mediated gene transfer into canine X-linked muscular dystrophy in Japan (CXMDJ)

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A recombinant adeno-associated virus (AAV)-mediated gene transfer is one of the attractive approaches to the treatment of Duchenne muscular dystrophy (DMD). It is important to examine therapeutic effects and the safety issue of the approach in larger animal models, such as dystrophic dogs. We recently established a Beagle-based dystrophic dog colony in Japan, CXMDJ (Exp Anim, 52: 93-97, 2003) and the dogs show similar symptoms seen in DMD (Acta Myologica, XXIV: 145-154, 2005; BMC Cardiovasc Disord. 2006;6:47). First, we injected a recombinant AAV2 encoding the LacZ gene into skeletal muscles of normal Beagles. β -galactosidase (β -gal) was expressed only in a few fibers, but instead marked cellular infiltration was appeared. Immunosuppressive treatment improved β -gal expression, though the effect was not complete. We, therefore, generated a type 8 recombinant AAV (AAV8) encoding the LacZ gene. Recombinant AAV8 encoding the LacZ gene driven by a CMV promoter was injected into tibialis anterior and extensor carpi ulnaris of normal Beagles. We found more β -gal positive fibers in AAV8-injected canine skeletal muscle than those in AAV2-injected muscle. Moreover, cellular infiltration in AAV8-injected muscle was much less than the AAV2-injected muscles. We also performed the in vitro interferon- γ release assay against β -gal, AAV2 or AAV8 particle utilizing canine peripheral blood mononuclear cells and IFN- γ secretion against AAV8 showed lower response than that against AAV2. We, therefore, injected recombinant AAV8 encoding canine micro-dystrophin (c Δ CS1) gene into skeletal muscle of CXMDJ skeletal muscle and confirmed the expression of micro-dystrophin. The injection of recombinant AAV8 encoding c Δ CS1 through limb perfusion or systemic injection would be favorable in dystrophic dog. Recently, we introduced Morpholino antisense oligonucleotides (AO) to induce exon skipping of the mutated canine DMD gene in CXMDJ, in collaboration with Dr. Eric Hoffman's research group (Yokota T, Partridge TA, and Lu QL). Results of on-going experiments in CXMDJ can be presented at the meeting.

Wednesday, 17 January 2007

Functional evaluations, imaging, delivery methods

8:30 - 10:45: Session 5 / Functional evaluation - Standardization

General aspects of functional evaluation in NMD patients

Francesco Muntoni,

Hammersmith Hospital, Imperial College, London UK.

Neuromuscular disorders in children are a heterogeneous group of conditions which result in muscle weakness, typically affecting the proximal and axial muscles more severely than distal muscles. Maximal functional abilities vary, some children never acquiring the ability to walk. Progression of weakness is also variable: rapidly progressive in some, slowly or non-progressive in others. This heterogeneity coupled with the variable degrees of ability to collaborate and the fatigability which characterises the weakest children is a challenge to the development of robust assessment tools to measure strength and disease progression. In addition, differences in management strategies and standard of cares between different centres, represent additional aspects that need to be considered especially when designing multicenter trials. Finally, there is increasing recognition of the limitations of measuring strength in isolation, not only because of the complexity of determining muscle strength and its change in severely weak children, but also because functional outcomes can be appreciated more readily by patients and physicians. Bearing all these issues in mind, our group has been involved in the organisation of clinical networks aimed at developing, validating and implementing functional scales to monitor disease progression of ambulant children with DMD and, more recently, for non ambulant SMA children. Seventeen specialist centres across UK have collaborated for a period of 3 years and data on more than 400 ambulant DMD boys regularly followed up by these centres using an agreed and standardised protocol have been gathered. The protocol was developed specifically with the aim to facilitate multicentre clinical audit and trials; a similar assessment tool has also been developed for non ambulant SMA children and already used in clinical trials, and is currently undergoing further modifications to take into account some of the limitations. The limitations and strengths of these functional assessment tools will be discussed.

Acknowledgements.

The help of Elaine Scott and Anna Mayhew, Coordinators of the UK North Star and SMARTnet Networks for DMD and SMA (funded by MDC and Jennifer Trust) is gratefully acknowledged.

Molecular characterization of the the SMN complex, development of assays for drug discovery and biomarkers for SMA

Lili Wan, Daniel Battle, Jeongsik Yong, Francesco Lotti, Mumtaz Kasim, Stephen Kolb, Terrence Lau, John Mouaikel, Francesco Niola, Congli Wang, Liz Ottinger, Ihab Younis, Tina Glisovic, Zhengxi Zhang and **Gideon Dreyfuss**

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The SMN complex, comprised of SMN and the Gemins (2-8), is a central orchestrator of cellular RNA metabolism. The SMN complex is a molecular machine that is essential in all cells and functions in the biogenesis of spliceosomal small nuclear ribonucleoproteins (snRNPs, the major components of the cell's mRNA splicing machinery) and likely other RNPs. Reduction in functional SMN causes spinal muscular atrophy (SMA). The activity of the SMN complex can be recapitulated in vitro. To study the molecular mechanism and regulation of the SMN complex, we developed a sensitive and quantitative high throughput in vitro assay that measures the formation of snRNPs. The assay reveals a measurable biochemical deficiency in the capacity of SMA cells to form snRNPs. Future progress towards finding a therapy for SMA will likely come from detailed understanding of the function, expression and regulation of the SMN complex, and from direct screens for small molecules that affect these processes. In practical terms, several approaches can be envisioned as potential therapeutic approaches for SMA. Two, in particular, that we consider, based on current knowledge, to be most direct and have focused on are screens for small molecules that increase the amount of SMN in cells or the activity (the downstream products) of the SMN complex. Towards this, we developed high throughput assays that allow for the screening and characterization of such molecules. In addition, we developed assays to measure SMN protein levels and SMN complex activity in blood samples, which can be used as surrogate assays (biomarkers) to monitor efficacy of treatment patients.

EVALUATION OF THE MOTOR FUNCTION IN NEUROMUSCULAR DISEASES WITH THE MOTOR FUNCTION MEASURE. THE CASE OF THE DUCHENNE MUSCULAR DYSTROPHY.

C Bérard MD and the MFM collaborative study group.

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The Motor Function Measure scale (MFM) was validated for the evaluation of patients, 6 to 60 years of age, with neuromuscular disorders: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, limb-girdle muscular dystrophy, facio-scapulo-humeral dystrophy, myotonic dystrophy, congenital myopathy, congenital muscular dystrophy, spinal muscular atrophy and hereditary neuropathy. The scale structure has three dimensions: D1: standing position and transfers, D2: axial and proximal motor function, D3: distal motor function. The scale comprises 32 items, with ratings ranging from 0=no movement to 3=complete movement and tests a variety of movements (in standing and sitting positions). The cotations of the items are described very precisely in a user's manual. Sensitivity to change was tested among 152 patients.

This scale is reliable, lasts an average of 36', does not require any special equipment and is well-accepted by patients.

The scale was used to evaluate the evolution of 41 DMD patients over 6 years of age. In a one-year period, all the scores showed significant differences with a decrease of: D1 4.9% [- 9.9; 0.2], D2 7.7%

[-12.4; - 2.9], D3 4.3% [- 9.5; 1.0] and Total Score 5.8% [- 9.1; - 2.4]. For the 11 ambulant patients at the beginning of the study, the average value of D1 annual decrease was 26.1%. For the non-ambulant patients, the annual average decrease was 11.8% for D2 and 6.3% for Total Score. Over the year, a few patients showed stable or even improved results in each dimension and for the total score. An average curve of the motor function degradation in relation to age may be constructed. The MFM was sensitive for a one-year follow-up of DMD patients. A sensitive threshold value for the loss of the ability to walk and a predictive value a year before loss of ambulation may be estimated. Correlations between phenotypes and genotypes as well as the determination of beneficial effects of future therapeutics will therefore be facilitated. Having a unique scale covering all the evolution of the disease is an advantage.

Acknowledgements

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References

Bérard C, Payan C, Hodgkinson I, Fermanian J, and the MFM collaborative study group : A motor function

measure scale for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord* 2005;15 : 463-70.

Bérard C, Payan C, Fermanian J, Girardot F ad the MFM collaborative study group . The Motor Function Measure, a tool of clinical evaluation for neuromuscular diseases. Validation study. (in French). *Rev Neurol* 2006;162:485-93.

Motor Function Measure : user's manual. MFM study group AFM editor, Evry France, 2006.

Assessment of muscle strength in NMD patients

Jean-Yves Hogrel

Institut de Myologie - Paris - France

Any pathology involving the neuromuscular system can be longitudinally investigated with one or several methods to follow degenerative effects on muscle strength during the natural history of the disease or to detect small changes during therapeutic trials. This is mainly critical in the latter case for diverse ethical, scientific and economical reasons. Methodological issues are fundamental because the patients may present different motor capacities, the changes in strength may be fairly small over the duration of the trial, and different evaluators may be involved in different clinical centres.

Several evaluation tools for assessing muscle strength are available. There are usually classified into two groups: manual muscle testing (MMT) methods and quantified muscle testing (QMT) methods. The presentation will quickly present the principles of strength measurements, and the different tools and materials that are commonly used in clinical settings. Their limitations and drawbacks will be illustrated through several examples. No consensus exists on the various methods to use. No methods are perfect or ideal yet and none will never probably be. Beyond the evaluation method itself, strength measurement is influenced by many technical, methodological, environmental and human factors. The challenge is to provide each clinical trial with appropriate, standardized, reliable and sensitive outcome measurements. Specific technical developments may be useful to optimize strength measurement of the muscle function, which has to be assessed.

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Since therapeutic trials may concern rare disorders, multiple centres are often involved to reach the required statistical power to demonstrate treatment efficacy. Thus, it is fundamental that all the centres use the same methodological procedures to assess the outcome measures. This is true for strength but also for all the evaluation criteria chosen. Rigorous training and monitoring are required before and during any therapeutic trial in order to not compromise the quality of its results.

Evaluation of ventilatory function in Neuromuscular Disorders

Christian Dohna-Schwake,

Dpt. of Pediatrics, University Hospital of Essen, Germany

Compromised ventilatory function determines a major part of quality of life, morbidity and mortality in patients with neuromuscular disorders (NMD). An accurate assessment of the individuals' ventilatory function is part of each clinical evaluation, a measure in clinical trials and can predict or detect respiratory complications such as sleep disordered breathing, hypoventilation and respiratory tract infections. Widely used methods are clinical examination, spirometry, peak cough flow, respiratory muscle function, blood gases and sleep studies (pulse oximetry, polysomnography). Recommendations for the routine use of these methods are: 1) easy to perform and interpret, 2) reliable, 3) highly predictive for respiratory complications. The most important role in ventilatory assessment plays spirometry: 1) It is easy to perform and interpret; 2) a decrease of the Vital Capacity (VC) from erect to supine reveals diaphragm weakness; 3) a Vital Capacity of 40% predicts the occurrence of nocturnal hypoventilation with a sensitivity of 88% and a specificity of 96%; 4) VC highly correlates with Peak Cough Flow (PCF), which is a measure of the ability to clear airway secretions. The routine evaluation of the ventilatory function should be done as part of a diagnostic algorithm with spirometry as the first step (as discussed in the presentation). Other methods of ventilatory evaluation such as diaphragm EMG, esophageal and gastric pressures, multiple breath wash-out techniques, static and dynamic compliance are not part of routine evaluation but of scientific value.

The CHOP INTEND: a motor scale for infants with neuromuscular disease

RS Finkel, AM Glanzman, M Main, E Bertini, E Mercuri

Hypothesis: A reliable, valid, sensitive and well-tolerated measure of motor skills for weak infants with neuromuscular disorders is needed as an outcome measure for clinical trials.

Aim: To develop a motor scale for weak infants with neuromuscular disease, using SMA type I as the initial focus.

Methods: Most existing infant motor scales for infants with SMA-I were determined to be unsatisfactory, due either to not capturing motor function in this group effectively, not being sensitive to small change or being too lengthy and not well tolerated by the infant. We first studied the Test of Infant Motor Performance (TIMP) and compared it to a newly created test termed the CHOP TOSS (Test of Strength in SMA). Both were shown to be reliable and the TOSS was found to be better tolerated in SMA-I infants. The TOSS was then modified significantly and incorporated 4 items from the TIMP. This new test is

termed the CHOP INTEND (INfant TEst for Neuromuscular Disorders). It was designed to be useful also in congenital myopathy/dystrophy and other conditions of infancy with hypotonia/weakness. 14 elicited and 2 observational items capture neck, trunk, proximal and distal limb strength. Testing takes about ten minutes. Each item is scored on a 0 to 4 scale.

Results: Initial training sessions in Rome and Philadelphia demonstrated good interrater reliability among trained physical therapists (ICC 0.97 and 0.98). The test is well tolerated by infants with SMA-I and informally in those with congenital myopathy and Prader-Willi syndrome. Validation of this scale is currently in progress.

Conclusion: The CHOP INTEND is a reliable and well-tolerated motor scale for infants with neuromuscular disease.

A copy of the test is available at this AFM meeting or you can request it from Richard Finkel at HYPERLINK "mailto:finkel@email.chop.edu" finkel@email.chop.edu. This project has been supported by the Families of SMA (US and Italy), the SMA Foundation and The Children's Hospital of Philadelphia GCRC.

11:00 - 12:30: Session 6 / Imaging

Contrast agent-enhanced MRI of skeletal muscle damage in animal models of muscular dystrophy **Volker STRAUB,**

Institute of Human Genetics, Newcastle University, Newcastle, UK

One of the critical events in the degeneration of skeletal muscle fibres in a number of muscular dystrophies is the breakdown of the sarcolemma. It is assumed that the increase in sarcolemmal permeability occurs when either the linkage between the cytoskeleton and the extracellular matrix is disrupted or membrane repair mechanisms are defective. A better understanding of this process will enable us to develop new diagnostic and therapeutic approaches. In previous studies we were able to illustrate that damaged muscle fibres can be specifically targeted using albumin as a carrier. Using a new albumin-targeted contrast agent sarcolemmal integrity of two animal models for muscular dystrophy was studied by MRI. Both the mdx mouse and a sarcoglycan-deficient mouse model showed significant accumulation of contrast agent in skeletal muscle. These first results suggested that contrast agent enhanced MRI could serve as a common, non-invasive imaging procedure for evaluating the localization, extent, and mechanisms of skeletal muscle damage in muscular dystrophy. Subsequently we conjugated human serum albumin with Gadolinium and systemically applied this compound (Gd-DTPA-hsa) to wild type and mdx mice. We found localised signal enhancement in mdx skeletal muscle and also demonstrated an intracellular accumulation of Gd-DTPA-hsa in clusters of damaged mdx muscle fibres by immunohistochemistry. Comparison of MRI and histological data emphasised the value of contrast agent enhanced MRI for the assessment of fibre damage in muscular dystrophies. Furthermore, our data provide evidence that albumin can be used as a carrier to target conjugated molecules to degenerating muscle fibres.

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QUANTITATIVE NMR INVESTIGATIONS OF SKELETAL MUSCLE

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Modern high-performance NMR imagers can produce thousands of images per operational hour. There is a striking imbalance between our capacity to generate high-quality medical images and our incapacity to process them rapidly, thoroughly and objectively. This is THE bottleneck towards real quantitative medical imaging and it holds particularly true when dealing with muscle imaging.

First and foremost, automatic segmentation of individual muscles is needed. Muscle contour delineation is a prerequisite to make relevant most processes aiming at muscle characterization. This apparently simple task appears to be the most challenging and is clearly the limiting step today.

Standard T1-weighted images provide sufficient contrast between muscle and fat (subcutaneous, intramedullary, inter- or intra-muscular) and between muscle and cortical bone. Using highly homogenous RF coils, quantification of these compartments by simple pixel counting is trivial. Modern NMR imagers make use of arrays of surface receiver coils with quite heterogeneous spatial profile. Correction of inhomogeneity bias must be performed ideally based on actual measurement of coil responses or alternatively based on post-processing only. Then delineation of muscle volume as a whole can often be achieved

The appropriate solution for automatic segmentation of individual muscles is unknown and will be the main topic of collaborative efforts in the immediate future. All reasonable options must be considered and tested. Clearly, imaging protocols ought to be modified to provide better delineation of aponeurosis and fascia.

Limb or segment registration is mandatory in longitudinal studies. High deformability of muscle structures set an irreducible limit to the local spatial information that can be derived from registration.

Once automatic muscle segmentation will be achieved, muscle characterization tools will be implemented and refined. Texture analysis may reveal information inaccessible to visual inspection. The disorganization of muscle architecture by diseases and the frequently heterogeneous distribution of histological lesions are likely to result in patterns of voxels, whose specificity and clinical value need to be investigated.

IN VIVO Fluorescence Imaging of SKELETAL MUSCLE:

Future improvements through imaging of genetically ENGINEERED MICE

Daniel STOCKHOLM(1) , Marc BARTOLI(1), Nathalie BOURG(1), Corinne LAPLACE-BUILHE(1), and Isabelle RICHARD(1)

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New approaches, such as in vivo imaging utilizing intra-vital multi-photon microscopy, are showing a vital role in providing the pharmaceutical industry quantitative high-resolution analyses of drug activity, distribution and function. These approaches combined with genetically engineered disease model will help drug discovery and development.

The observation of cells directly in living animal confer a number of advantages. The integrity of the cells are preserved and physiological conditions can be tested. Furthermore, the possibility to follow the same animal in a longitudinal study highly increases the statistical significance of the results. However, monitoring of cells, subcellular compartments or molecular activity into organs of living animals requires both high spatial and temporal resolutions. Fluorescence imaging is a method of choice for such requirement. Genetically encoded fluorescent (Green Fluorescent Proteins and their variants) can be used to target specific compartments and are easily detectable without harmful staining processes.

FRET microscopy detects the transfer of excitation energy from donor to acceptor fluorophores that can occur only when the distance of the two fluorophores are less than about 10nm. It allows to partially overcome a limitation of optical microscopy that have a resolution of approximately 200nm due to the diffraction of light. With this technique it is possible to monitor molecular processes like protein-protein interactions and enzymatic activities into organs of living animals with both high spatial and temporal resolutions. We have combined multiphoton microscopy, which is a method of choice for non-destructive living tissue inspection, and FRET imaging to monitor calpain proteolytic activity in living mouse muscle. This 3D intravital imaging was applied to a mouse model that expresses ubiquitously a fluorescent reporter consisting of eCFP and eYFP separated by a linker cleavable by ubiquitous calpains. The decrease in FRET efficiency can be measured and is correlated with the activity of calpains. After performing validation studies, this model was used to quantify the proteolytic activity of ubiquitous calpains during several biological processes in skeletal muscle. Calpain activity was visualized in vivo in exercise-induced damages, after denervation, after fasting, during the degeneration and regeneration processes following injury and during ischemia. We also crossed this model with a model deficient in α -sarcoglycan and demonstrated an elevation of basal calpain activity compared to normal muscle. Our data showed that our model can be useful for real-time visualization of spatial and temporal activity of calpains in physiological and pathological conditions.

Combining intravital fluorescent imaging techniques and genetically engineered mice allows the monitoring of skeletal muscle at high spatial and temporal resolutions. Such combinations of technologies open considerable promises for the study of physiological and pathological processes as well as for the evaluation of therapeutics directly in vivo in small animals.

2:30 - 4:00: Session 7 / Delivery methods / Biodistribution

Hydrodynamic Limb Vein Injection for Delivery of Plasmid DNA to Skeletal Muscle

Christine Wooddell, Stephane Blot, Julia Hegge, Guofeng Zhang, Mark Noble, Karl Vigen, Thomas Grist, Vladimir Subbotin, Magdolna Sebestyen, Filippo Adamo, Jacob Griffin, Hans Herweijer, Thierry Huss, Martine Baudin, Christine Thioudellet, Patrica Kleinpeter, Serge Braun, James Hagstrom, and Jon Wolff

Hydrodynamic limb vein injection (Pathway IV) is a safe, efficient procedure to deliver nucleic acids

Workshop on overcoming bottlenecks in the development of therapeutics for rare neuromuscular disorders

and viral vectors to skeletal muscle cells. To deliver plasmid DNA (pDNA) to a limb, the treatment zone target area is isolated using a tourniquet. Then the pDNA in a saline solution is injected intravenously at a controlled, rapid rate. Elevated pressure transiently enhances permeability. This robust procedure works well in rodents and in primates. Limb observation, muscle histology, MRI, EMG, MR angiography, laser Doppler, and serum analysis confirm the safety of the procedure in primates.

A single injection of LacZ reporter plasmid in normal mice transfected 20-30% of the myofibers in the hind limb. The percentage of transfected myofibers increased as the dose was increased, whereas the efficiency of transfection decreased as the size of the plasmid increased (approximately 4% less efficiency per kilobase). Multiple injections gave additive increases in transgene expression. Efficient gene expression depended on the mass of pDNA per gram of target muscle being above a threshold dose that was similar in rats and in monkeys. In rodents, the CMV promoter as well as muscle-specific promoters enabled stable expression for over a year. In rhesus monkeys, however, expression from the muscle creatine kinase promoter was more stable than from the CMV promoter.

In the mdx mouse model for muscular dystrophy, multiple injections of dystrophin expression plasmids increased the percentages of expressing myofibers and the amount of dystrophin protein. Expression persisted for at least one year without any immunosuppression. Mdx mice that had been injected with this plasmid were exercised to challenge their muscles and thereby functionally assess the effectiveness of the expressed dystrophin gene product. We show by two-fold greater resistance to Evans blue dye that the myofiber integrity was preserved. Results with the GRMD dog model will be presented.

The potential of Electrotransfer

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Several techniques have been investigated to deliver genes by physical techniques. They include: 1) Naked DNA; 2) Gene gun; 3) Hydrodynamic (tail vein, arterial or leg vein injection); 4) Electrotransfer; 5) Focused ultrasound mediated gene delivery; 6) Laser induced plasmid delivery. After a brief description of these different techniques, the lecture will focus on electrotransfer.

Gene delivery to skeletal muscle and to tumors is a promising strategy for the treatment of muscle disorders or cancer, and for the systemic secretion by muscle of therapeutic proteins. We and others had reported very efficient plasmid DNA transfer in muscle fibers using square-wave electric pulses of low field strength and of long duration. This intramuscular « electrotransfer » method increases reporter and therapeutic gene expression by several orders of magnitude in various muscles and species, and decreases inter-individual variability.

The five following aspects will be reviewed: 1) Mechanism; 2) Sustained plasmatic protein secretion; 3) I.M. electrotransfer of EPO encoding plasmid in beta-thalassemic mouse; 4) Non viral gene therapy by electrotransfer of hTNF- α soluble receptor-I variants and its application to the treatment of experimental arthritis; 5) Applications for eye gene therapy.

The potential applications for the treatment of rare genetic diseases will be discussed.

AAV-Mediated Delivery of IGF-1 to the CNS by Deep Cerebellar Nuclei Injection Improves Motor Function and Prolongs Survival in Symptomatic ALS Mice.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the motor system. Although the etiology of ALS remains unclear, recent evidence suggests that multiple cell types contribute to disease pathogenesis. Neurotrophic factors have potent effects in animal models of ALS but their delivery to the affected regions remain a challenge. We report a novel, single-injection delivery strategy that achieved neurotrophic factor expression in all regions of the brainstem and spinal cord without directly targeting the muscle or neuromuscular junction. Injection of adeno-associated virus (AAV) encoding insulin-like growth factor-1 (IGF-1) into the deep cerebellar nuclei (DCN) of symptomatic ALS mice resulted in retrograde delivery of IGF-1 to the brainstem and spinal cord, a reduction in neuropathology, improved muscle strength and a significant extension of lifespan. These results demonstrate that a single injection delivery strategy of IGF-1 can significantly modify disease progression by influencing motor neurons and their environment at multiple levels of the CNS undergoing neurodegeneration.

Cell therapy of Muscular Dystrophy: from pre-clinical animal models to clinical experimentation.

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Mesoangioblasts are recently characterized stem cells that are associated with the vasculature and can differentiate in different types of solid mesoderm including skeletal muscle (Minasi et al. *Development* 129, 2773, 2002). When both wild type or dystrophic, genetically corrected, mesoangioblasts were delivered intra-arterially to dystrophic muscle of α -sarcoglycan null mice (a model for limb girdle muscular dystrophy), they resulted in a dramatic functional amelioration of the dystrophic phenotype (Sampaolesi et al. *Science* 301, 487, 2003).

Intra-arterial or systemic delivery of wild type, non DLA matched mesoangioblasts resulted in a partial recovery of muscle morphology and function, dystrophin expression and clinical amelioration, which persisted for a few months after removal of immune suppression. Delivery of autologous mesoangioblasts expressing human micro-dystrophin did not cause a comparable amelioration, despite widespread micro-dystrophin expression (Sampaolesi et al. *Nature* 444, 574, 2006). These results show efficacy of cell therapy in a large, immuno-competent animal and set the rationale for a future clinical trial, using donor cells from an HLA-matched donor under immune suppression. Problems still facing this approach and possible strategies to overcome them will be discussed.

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