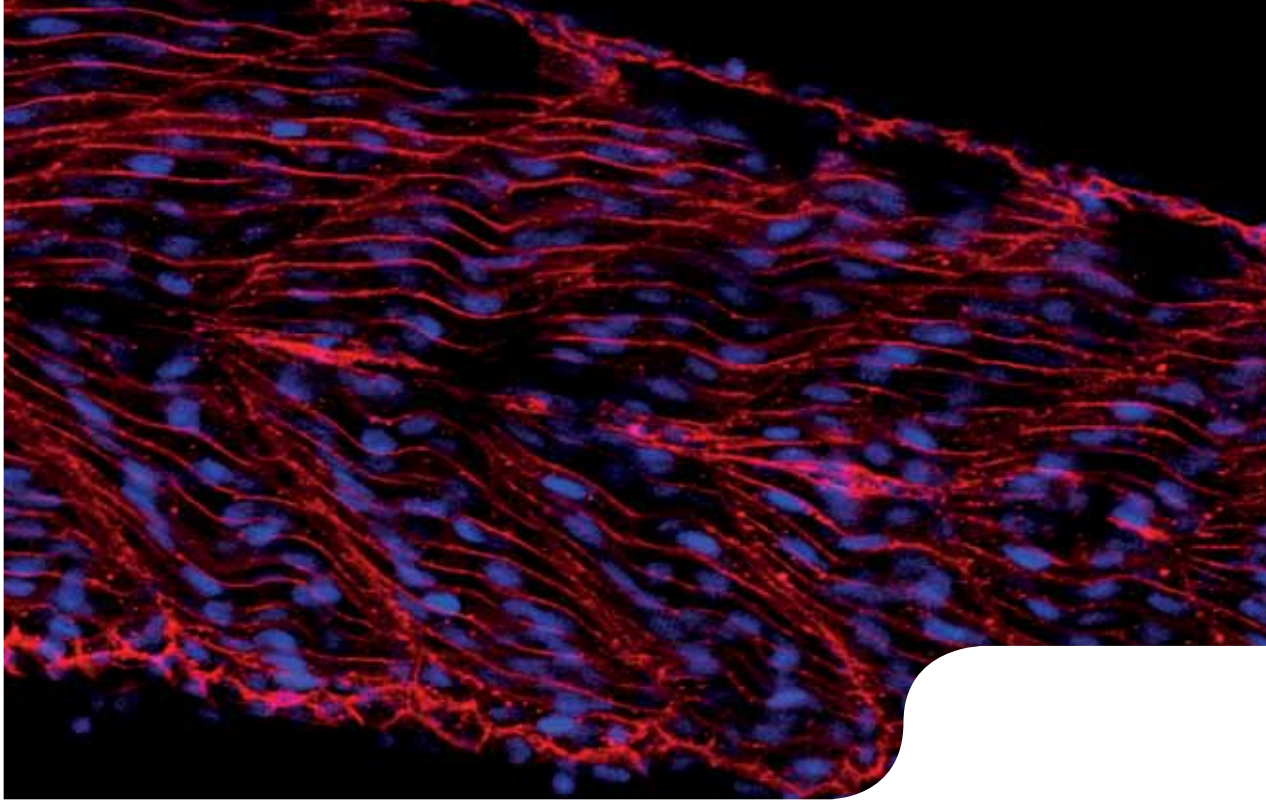
2011

March 13-16

Las Vegas, Nevada



MDA NATIONAL SCIENTIFIC CONFERENCE

Neuromuscular Therapeutic Strategies: Overcoming the Barriers from Microscope to Marketplace

MDA Muscular Dystrophy Association

Fighting Muscle Disease

Table of Contents

Agenda

Sunday – March 13

2:00 p.m. ..... REGISTRATION
6:00 – 8:00 p.m. ..... EVENING RECEPTION

Monday – March 14

R. Rodney Howell, M.D.
MDA Chairman of the Board

Charles Thornton
Conference Co-Chair

Robert Mattaliano
Conference Co-Chair

8:15 a.m. ..... Welcome

OLIGONUCLEOTIDE SESSION

CHAIRS: CARMEN BERTONI, PH.D. AND FRANK BENNETT, PH.D.

8:30-9:00 a.m. ..... Keynote speaker: Steve Wilton, Ph.D.
Splice switching therapies for Neuromuscular Diseases

9:00-9:30 a.m. ..... Thurman Wheeler, M.D.
Antisense oligonucleotide therapy for myotonic dystrophy

9:30-9:50 a.m. ..... Alessandra Belayew, Ph.D.
Suppression of DUX4 or DUX4c protein expression by antisense strategies in a therapeutic approach for FSHD

9:50-10:10 a.m. ..... Carmen Bertoni, Ph.D.
Oligonucleotide-mediated gene editing of the dystrophin gene using methyl-CpG ssODN in the mdx mouse model for Duchenne muscular dystrophy

10:10-10:30 a.m. COFFEE BREAK

10:30-11:00 a.m. ..... Don Cleveland, Ph.D.
Antisense oligonucleotide therapy for motor neuron diseases

11:00-11:20 a.m. ..... Paul Porensky, M.D.
Antisense morpholino against ISS-N1 corrects SMA mice

11:20-11:40 a.m. ..... Adrian Krainer, Ph.D.
Correction of SMN2 RNA splicing in the CNS with antisense oligonucleotide as a therapeutic strategy for spinal muscular atrophy

11:40 a.m.-12:00 p.m. ..... Aurelie Goyenvalle, Ph.D.
AAV-U7snRNA mediated multi exon-skipping for Duchenne Muscular Dystrophy

12:00-12:30 p.m. ..... *Discussion: What are the hurdles to developing oligonucleotide therapies and how do we overcome those barriers?*

12:30-1:30 p.m. LUNCH

STEM CELL SESSION

CHAIRS: CLIVE SVENDSEN, PH.D. AND JOHNNY HUARD, PH.D.

1:30-1:50 p.m. . . . . Introduction: Clive Svendsen, Ph.D.

1:50-2:10 p.m. . . . . Nicholas Boulis, M.D.
Practical Considerations in Human Spinal Cord Stem Cell Transplantation

2:10-2:30 p.m. . . . . Letizia Mazzini, M.D.
Long- term follow -up after mesenchymal stem cells transplantation in Amyotrophic Lateral Sclerosis

2:30-2:50 p.m. . . . . Daniel Miller, M.D., Ph.D.
FSHD-patient Derived IPS cells as a Model to Study Developmentally regulated DUX4 Expression and Test the affect of Potential Therapies

2:50-3:10 p.m. . . . . Svitlana Garbuzova-Davis, Ph.D., D.Sc.
Blood-Brain/Spinal Cord Barrier Impairment in ALS: How to Repair?

3:10-3:30 p.m. COFFEE BREAK

3:30-3:50 p.m. . . . . Introduction: Johnny Huard, Ph.D.

3:50-4:10 p.m. . . . . Terence Partridge, Ph.D.
Effects of the dystrophin mutation on growth and regeneration in mdx mouse muscle

4:10-4:30 p.m. . . . . Giulio Cossu, M.D.
A Phase I/II cell therapy trial for Duchenne Muscular Dystrophy

4:30-4:50 p.m. . . . . Alessandra Sacco, Ph.D.
Short Telomeres and Stem Cell Exhaustion Model Duchenne Muscular Dystrophy in mdx/mTR Mice

4:50-5:10 p.m. . . . . Ilona Skerjanc, Ph.D.
Retinoic Acid Enhances Skeletal Myogenesis in Human Embryonic Stem Cells by Expanding the Premyogenic Progenitor Population

5:10-5:40 p.m. . . . . *Discussion: Taking stem cells to the clinic for ALS and MD – where are we now?*

5:40-7:30 p.m. POSTER SESSION A

7:30 p.m. DINNER

Tuesday – March 15

8:15 a.m. . . . . Introduction

SMALL MOLECULE SESSION

CHAIRS: JASBIR SEEHRA, PH.D. AND JEFFREY ROTHSTEIN, M.D., PH.D.

8:30-9:00 a.m. . . . . James Rusche, Ph.D.
Developing drugs that target gene expression to replace key proteins in single gene neurodegenerative diseases

9:00-9:30 a.m. . . . . John McCall, Ph.D.
Small molecule drug discovery in muscular dystrophy: what's involved?

9:30-9:50 a.m. . . . . Stanley Froehner, Ph.D.
Sildenafil Improves Skeletal and Cardiac Muscle Function in the mdx Mouse

9:50-10:10 a.m. . . . . Gino Cortopassi, Ph.D.
Alterations in Thioredoxin-related antioxidants in Friedriech's ataxia models, and High-throughput screening

10:10-10:30 a.m. COFFEE BREAK

10:30-11:00 a.m. . . . . Jeffrey Rothstein, M.D., Ph.D.
Small molecules for ALS: Assessing candidate targets and optimizing clinical discovery

11:00-11:20 a.m. . . . . Jiming Kong, M.D., Ph.D.
Rescuing motor neurons by targeting the BNIP3 cell death pathway

11:20-11:40 a.m. . . . . Laxman Gangwani, Ph.D.
JNK is required for neuronal degeneration in spinal muscular atrophy

11:40 a.m.-12:00 p.m. . . . . James Dowling, M.D., Ph.D.
Myotubular Myopathy and the Neuromuscular Junction: a Novel Therapeutic Approach?

12:00-12:30 p.m. . . . . *Discussion: Developing Small Molecule Therapies.*

12:30-1:30 p.m. LUNCH

PROTEIN THERAPY SESSION

CHAIRS: ROBERT MATTALIANO, PH.D. AND JUSTIN FALLON, PH.D.

1:30-2:00 p.m. . . . . Keynote Speaker: Robert Mattaliano, Ph.D.
Developing a Muscular Dystrophy Therapy – Overcoming Barriers in Pompe Disease

2:00-2:30 p.m. . . . . Jasbir Seehra, Ph.D.
Inhibition of the myostatin pathway for treatment of neuromuscular diseases

2:30-2:50 p.m. . . . . Dennis Guttridge, Ph.D.
Understanding NF-kB Function and Its Therapeutic Potential in DMD

2:50-3:10 p.m. . . . . Jachinta Rooney, Ph.D,
Laminin-111 protein therapy restores viability, reduces pathology and improves muscle strength in the dyW mouse model of Merosin Deficient Congenital Muscular Dystrophy Type 1A

3:10-3:30 p.m. COFFEE BREAK

3:30-4:00 p.m. . . . . Justin Fallon, Ph.D.
Biglycan as a therapeutic for DMD

4:00-4:20 p.m. . . . . Thomas Thompson, Ph.D.
How structures of myostatin can help with inhibitor design

4:20-4:40 p.m. . . . . Michael Lawlor, M.D., Ph.D.
Weakness and Responses to Treatment Are Dependent on Fiber Type and Mutation in Murine Models of Myotubularin Deficiency

4:40-5:00 p.m. . . . . Thien Nguyen, M.D., Ph.D.
Axonal protective effects of netrin-1

5:00-5:30 p.m. . . . . *Discussion: Why choose a protein therapy — what are the advantages and disadvantages of this modality?*

5:30-7:30 p.m. POSTER SESSION B

Wednesday – March 16

8:15-8:25 a.m. Presentation of Poster Awards

8:25-9:10 a.m. . . . . Keynote speaker: James Wilson, M.D., Ph.D.
Immunology and delivery – the key challenges for in vivo gene therapy

9:10-9:40 a.m. . . . . Jeffrey Chamberlain, Ph.D.
Gene therapy for the muscular dystrophies: overcoming the remaining challenges

9:40-10:00 a.m. . . . . Joel Chamberlain, Ph.D.
Validity of RNAi-based therapeutics as a treatment for FSHD as demonstrated in a mouse model of muscular dystrophy

10:00-10:20 a.m. . . . . Dawn Delfin, Ph.D.
A novel treatment strategy for muscular dystrophy-based cardiomyopathy and heart failure: gene therapy using the tight junction protein claudin-5.

10:20-10:50 a.m. COFFEE BREAK

10:50-11:20 a.m. . . . . Brian Kaspar, Ph.D.
Gene Delivery for Motor Neuron Disease: Pathway to the Clinic

11:20- 11:40 a.m. . . . . Zejing Wang, M.D., Ph.D.
Long term transgene expression and amelioration of muscle function following large scale rAAV-micro-dystrophin treatment in dogs with Duchenne muscular dystrophy

11:40 –12:00 p.m. . . . . Louise Rodino-Klapac, Ph.D.
rAAV5 Mediated Delivery of Dysferlin as a Therapeutic Strategy for LGMD2B and Miyoshi Myopathy

12:00-12:30 p.m. . . . . Discussion

12:30 p.m. LUNCH

Invited Speakers

OLIGONUCLEOTIDE SESSION:

CHAIRS: CARMEN BERTONI, PH.D. AND FRANK BENNETT, PH.D.
Monday, March 14th 8:30 a.m. – 12:30 p.m.

8:30-9:00 a.m.: Steve Wilton, University of Western Australia (Keynote speaker)
Splice switching therapies for Neuromuscular Diseases.

Steve Wilton

Most of our genetic make-up was previously considered "junk", with less than 2% encoding protein (exons). The rest of our genome is composed of introns (non-coding sequences found between the protein-encoding exons), repeated elements, RNA genes, and intergenic spacers. It is now known that the majority of our genome is transcribed into RNA and some of the non-coding RNA transcripts are used to regulate gene expression.

The complexity of gene expression offers opportunities for intervention, and exon skipping, the ability to target and remove one or two exons from the dystrophin gene transcript to skip over a Duchenne MD-causing mutation, has emerged as a most promising therapy. Splice switching antisense oligomers (AOs) are chemically synthesized RNA-like genetic bandaids that can modify gene expression, by binding to a target exon and masking it from the cellular processing machinery. Appropriately designed compounds can remove an exon carrying or associated with a protein-truncating mutation in the dystrophin gene transcript to induce a Becker MD-like isoform that retains some function and reduces the pathology. From characterization of BMD cases showing no or mild symptoms, it was found that substantial portions of the dystrophin protein were largely redundant. Independent clinical trials are evaluating 2'-O-Methyl AOs and the phosphorodiamidate morpholino oligomers (PMOs) as splice switching compounds. Both studies have reported restoration of dystrophin synthesis in DMD individuals whose frame-shifting deletion would be restored by skipping of exon 51. Although encouraging results from both trials have been reported, there are many challenges ahead:

- establishing appropriate dosing regimens.
- predicting variable responses to the same splice switching AO between DMD boys
- bringing many different dystrophin splice switching oligomers to the clinic.
- developing effective safety and toxicology testing for each oligomer.
- gaining regulatory approval for many different compounds.
- consequences of long term administration.
- selection of the next targets/clinical trials.
- affordability and long term sustainability.

Finally, if this form of genetic therapy is shown to benefit many different DMD individuals, we believe that the splice switching technology can be applied to many other neuromuscular conditions.

Spinal muscular atrophy arises from the loss of SMN1 and inappropriate splicing of a near identical gene, SMN2, so there is greatly reduced levels of the SMN protein. In this situation, it is necessary to do the opposite of exon skipping and attempt to re-enforce SMN2 exon 7 recognition and inclusion in the mature SMN2 transcript. Facioscapulohumeral MD is associated from the loss of the D4Z4 repeats at the end of chromosome 4, although the precise mechanism has not yet been established. Up-regulated and inappropriate expression of several candidate genes has been proposed in the pathogenesis of this condition and AOs can be used to modify expression of these candidate genes. New therapeutic windows are being opened on a variety of neuromuscular conditions and future splice switching applications may only be limited by our imagination.

9:00-9:30 a.m.: Thurman Wheeler, M.D., University of Rochester
Antisense oligonucleotide therapy for myotonic dystrophy

Thurman Wheeler, M.D.
University of Rochester

9:30-9:50 a.m.: Alessandra Belayew, Ph.D., University of Mons

Suppression of DUX4 or DUX4c protein expression by antisense strategies in a therapeutic approach for FSHD.

C. Vanderplanck1, F. Coppée1, E. Ansseau1, A. Tassin1, S.Charron1, D. Laoudj-Chenivesse2, S.D. Wilton3 and A. Belayew1.
1.Lab. Molecular Biology, University of Mons, Belgium ; 2. INSERM U1046, University of Montpellier, France ; 3. Australian Neuromuscular Research Institute, Nedlands, WA, Australia.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder with a prevalence of 7/100,000 birth. It is characterized by an antero-posterior and often asymmetric progression of muscle weakness first affecting the face, the scapulae and the foot dorsiflexors. FSHD is linked to contractions of the D4Z4 repeat array in 4q35. We have identified the double homeobox 4 (DUX4) gene within each D4Z4 unit and shown it encodes a transcription factor that is expressed in FSHD but not control primary myoblasts or muscle biopsies. DUX4 activation initiates a large transcription deregulation cascade leading to muscle atrophy and differentiation defects, inflammation and oxidative stress which are key features of the disease. We found that stable mRNAs comprising the full DUX4 ORF only derived from the most distal unit and unexpectedly extended within the flanking pLAM region that provided an intron and a polyadenylation signal. Other groups recently showed this signal was required to develop FSHD. We also characterized the homologous DUX4c gene located 42-kb centromeric of the D4Z4 locus. It is expressed in muscles from healthy individuals, is increased in DMD and FSHD, and its over-expression induces human myoblast proliferation. We now found that DUX4 expression in human myoblast cultures induced E3 ubiquitin ligases associated with muscle atrophy and the formation of atrophic myotubes. In contrast DUX4c expression induced β-catenin stabilization and disorganised myotubes with large clusters of myonuclei. Interestingly, FSHD primary myotubes present different proportions of these atrophic/disorganized phenotypes. We reasoned that inhibition of DUX4 or DUX4c expression should prevent the transcription deregulation cascade and restore a healthy myotube phenotype. We used different antisense approaches in human myoblast cultures to either induce mRNA destruction by RNA interference (siRNAs) or affect splicing by specific antisense oligomers. Decrease in DUX4 or DUX4c protein expression was confirmed by immunodetection on western blot and the biomarker and myotube phenotypes changed as expected. These strategies seem promising and could contribute to future development of therapeutic approaches for FSHD. We acknowledge funding from the AFM. CV had a FRIA (Belgium) graduate fellowship, AT and EA were post-doctoral FNRS (Belgium) associates.

9:50-10:10 a.m.: Carmen Bertoni, Ph.D., University of California, Los Angeles
Oligonucleotide-mediated gene editing of the dystrophin gene using methyl-CpG ssODN in the mdx mouse model for Duchenne muscular dystrophy.

Farnoosh Nik-Ahd1, Refik Kayali1, Arjun Rustagi2 Thomas A. Rando2 and Carmen Bertoni1
1Department of Neurology , University of California Los Angeles, CA 90095, USA and 2 Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305-5235, USA.

Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder characterized by complete absence of dystrophin expression in skeletal muscles. Gene editing mediated by single stranded oligodeoxynucleotides (ssODNs) has the potential to treat both single point mutations as well as deletions that cause frame shift of the dystrophin mRNA.

We have focused on the development of new vectors capable of activating specific repair mechanisms to direct the repair process specifically on the sequence of the genomic DNA targeted for correction. The Methyl Binding Protein 4 (MBD4) is a glycosylase capable of recognizing a T to G transversion at Methyl-CpG sites and direct the conversion of the thymine into methylcytosine. Methyl-CpG modifications were introduced on the mutating base of the targeting oligonucleotide in the attempt to mimic a deamination of methylcytosine and activate MBD4.

The feasibility of using modified ssODNs for the treatment of DMD was tested in the mdx mouse. We have designed ssODNs complimentary to the coding or the non-coding strand of the donor site of exon 23 to induce skipping of the exon responsible for the lack of dystrophin in mdx and restore its expression. The ability of ssODNs containing Methyl-CpG modifications to increase gene repair was studied in vitro and in vivo. The amount of dystrophin protein restored was significantly increased by the use of ssODNs designed to activate MBD4. Studies conducted on muscle cells in culture demonstrated up-regulation of MBD4 mRNA and the activation of the base excision repair mechanism through which MBD4 acts. Correction of the dystrophin gene was shown to occur at the genomic level and was stable over prolonged periods of time. In muscle cells in culture, restoration of dystrophin expression was analyzed at the protein level by western blot and immunohistochemistry and at the mRNA level by RT-PCR. Immunostaining analysis of mdx-injected muscles demonstrated the efficacy of ssODN containing Methyl-CpG modifications of increasing the expression of functional dystrophin in vivo. The single base pair alteration was confirmed at the genomic level using restriction endonuclease analysis of total DNA isolated from muscles injected with targeting ssODN. Dystrophin expression was stable for at least four months after injection (the latest time point analyzed). Control oligonucleotides homologous to the region of the genomic DNA targeted for repair but lacking the mismatch had no effects.

10:30-11:00 a.m.: Don Cleveland, Ph.D., Ludwig Institute for Cancer Research/UCSD
Antisense oligonucleotide therapy for motor neuron diseases

11:00-11:20 a.m.: Paul Porensky, M.D., Ohio State University

Antisense morpholino against ISS-N1 corrects SMA mice

Paul Porensky1, Wai Mitrpant3, Steve Wilton3 and Arthur Burghes2 Dept of (1) Neurosurgery and (2) Molecular and Cellular Biochemistry Ohio State University. Columbus, OH. (3) Australian Neuromuscular Research Institute, University of Western Australia, Perth, Western Australia

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by alpha motor neuron loss in the anterior horn of the spinal cord. SMA results due to deletion or mutation of the survival motor neuron 1 gene (SMN1) and retention of SMN2. A single nucleotide difference between SMN1 and SMN2 results in the majority of transcript from SMN2 excluding exon 7, leading to markedly decreased SMN protein levels and the development of SMA. A series of splice enhancers and silencers regulate the incorporation of SMN2 exon 7; these sites can be blocked or enhanced with antisense oligomers (ASOs) so as to alter the SMN2 transcript splicing. We have developed a morpholino chemistry ASO against ISS-N1, and delivered the morpholino to postnatal day 0 SMA pups (mSmn -/-, hSMN2+/+, Δ7 +/+; "SMA mouse") by transcranial-intraventricular delivery. 2µl of 2,4 or 6mM morpholino was delivered to 10 SMA mice for each dosage. A remarkable phenotypic modification was obtained for all dose levels, with life-span extension from an average of 14 days to well over 100 days after a single therapeutic injection. To date the corrected animals have shown no motor abnormalities. Delivery after P0 has decreased efficacy, though postnatal day 4 injection still extended survival to greater than 40 days. Control animals show no adverse effects from either the morpholino or intraventricular delivery. We have analyzed the ability of the ASO to alter splicing of SMN2 in various mouse tissue; ASO treatment by ICV resulted in a marked increase in full length SMN transcript as well as SMN protein in neural tissue, but not in tissue outside of the nervous system. Interval analysis shows a decrease in alternative splice modification of SMN2 RNA at later time-points. Indeed, 50 day old injected mice developed necrosis of peripheral structures (tail, pinna, snout). We are testing whether this necrosis can be prevented by subsequent dosing with morpholino. We suggest that CNS increases of SMN will have a major impact on SMA, and that a time-limited correction of SMN level results in correction of motor phenotypes. Last, the early introduction by intrathecal delivery of morpholino ASOs early is a potential treatment for SMA.

11:20-11:40 a.m.: Adrian Krainer, Ph.D., Cold Spring Harbor Laboratory

Correction of SMN2 RNA splicing in the CNS with antisense ologonucleotide as a therapeutic strategy for spinal muscular atrophy

Yimin Hua1, Kentaro Sahashi1, Frank Rigo2, John Matson2, Gene Hung2, Marco A. Passini3, Seng H. Cheng3, C. Frank Bennett2, and Adrian R. Krainer1
1Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY 11724; 2Isis Pharmaceuticals, 1896 Rutherford Rd, Carlsbad, CA 92008; 3Genzyme Corporation, 49 New York Ave, Framingham, MA 01701

Spinal Muscular Atrophy (SMA) is a genetic disease characterized by progressive degeneration of motor neurons in the anterior horn of the spinal cord, which in turn leads to severe muscle weakness and atrophy. SMA is caused by deletion or loss-of-function mutations in the Survival-of-motor neuron (SMN1) gene. The paralogous SMN2 gene, present in one or more copies in all SMA patients, attenuates the severity of SMA, but expresses only a low level of 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 an optimal 2′-O-(2-methoxyethyl) (MOE) phosphorothioate 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 liver and kidneys 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 (dubbed ISIS-SMN_{Rx}) was delivered into cerebrospinal fluid through the right lateral ventricle in adult $Smn^{-/-}$ type-III SMA mice carrying a human SMN2 transgene. Dose-response studies revealed that intracerebroventricular (ICV) infusion of the 18mer ASO increased SMN2 exon 7 inclusion in spinal cord to ~90%, compared to ~10% in saline-treated mice. Western blotting and immunohistochemical analysis demonstrated a robust increase of the human transgenic SMN protein levels in spinal-cord motor neurons. We have also used ICV bolus injection in embryonic, neonate, or adult mild or severe SMA mouse models to optimize the effectiveness of the ASO, characterize phenotypic improvement, and establish a time window for effective treatment. Recent results in adult transgenic mice demonstrate that ICV bolus injection is a more efficient method of delivery for the antisense drug than ICV infusion. In addition, studies in non-human primates support IT bolus injection as a feasible route of delivery. These data show that ISIS-SMN_{Rx} is a promising drug candidate for SMA therapy.

11:40-12:00 p.m.: Aurélie Goyenvalle, Ph.D., University of Oxford

AAV-U7snRNA mediated multi exon-skipping for Duchenne Muscular Dystrophy

Aurélie Goyenvalle1, Arran Babbs1, Jordan Wright1, Luis Garcia2 and Kay E. Davies1
1MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK, e-mail: aurelie.goyenvalle@dpag.ox.ac.uk 2 Institut de Myologie, UPMC UMR S 974, Biotherapies des maladies Neuromusculaires, Paris, France

Most cases of Duchenne muscular dystrophy (DMD) are caused by mutations that disrupt the dystrophin mRNA reading frame. In many cases, skipping of a single exon could restore the reading frame, giving rise to a shorter but still functional quasi-dystrophin protein. It has previously been proposed to use small nuclear RNAs, especially U7snRNA, to shuttle antisense sequences designed to mask key elements involved in the splicing of targeted exons.

Our present project focuses on the optimisation of U7snRNA constructs to complete rescue of dystrophin by exon-skipping in DMD patients. In particular, we are investigating the multi exon-skipping of exons 45 to 55, which could rescue up to 63% of DMD patients with a deletion.

In order to achieve this multi-skipping, we have first developed U7snRNA constructs targeting every exon between 45 and 55. Each construct has been inserted into lentiviral vectors for in vitro analysis in myoblasts from DMD patients. After transduction of these cells with lentiviral vectors encoding the various U7 constructs, specific skipping of the targeted exon was confirmed by RT-PCR. In parallel, we demonstrated the efficacy of these constructs in vivo in transgenic mice carrying the entire human DMD locus (hDMD mice) after intramuscular injection of AAV vectors encoding the U7snRNAs. Based on in vitro and in vivo results, the U7snRNA constructs inducing the most efficient skipping for each targeted exon were selected and combined into multi-skipping vectors. These AAV vectors are currently being tested in DMD myoblasts and in hDMD mice and efficient skipping of up to six exons has been demonstrated thus far.

These very encouraging results provide evidence that efficient multi exon-skipping can be achieved using AAV vectors encoding multiple U7snRNAs. These new constructs offer therefore very promising tools for clinical treatment of DMD.

STEM CELL THERAPY SESSION:

CHAIRS: CLIVE SVENDSEN, PH.D. AND JOHNNY HUARD, PH.D.
Monday, March 14th 1:30 p.m. – 5:40 p.m.

1:30-1:50 p.m.: Nicholas Boulis, M.D., Emory University

Development of Surgical Techniques for Spinal Cord Transplantation in ALS

Nicholas Boulis MD, Thais Federici PhD, Clive Svendsen PhD., and Jonathon Riley MD,

The emergence of proof of principle data supporting the ability of cell based spinal cord therapies to provide protection and repair in models of spinal cord injury and motor neuron disease prompted a variety of efforts to launch human spinal cord transplantation trials. Our initial Pre-preIND discussions with the FDA in 2004 required us to articulate principles for safe and accurate spinal cord transplantation. First, cell suspensions must be injected into the spinal cord at a slow controlled rate to minimize graft reflux and pressure in cord parenchyma. Second, the injection apparatus must be stabilized with respect to the cord to allow for reproducible targeting and to prevent sheering of white matter. Third, we believe that stabilization must prevent movement of the injector with respect to the patient in the context of ventilation and inadvertent jostling (macro movement). Finally, movement of the spinal cord within the canal with ventilation and pulse (micro movement) may pose a risk in the context of rigid cannulas. To satisfy these principles, we have designed a series of spine mounted platforms and cannulas, that have been improved in successive versions over the past 7 years. The most recent version is currently in use in the first spinal cord stem cell transplantation trial for ALS. To date, patients undergoing this procedure, using this device have suffered no major neurological morbidity.

1:50-2:10 p.m.: Letizia Mazzini, M.D., Eastern Piedmont University

Long- term follow -up after mesenchymal stem cells transplantation in Amyotrophic Lateral Sclerosis

Letizia Mazzini1, Katia Mareschi2, Ivana Ferrero2, Massimo Miglioretti3, Alessandro Stecco4, Franca Fagioli2
1ALS Centre Department of Neurology "Eastern Piedmont" University, "Maggiore della Carità" Hospital, Novara,
2Stem Cell Transplantation and Cellular Therapy Unit; Pediatric Onco-Hematology Division, "Regina Margherita" Children's Hospital, University of Torino
3Department of Psychology, University of Milano-Bicocca, Milano
4Department of Diagnostic and Interventional Radiology, "Eastern Piedmont" University, "Maggiore della Carità" Hospital, Novara

Stem-cell-based therapies represent a new possible strategy for Amyotrophic Lateral Sclerosis (ALS) clinical research. Long-term follow-up represents a necessary part of the design of any initial trial of the safety of stem cell transplantation to assess the possibility of the development of tumor, cyst or syrinx at the site of transplantation. This is the first report of very long term follow up (up to 9 years) of intraparenchymal transplantation of Mesenchymal Stem Cells into the spinal cord of ALS patients. 19 patients with ALS were enrolled in two consecutively phase 1 clinical trials. The trials were approved and monitored by the Ethic Committees and by the National Institute of Health. Clinical, laboratory, and radiographic evaluations of the patients showed no deaths or serious treatment related adverse events in the short and long-term follow-up. No evidence of new masses at the injection site or anywhere else in the neuraxis were visible in any of the MRI images of the whole follow up. DTI tractography did not detect any structural changes in the corticospinal tracts. No negative reactions on mood and quality of life were detected . The main points that will be discussed are:

1. Stem cell trials represent a new scenario in ALS clinical research. Our results show that a surgical clinical trial with stem cells transplantation may be proposed in patients with ALS, but many questions related to the site of transplantation, the surgical techniques, the type of cells and the inclusion criteria of patients are still open and need a comment.
2. Due to the novelty of our clinical research, the great hopes of patients and the large media interest in stem cells we were faced with many pressures. Institutional oversight by the scientific committee of the National Institute of Health and Ethic Committees guaranteed the transparency of Research Procedures and interpretation and communication of the results. A careful psychological selection and monitoring of patients avoided any deterioration in psychosocial status.
3. Our research was facilitated by a large, highly competent multidisciplinary team having experience in cell therapy and in the treatment of ALS. The clinical team was also assisted by a multidisciplinary group of basic researchers with documented experience in working with stem cells in vitro and in experimental animal models.
4. Recruitment and selection of appropriate patients for larger trials will be a challenge and will require national and/or international multi-center collaboration.

2:30-2:50 p.m.: Daniel Miller, M.D., Ph.D., University of Washington

FSHD-patient Derived IPS cells as a Model to Study Developmentally regulated DUX4 Expression and Test the affect of Potential Therapies.

Gregory J. Block1, Lisa M. Petek1, Linda N. Geng2, Lauren Snider2, Natalia Rabaia2, Stephen J. Tapscott2, Galina N. Filippova2, Rabi N.Tawil3, Silvere Van Der Maarel4 and Daniel G. Miller1
1Department of Pediatrics, Seattle Childrens Hospital, Institute for Stem Cell and Regenerative Medicine, University of Washington. 2Fred Hutchinson Cancer Research Center, Seattle, WA 3University of Rochester, Rochester, New York 4Leiden University Medical Center, Leiden, Netherlands.

Developmentally regulated DUX4 transcription is an important determinant of FSHD pathology. It was previously shown that FSHD symptoms correlate with D4Z4-associated histone modifications characteristic of chromatin relaxation and transcriptional activation in both FSHD1 and FSHD2-affected individuals. To determine if this epigenetic profile correlates with expression of DUX4 transcripts during development, we assessed DUX4 expression and associated histone modifications before and after induced differentiation of FSHD IPS cells. The presence of DUX4 transcripts correlated with histone modifications typical of euchromatin in FSHD IPS cells and differentiated embryoid bodies. In contrast, a transition to histone modifications characteristic of heterochromatin and transcriptional repression occurred as normal IPS cells were induced to differentiate. Because the tissue and regional distribution of FSHD pathology suggests there are additional levels of regulatory control, we constructed LacZ reporters and delivered them to Human ES cells using a lentivirus vector so that promoter activity could be evaluated without array-mediated transcriptional influences. We show that transcription from the DUX4 promoter in this context is repressed in undifferentiated embryonic stem cells but becomes active in a restricted pattern as cells begin to differentiate. These experiments demonstrate that DUX4 transcription and toxicity likely requires the presence of factors restricted to specific cell types in addition to a transcriptionally permissive chromatin environment. Finally, we have identified the WNT pathway as a potent mediator of DUX4 expression and demonstrate that WNT pathway inhibitors suppress DUX4 expression in myoblasts from FSHD patients suggesting a potential therapeutic strategy for FSHD treatment.

2:50-3:10 p.m.: Svitlana Garbuzova-Davis, Ph.D., D.Sc., University of South Florida
Blood-Brain/Spinal Cord Barrier Impairment in ALS: How to Repair?

Svitlana Garbuzova-Davis1,2, Maria CO Rodrigues1, Diana G Hernandez-Ontiveros1, Sanjeev Rattan1, Cesario V Borlongan1,2, and Paul R Sanberg1,2
1Center of Excellence for Aging and Brain Repair, 2Department of Neurosurgery and Brain Repair, University of South Florida, College of Medicine, Tampa, USA.

Development of an effective treatment for Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disorder, is complicated by various underlying disease mechanisms and the diffuse nature of motor neuron death. One possible mechanism involved in ALS pathogenesis is impairment of the blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB), aggravating motor neuron damage. Recent findings indicate that these barriers are compromised in an animal model of ALS. Structural and functional impairments of BBB/BSCB were detected in G93A SOD1 mice at different disease stages. Of interest, capillary ultrastructural examination revealed endothelial cell degeneration, which, along with astrocyte degeneration, compromised the BBB/BSCB, resulting in vascular leakage. In autopsied spinal cords from ALS patients, decreased tight junction proteins were indicated, strengthening the likelihood that barrier disruption contributes to disease progression. The present study investigated BBB/BSCB integrity in post-mortem gray matter of brainstems (medulla) and spinal cords (cervical and lumbar) from 26 sporadic ALS patients and 18 age-matched controls without neurological or immunological pathology, obtained from human tissue banks (Human Brain and Spinal Fluid Resource Center, Los Angeles, CA, and the NICHD Brain and Tissue Bank, Baltimore, MD). Neuropathological examinations of tissue from ALS patients provided by banks confirmed clinical ALS diagnoses and that patients died as a result of complications from this disorder. Ultrastructural capillary analysis in ALS tissues showed endothelial and pericyte cell damage. Significant extra- and intracellular edema was evident. A large accumulation of collagen was observed between the basement membrane covering the endothelial cells and the limiting basement membrane forming the blood-neural tissue barrier. Immunohistochemical analysis showed decreased basement membrane laminin associated with albumin extravasation and IgG leakage in capillaries in ALS tissues. Also, CD31 (PECAM-1) and CD105 (endoglin) detected ill-defined vessel margins and discontinuities in the endothelial lining, along with an asymmetrical increase in the capillary wall extensions in ALS tissues. These results demonstrated substantial BBB/BSCB dysfunction in ALS and revealed ALS to be a neurovascular disease. One possibility for repairing the vascular damage is endothelial cell replacement. Our preliminary study demonstrated that administration of human bone marrow endothelial precursor cells into G93A mice might restore BSCB integrity. Administered cells incorporated and engrafted into the vascular wall in a majority of blood vessels in the spinal cord. Restoration of barrier competence may be critical to prevent further motor neuron degeneration. Detailed mechanisms of the BBB/BSCB impairment and re-establishment are discussed.

Supported in part by the Muscular Dystrophy Association (Grant #92452) and by the USF Department of Neurosurgery and Brain Repair.

3:30-3:50 p.m.: Terence Partridge, Ph.D., Children's National Medical Center
Effects of the dystrophin mutation on growth and regeneration in mdx mouse muscle.

Terence Partridge, Ph.D.

The generally accepted model of dystrophinopathy is that lack of dystrophin renders the muscle fibers susceptible to activity-associated necrosis which drives acute and chronic inflammation and activation of satellite cells to repair the lesion. Our recent research modifies this view by demonstrating more generally pervasive effects of the lack of dystrophin. Analysis of the proteins secreted from mdx muscle fibers in vitro show excessive efflux of a large number of proteins and peptides in the absence of any sign of cell damage or generalized leakiness. This is combined with activation of intrinsic inflammatory pathways and with a generalized disturbance of fibre metabolism. These differences are echoed in vivo in the growing muscle of the pre-pathological mdx mouse both in the mode of growth of the muscle fibers and in the behaviour of the satellite cells. These early signals of malfunction present us with a new set of biomarkers for assessment of the efficacy of putative therapeutic agents and may also represent new therapeutic targets for early intervention, that would avoid the consequences of the pathological cascade that is unleashed by subsequent myonecrosis.

4:10-4:30 p.m.: Giulio Cossu, M.D., San Raffaele Scientific Institute

Novel strategies for the cell therapy of muscular dystrophy

Cossu, G.
Division of Regenerative Medicine, San Raffaele Scientific Institute

Mesoangioblasts are recently characterized progenitor cells, associated with the vasculature and able to differentiate into different types of solid mesoderm, including skeletal muscle (1).

When wild type or dystrophic, genetically corrected, mesoangioblasts were delivered intra-arterially to dystrophic muscle of α-sarcoglycan KO mice (a model for limb girdle muscular dystrophy), they resulted in a significant functional amelioration of the dystrophic phenotype (2).

Intra-arterial delivery of wt mesoangioblasts, non DLA matched to GRMD dystrophic dogs resulted in a partial recovery of muscle morphology and function, dystrophin expression and clinical amelioration. Delivery of autologous mesoangioblasts expressing human micro-dystrophin did not cause a comparable amelioration (3). Human adult mesoangioblasts were isolated and expanded in vitro from muscle biopsies: they were shown to correspond to a subset of pericytes (4).

Based on these results, a monocenter, prospective, non-randomised, clinical phase I/II study of cell therapy with HLA-matched donor human mesoangioblasts in DMD patients started in June 2009, with a one year preliminary study (involving 28 DMD patients, aged 5-10), required to validate outcome measures. Six out of these patients will undergo successive intra-arterial transplantations at escalating doses of cells under a continuous regime of immune suppression. Safety will be the primary objective of the study. A possible increase in muscle strength as a consequence of mesoangioblast transplantation will also be evaluated.

Future strategies for autologous cell therapy based upon reversible immortalization of human mesoangioblasts and transfer of a human artificial chromosome containing the whole dystrophin gene, will be discussed.

4:30-4:50 p.m.: Alessandra Sacco, Ph.D., Sanford-Burnham Medical Research Institute
Short Telomeres and Stem Cell Exhaustion Model Duchenne Muscular Dystrophy in mdx/mTR Mice

Alessandra Sacco*1,2, Foteini Mourkioti*1, Rose Tran1, Jinkuk Choi3, Michael Llewellyn4, Peggy Kraft1, Marina Shkreli3, Scott Delp 4, Jason H. Pomerantz1,5, Steven E. Artandi3 and Helen M. Blau1
1 Baxter Laboratory for Stem Cell Biology, Department of Microbiology and Immunology, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
2 Current Affiliation: Muscle Development and Regeneration Program, Sanford-Burnham Medical Research Institute, 10901 N Torrey Pines Rd, La Jolla, CA 92037, USA
3 Department of Medicine, Cancer Biology Program, Stanford University School of Medicine, Stanford, CA 94305, USA
4 BioX Program, James H. Clark Center for Biomedical Engineering and Science, Stanford University, CA 94305, USA
5 Current Affiliation: Departments of Surgery and Orofacial Sciences, Division of Plastic and Reconstructive Surgery, Craniofacial and Mesenchymal Biology Program, Eli and Edythe Broad Center of Regeneration Medicine, University of California, San Francisco, 94143
* These authors contributed equally to this work.

In Duchenne Muscular Dystrophy (DMD), dystrophin mutation leads to progressive lethal skeletal muscle degeneration. Eventually in patients functional muscle tissue is supplanted by fibrosis, calcium deposits and adipose accumulation that coincides with clinical manifestations. Although clearly initiated by dystrophin deficiency, the pathophysiological cause of eventual failure of the tissue repair process is unknown. Unfortunately dystrophin deficiency does not recapitulate DMD in mice (mdx), which have mild skeletal muscle defects and potent regenerative capacity. The absence of a mouse model for DMD that faithfully mimics key features of the human disease has limited our understanding of its pathophysiology and tests of potential therapies. We postulated that human DMD progression is a consequence of loss of muscle stem cell function and that the mild mouse mdx phenotype results from greater reserve fueled by longer telomeres. To test this hypothesis, we crossed dystrophic mdx mice with mice lacking the RNA component of the telomerase enzyme Terc. We report that this novel mouse model has severe muscular dystrophy with profound muscle weakness, elevated serum enzyme levels, and increased muscle fibrosis and calcium deposits. Their muscle stem cells exhibit reduced proliferative and regenerative capacity in vitro and in vivo upon transplantation and their progeny has shortened telomeres. These data suggest that DMD progression results from a cell autonomous failure of muscle stem cells to maintain the damage-repair cycle initiated by dystrophin deficiency. The essential role of muscle stem cell function has implications for treatment approaches for DMD.

4:50-5:10 p.m.: Ilona Skerjanc, Ph.D., University of Ottawa

Retinoic Acid Enhances Skeletal Myogenesis in Human Embryonic Stem Cells by Expanding the Premyogenic Progenitor Population,

Tammy Ryan, Jun Liu, Alphonse Chu, Lisheng Wang, Alexandre Blais and Ilona S. Skerjanc
Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Rd, Ottawa, Ontario, Canada, K1H 8M5.

Human embryonic stem cells (hESCs) are a potential source of cells for cell therapy of muscle diseases. To date, it has proven difficult to generate skeletal muscle from hESCs in high yields and within a reasonable timeframe. Further, a human ES-derived Pax3/7-positive skeletal muscle progenitor population has not yet been described. Previous studies have shown that Pax3/7-positive progenitor cells can repopulate the satellite cell niche, indicating the importance of this population for therapy. We sought to optimize the differentiation of hESCs into skeletal muscle with a view to identifying distinct stages of myogenesis and shortening the time course. We treated hESCs with retinoic acid (RA) and found that skeletal myogenesis, and the expression of the myogenic regulatory factors (MRFs) Myf5, MyoD and myogenin were enhanced. Furthermore, we found that RA treatment expanded the muscle progenitor pool, which occurred as a distinct Pax3+ve/Meox1+ve population prior to MRF expression. Non-skeletal muscle tissue types, known to be enhanced by RA, were not significantly affected. We have identified a differentiation pathway in hESCs that provides a skeletal muscle progenitor population and a shorter time course of myogenesis. We propose that RA could fit into a directed culture method for deriving skeletal muscle from hESCs.

SMALL MOLECULE SESSION:

CHAIRS: JASBIR SEEHRA, PH.D. AND JEFFREY ROTHSTEIN, M.D., PH.D.
Tuesday, March 15 8:30 a.m. – 12:30 p.m.

8:30-9:00 a.m.: James Rusche, Ph.D., Repligen Corporation

Developing drugs that target gene expression to replace key proteins in single gene neurodegenerative diseases

James R Rusche1, Charlotte Sumner2, Christine DiDonato3, Heather Plasterer1, Joel Gottesfeld4, David Jacoby1
1Repligen Corporation, Research and Development, Waltham, MA, 02453, 2Johns Hopkins, Neurology, Balltimore, MD, 21287, 3Northwestern University, Pediatrics, Chicago, IL, 60614, 4 The Scripps Research Institute, Molecular Biology, La Jolla, CA, 92037

Spinal Muscular Atrophy (SMA) and Friedreich's ataxia (FRDA) each have unique genetic causes, neuropathologies, and disease presentations. However, these two diseases also share some striking similarities. They are both autosomal recessively inherited disorders that are caused by reduced expression of a single protein product. SMA is the consequence of mutation of the survival motor neuron 1 (SMN1) gene, reduced expression of full length SMN transcripts arising from a duplicate gene (SMN2), and consequently reduced levels of SMN protein. FRDA is caused by a GAA-CTT triplet repeat insertion in an intron of the frataxin gene that interferes with transcription elongation resulting in reduced amounts of the frataxin protein. An increase in gene expression can potentially be therapeutic since both SMN2 and mutant FXN would produce a native, fully functional protein. We are developing orally active, small molecules that can achieve gene expression changes and positive effects in animal models of disease. This presentation will summarize the pre-clinical characterization of the molecules and their targets and discuss issues that arise in advancing these experimental therapeutics through clinical development. Frataxin expression can be increased in patient derived cells and transgenic mice with an HDAC inhibitor of the benzamide structural group. Biomarkers of frataxin gene and protein changes have been developed to aid in assessing drug effects early in clinical research. Challenges also exist in how to determine optimal dosing to achieve target tissue changes of frataxin expression and how to measure treatment effects in FA patients. A variety of targets are being evaluated to achieve increased expression of SMN protein to treat SMA including HDAC inhibitors, splicing modulators, and translation termination blockers. A screen for increasing SMN2 promoter activity using production of a reporter gene product identified a compound that appears to result in modest increases in SMN protein and prolonged survival in mouse models of SMA. The protein target of these compounds is the mRNA cap degrading enzyme DcpS.

9:00-9:30 a.m.: John McCall, Ph.D., Pharmac LLC
Small molecule drug discovery in muscular dystrophy: what's involved?

John McCall, Ph.D.
Pharmac LLC

Translational research and development has as its ultimate goal registration of new therapies for particular diseases. Small molecure drug discovery proceeds through a series of well recognized steps. These milestone gated steps manage controllable risk and increase chances for future success. Chemical matter with drug potential can be identified in a variety of ways. High throughput screening, de novo design, repurposing, and blind luck can all play parts. The process of drug discovery will be reviewed and exemplified by a NFκB inhibitor that Validus Biopharma is developing for Duchenne.

9:30-9:50 a.m.: Stan Froehner, Ph.D., University of Washington

Sildenafil Improves Skeletal and Cardiac Muscle Function in the mdx Mouse

Justin M. Percival1, Nicholas P. Whitehead1, Heidi N. Gray2, Candace M. Adamo2, Joseph A. Beavo2 and Stanley C. Froehner1
1Departments of Physiology & Biophysics and 2Pharmacology, University of Washington, Seattle, WA.

Nitric oxide (NO) is indispensable for skeletal and cardiac muscle health. Disruption of NO signaling is associated with several muscular dystrophies including Duchenne and Becker dystrophies and certain Limb Girdle muscular dystrophies. In muscle tissue, NO signaling can lead to the production of the second messenger cGMP, switching on cGMP-activated serine/threonine kinase (PKG) that is thought to mediate many of the cytoprotective effects of NO. cGMP levels are decreased by the activity of phosphodiesterase 5 (PDE5) and the inhibition of PDE5 with sildenafil, an FDA-approved drug (Viagra/Revatio) has cardioprotective effects. Given reduced muscle NO signaling in many dystrophies, we reasoned that phosphodiesterase 5 (PDE5) inhibition may have significant potential as a relatively safe and rapidly implementable therapeutic approach. Our noninvasive echocardiographic studies of cardiac function showed that sildenafil administered at 12 months of age (after cardiomyopathy has already developed) caused an almost complete reversal of left ventricle dysfunction within a few days of treatment initiation (Adamo et al., PNAS 2010, 107:19079-83). Here, we report results on diaphragm, the skeletal muscle in mdx mice that best recapitulates the pathology and dysfunction seen in DMD patient muscles. Chronic oral administration of sildenafil to mdx mice significantly reduced fibrosis in the diaphragm at 4 months of age (as quantified by fibronectin levels). This pathological improvement was accompanied by a highly significant increase in specific force. These preclinical findings suggest that the PDE5 inhibitor, sildenafil, may be a useful therapeutic agent for the amelioration of both skeletal and cardiac muscle dysfunction in DMD boys. Research supported by NINDS, NIAMS, MDA, Parent Project Muscular Dystrophy and Charlie's Fund.

9:50-10:10 a.m.: Gino Cortopassi, Ph.D., UC Davis

Alterations in Thiredoxin-related antioxidants in Friedriech's ataxia models, and High-throughput screening

Gino Cortopassi, Yuxi Shan, Robert Schoenfeld, Mark Pook and Sunil Sahdeo
University of California, Davis
Gcortopassi@ucdavis.edu

Background/Hypothesis: Our hypothesis is that frataxin-mediated iron-sulfur dysfunction causes sensitivity to oxidative stress through deficiency of thioredoxin-related antioxidants. Methods: Our methods include microarray of neural tissues, QRTPCR and Western analysis of protein expression, biochemical and neurite extension and toxicitiy of dorsal root ganglion neurites, and high-throughput drug screening. Results. Microarrays of DRG neurons of the YG8 mouse model indicated decreases in thioredoxin-related transcripts, and in myelination transcripts, and increases in axonal transport transcripts. Biochemical analysis in frataxin-knockdown cells and DRG neurons indicated alterations in peroxiredoxin redox state and expression level. Frataxin knockdown cells have less thioredoxin reductase activity, and frataxin knockdown cells and DRG cells are less viable in the context of challenge with a thioredoxin reductase inhibitor. Screening of 12 inhibitors of thiol-antioxidants in Friedreich's fibroblasts and knockdown cells identified a differential sensitivity to diamide, a specific oxidizer of glutathione. Screening a library of 1060 FDA approved drugs identified several hits, which rescued from diamide sensitivity. These are now being tested in secondary and tertiary screens for function. Conclusions: Frataxin interacts with iron-sulfur related proteins and alters sulfur-amino acid and iron-sulfur biogenesis. How this may impact antioxidant status has not been clear. Our results imply that iron-sulfur deficiency alters redox state of thioredoxin-related antioxidants, decreasing the protection of DRG neurons and Schwann cells from oxidative stress, leading to neurodegeneration. A screen of a 1060-compound library based on diamide sensitivity identifies multiple leads of potential benefit for further testing as therapeutics.

10:30-11:00 a.m.: Jeffrey Rothstein, M.D., Ph.D, Johns Hopkins School of Medicine
Small molecules for ALS: Assessing candidate targets and optimizing clinical discovery

Jeffrey Rothstein, M.D., Ph.D.
Johns Hopkins School of Medicine

There has been great progress in the development of new small molecules and imaging agents that may be useful for ALS treatment and management. We will review several of the newer candidates drugs including muscle contraction enhancement (e.g. Cytokinetics), agents that alter neurotransmission (e.g ceftriaxone), mitochondrial function/energy metabolism (e.g. R-pramipexole). In addition- new molecule or cellular targets for possible therapy in ALS include microglia, astroglial as well as oligodendroglial cells will be discussed. Unexpected biology involving oligodendroglia/astroglia progenitors, NG2 cell, could provide powerful disease altering therapeutic targets. Finally, the development of cellular and neurotransmitter based PET imaging agents, including mGluR5, glycine transporter and glutamate transporters, could provide exceptionally valuable tools in ALS clinical trial design and small molecule efficacy analysis for future studies.

11:00-11:20 a.m.: Jiming Kong, M.D., Ph.D., University of Manitoba
Rescuing motor neurons by targeting the BNIP3 cell death pathway

Jiming Kong, PhD
Associate Professor
Department of Human Anatomy and Cell Science University of Manitoba
745 Bannatyne Ave
Winnipeg, MB R3E 0J9, Canada

Oxidative stress, mitochondrial dysfunction and morphologically necrotic-like motor neuron death are major features in ALS. Previously we showed that oxidative stress provided a redox signal to activate hypoxia-inducible factor 1 alpha (HIF-1), which is the primary, if not the only, transcriptional factor for the death-inducing gene BNIP3. Expression of BNIP3 caused a caspase-independent form of neuronal cell death in vitro and in vivo. Here we show that BNIP3 was induced to express at the onset of the disease in transgenic mice expressing the G93A and the G37R mutations of SOD1. BNIP3 was not detectable in the brain of control animals and in the G93A and the G37R mice before the onset of disease. Levels of BNIP3 expression increased with disease progression as evidenced by immunohistochemistry, Western blotting and RT-PCR analyses. The expressed BNIP3 was found to be primarily localized in motor neurons. BNIP3 was not detectable in the liver, kidney and lung tissues from the same groups of G93A and G37R animals that showed high levels of BNIP3 in the spinal cord. BNIP3 was detected in the mitochondrial membranes after alkaline extract, indicating that the expressed BNIP3 was active because inactive BNIP3 is known to be dissociated from mitochondria after alkaline treatment. To further determine the role of BNIP3 in mutant SOD1-induced neuronal death, a lentiviral shRNA vector targeting the nucleotides 167-188 of the BNIP3 mRNA, which was able to almost completely inhibit BNIP3 expression, was injected into the lumbar spinal cord of the G93A mice at the age of 8 weeks. Animals injected with a scramble shRNA vector were used as controls. Inhibition of BNIP3 by RNAi significantly increased the number of axons in the L5 ventral roots ($p=0.015$). Analysis of axon size distribution showed clearly the protection of middle to large (larger than 6 mm in inner diameter) axons by the lentiviral BNIP3 shRNA vector. We further analyzed the BNIP3 pathway and found that BNIP3 interacted with the ion channel VDAC to induce mitochondrial release of endonuclease G leading to a caspase-independent apoptosis. To look for an inhibitor for the BNIP3 pathway, we identified the small chemical necrostatin-1 that was able to inhibit BNIP3 cell death pathway by preventing integration of BNIP3 to the outer membrane of mitochondria. The results demonstrate that BNIP3 plays a role in mediating mutant SOD1-induced motor neuron death. The BNIP3-induced cell death pathway provides a molecular linkage for mitochondrial degeneration, oxidative stress and caspase-independent neuronal death. Necrostatin-1 appears to be a potent inhibitor for the BNIP3 pathway and may be a new therapy for ALS.

11:20-11:40 a.m.: Laxman Gangwani, Ph.D., Texas Tech University Health Science Center

JNK is required for neuron degeneration in spinal muscular atrophy

Author: Laxman Gangwani*
Affiliation: Center of Excellence in Neurosciences, Department of Biomedical Sciences, Paul L. Foster School of Medicine, Texas Tech University Health Science Center, El Paso, Texas 79905.
*Presenting author and send correspondence to laxman.gangwani@ttuhsc.edu

Spinal muscular atrophy (SMA) is caused by mutations of the Survival Motor Neurons (SMN1) gene. SMA is characterized by degeneration of spinal motor neurons caused by low levels of SMN protein. The cellular and molecular mechanisms underlying motor neuron degeneration in SMA are unknown. No treatment is available to cure or prevent motor neuron degeneration to reduce the burden of illness for SMA patients. We report that the brain specific isoform (JNK3) of c-Jun NH2-terminal kinase (JNK) group of mitogen-activated protein kinases (MAPK) mediates the degeneration of spinal motor neurons caused by SMN deficiency in mice with SMA. Reduced expression of the zinc finger protein ZPR1 that is required for accumulation of SMN in the nucleus also results in activation of the JNK signaling pathway and causes neuron degeneration. The deficiency of JNK3 and treatment with JNK inhibitors reduce degeneration of neurons lacking SMN. These data suggest that the JNK signaling pathway may mediate the neurodegeneration caused by SMN deficiency and represents a potential therapeutic target for the treatment of SMA.

11:40-12:00 p.m.: James Dowling, M.D., Ph.D., University of Michigan
Myotubular Myopathy and the Neuromuscular Junction: a Novel Therapeutic Approach?

Dowling, JJ, Buj-Bello, A, and C. Pierson

Myotubular myopathy (XLMTM) is a severe congenital myopathy characterized by hypotonia, weakness, and significantly reduced life span. XLMTM is due to mutations in myotubularin, a lipid phosphatase implicated in the regulation of endosomal sorting. Much remains to be understood about XLMTM disease pathogenesis, and no therapies currently exist. Recently, vertebrate models that accurate reflect the clinical course and histopathology of XLMTM have been developed. Using a zebrafish model of XLMTM, we have identified abnormalities in neuromuscular junction organization, and demonstrated that motor deficits in our mutant fish are improved by treatment with the acetylcholinesterase inhibitor pyridostigmine. Based on these findings, we hypothesized that aberrant neuromuscular junction (NMJ) transmission is a critical aspect of the disease process. We tested this hypothesis using two murine models of XLMTM, one a well-characterized gene knockout model and the other a novel gene knock-in model. In these models, we identified clear "clinical" evidence of an NMJ disorder: fatigable weakness, improved strength with edrophonium, and electrodecrement with repetitive nerve stimulation testing. We also uncovered prominent histopathologic abnormalities in the appearance and size of individual neuromuscular junctions, and disturbances in the overall organization of these junctions. These abnormalities, in addition, correlated with changes in gene expression of NMJ components. We are currently studying the mechanism(s) underlying these changes by examining acetylcholine receptor recycling and clustering. Lastly, we performed a placebo controlled, randomized trial of pyridostigmine in our XLMTM mouse models. We observed significant improvement in our pyridostigmine group in several motor parameters including fatigable weakness and endurance. In all, we describe a novel pathologic abnormality in XLMTM, and identify a potential treatment to overcome this abnormality and improve clinical course in the disease.

PROTEIN THERAPY SESSION:

CHAIRS: ROBERT MATTALIANO, PH.D., AND JUSTIN FALLON, PH.D.
Tuesday, March 15th 1:30 p.m. – 5:30 p.m.

1:30-2:00 p.m.: Robert Mattaliano, Ph.D., Genzyme Corporation (Keynote Speaker)

Developing A Muscular Dystrophy Therapy; Overcoming Barriers in Pompe Disease

Robert J. Mattaliano, PhD
Biologics Development, Genzyme Corporation, Framingham, MA, USA

Alglucosidase alfa, a recombinant human acid alpha-glucosidase, represents the first scientific and clinical breakthrough in the treatment of a life-threatening human myopathy, Pompe disease. This enzyme replacement therapy (ERT) offers a means of correcting the disease primary biochemical defect, lysosomal glycogen accumulation. Clinical trials have demonstrated that alglucosidase alfa administration prolongs survival in infants, those patients with the most severe and immediately life-threatening form of the disease.1-3 In older patients, the presentation of Pompe disease is clinically diverse and the rate of functional decline more difficult to predict. A randomized study in adults indicated that alglucosidase alfa treatment was associated with improvements in walking distance and stabilization of pulmonary function.4 However, the clinical spectrum of Pompe disease, and the therapeutic response to ERT observed in patients, creates a desire for additional research in the pathological and clinical implications of GAA deficiency.

The availability of alglucosidase alfa is the result of more than a decade of partnership and persistence to achieve a common goal between academics, clinical investigators, patient advocacy groups, government regulators and Genzyme. Together, these groups worked to overcome multiple technical and clinical development barriers culminating in regulatory approvals. Currently, over 1400 patients in 59 countries are receiving this therapy. Improvements to the current standard of care will require a better understanding of Pompe disease biology, and the utilization of refined and/or complementary clinical strategies. This presentation will highlight some of the barriers encountered during the development of alglucisidase alfa, the means by which they were overcome, and challenges in the treatment of Pompe disease yet to be addressed.

1. Kishnani PS, Corzo D, Nicolino M, Byrne B, Mandel H, Hwu WL, et al. Recombinant human acid alpha glucosidase: major clinical benefits in infantile-onset Pompe disease. Neurology. 2007;68:99-109.

2. Kishnani PS, Corzo D, Leslie ND, Gruskin D, van der Ploeg A, Clancy JP, et al. Early treatment with alglucosidase alfa prolongs long-term survival of infants with Pompe disease. Pediatr Res. 2009;66:329-55.

3. Nicolino M, Byrne B, Wraith JE, Leslie N, Mandel H, Freyer DR, et al. Clinical outcomes after long-term treatment with alglucosidase alpha in infants and children with advanced Pompe disease. Genet Med. 2009;11:210-9.

4. van der Ploeg AT, Clemens P, Corzo D, Escolar D, Florence J, Groeneveld GJ et al. A Randomized Study of Alglucosidase Alfa in Late-Onset Pompe's Disease. N Engl J Med 2010;362:1396-1406.

2:00-2:30 p.m.: Jasbir Seehra, Ph.D., Co-founder, Acceleron

Inhibition of the myostatin pathway for treatment of neuromuscular diseases

Jasbir Seehra, Ph.D.

Myostatin, a ligand belonging to the TGF-β superfamily is a negative regulator of muscle growth. The protein sequence and function of myostatin have been conserved through evolution. Deletion of the gene in mice, cattle, dogs, humans and other species deletions results in the double muscling phenotype, amply demonstrating the conservation of function in evolution. Neutralizing antibodies, soluble decoy receptors and engineered forms of naturally occurring antagonists have demonstrated function increase in muscle in normal and disease bearing mice. Therapeutic approaches based upon the inhibitors of the myostatin pathway are currently in clinical development. These include myostatin neutralizing antibodies, soluble forms of the myostatin receptor, activin receptor IIB (ActRIIB) and neutralizing antibodies to ActRIIB. The presentation will review status of therapeutic development with molecules targeting this pathway.

The most advanced clinical program is ACE-031, a fusion protein comprised of the soluble ActIIb linked to the Fc region of IgG1 has completed single and multiple dose studies in healthy volunteers. Consistent with the preclinical studies in mice, treatment with multiple doses of ACE-031increased total lean mass (5.25% by DXA) and thigh muscle volume (4.1% by MRI). In addition, serum biomarkers and body composition by DXA suggest that ACE-03 treatment increases bone mass and decrease fat mass.

ACE-031 is phase II study in boys with DMD is ongoing in Canada.

2:30-2:50 p.m.: Dennis Guttridge, Ph.D., Ohio State University

Understanding NF-κB Function and Its Therapeutic Potential in DMD

J.M. Peterson, S. Acharyya, W. Kline, B. Canan, D. Delfin, D. Ricca, A. Baldwin, J.R. Mendell, J.A. Raphael-Fortney, P.M.L. Janssen, and D.C. Guttridge;
TheraLogics, Inc., Chapel Hill, NC, and The Ohio State University College of Medicine, Columbus, OH, USA.

The NF-κB signaling pathway is implicated as a contributing factor of dystrophic pathology. Based on genetic and pharmacological studies performed with mdx mice, NF-κB is thought to contribute to DMD by at least two major mechanisms. One is by acting in macrophages to promote an inflammatory repertoire of genes that contribute to myofiber necrosis. The second is by functioning in myofibers to regulate factors that limit the regeneration potential of satellite cells. Recently our laboratory deduced that one of these factors was TNF. This cytokine, secreted from dystrophic myofibers in an NF-κB dependent fashion, is proposed to inhibit satellite cell activation by repressing the synthesis of the Notch-1 receptor through the recruitment of a corepressor complex at the Notch-1 promoter. Taken together, the data support that NF-κB may be considered as potential therapeutic target for the treatment of DMD. Recent steps in our laboratory have been made to develop an anti-NF-κB inhibitor compound possessing both a favorable efficacy and safety profile in DMD animal models. We are currently testing an 11 amino acid peptide called NBD that functions by inhibiting the activity of the IKK kinase complex, which is the immediate upstream activator of NF-κB. The data show that NBD can be formulated as a GLP compound that when treated to mdx mice improves histological parameters (inflammation, necrosis, and regeneration) of hindlimb muscles by >50%, and significantly also increases diaphragm contractile force by >35% ($p < 0.05$). Impressively, dosing more severely affected dko mice with NBD rescued diaphragm function by 25%, as well as significantly improved cardiac dysfunction to levels of wild type mice in response to both frequency dependence and Î²-adrenergic stimulation. Finally, CBC and serum chemistry analysis at doses of 10 mg/kg NBD show no evidence of toxicity by IP or IV delivery. These favorable findings support continued efforts to pursue NBD therapy as a treatment for DMD.

2:50-3:10 p.m.: Jachinta E. Rooney. Ph.D., University of Nevada

Laminin-111 protein therapy restores viability, reduces pathology and improves muscle strength in the dyW mouse model of Merosin Deficient Congenital Muscular Dystrophy Type 1A

Jachinta E. Rooney and Dean J. Burkin
Department of Pharmacology, University of Nevada School of Medicine, Reno, NV 89557

Congenital muscular dystrophies (CMD) are a group of neuromuscular disorders characterized by severe muscle hypotonia, generalized muscle weakness, contractures of variable severity, delayed motor milestones and premature death. Merosin deficient CMD (MDC1A) is a severe form caused by mutations in the LAMA2 gene, resulting in loss of laminin-alpha2 protein. There is currently no effective treatment or cure for MDC1A. Laminin-alpha2 is required for the formation of laminin-211 (alpha2, beta1, gamma1) and laminin-221 (alpha2, beta2, gamma1) (merosin) which form the basal lamina in adult skeletal muscle. Normal skeletal muscle development in laminin-alpha2 deficient muscle in utero is due to the expression of laminin-111 (alpha1, beta1, gamma1) the predominant form in embryonic skeletal muscle. We examined if delivery of this embryonic protein into laminin-alpha2 deficient mouse muscle could alleviate the histological damage associated with MDC1A. Laminin-111 protein treatment significantly reduced the muscle pathology, increased muscle strength and dramatically increased life expectancy. In addition, we show that laminin-111 prevented programmed cell death associated with disease progression in both laminin-alpha2 null mouse muscle and cultured MDC1A primary myogenic cells. Our results demonstrate for the first time that laminin-111 can serve as an effective protein replacement to prevent muscle disease in a laminin-alpha2 deficient mouse model of MDC1A. Supported by NIH R01AR053697 and Prothelia Inc., Milford MA

3:30-4:00 p.m.: Justin Fallon, Ph.D., Brown University
Biglycan as a candidate therapeutic for DMD

Justin R. Fallon, Ph.D.
Dept. of Neuroscience, Brown University

Duchenne Muscular Dystrophy (DMD) is caused by mutations in dystrophin and the subsequent disruption of the dystrophin-associated protein complex (DAPC). Utrophin is a dystrophin homolog expressed at high levels in developing muscle that is an attractive target for DMD therapy. We have shown that the extracellular matrix protein biglycan regulates utrophin expression in immature muscle and that recombinant human biglycan (rhBGN) increases utrophin expression in cultured myotubes. Systemically-delivered rhBGN upregulates utrophin at the sarcolemma and reduces muscle pathology in the mdx mouse model of DMD. RhBGN treatment also improves muscle function as judged by reduced susceptibility to eccentric contraction-induced injury. Utrophin is required for the rhBGN therapeutic effect. Several lines of evidence indicate that biglycan acts by recruiting utrophin protein to the muscle membrane. RhBGN is well tolerated in animals dosed for as long as three months. These findins indicate that rhBGN could be a therapy for DMD. We are currently working to complete preclinical development with the goal of carrying out clinical trials of rhBGN in DMD patients.

4:00-4:20 p.m.: Tom Thompson, Ph.D., University of Cincinnati
How structures of myostatin can help with inhibitor design.

Thomas B. Thompson1,*, Jennifer Cash2, Betsy Angerman3, Henry Keutmann^
4Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Sciences Building,
Cincinnati, Ohio 45267
^Reproductive Endocrine Unit, Massachusetts General Hospital,
Boston, Massachusetts 02114
*Corresponding author:tom.thompson@uc.edu

Understanding the structure of myostatin and its antagonism Myostatin is a member of the TGFβ family of ligands and is a well-established inhibitor of muscle mass. Deletion of the myostatin gene or inhibitors of myostatin signaling results in animals with increased muscle mass. Therefore, therapies that target myostatin activity are in great demand and furthermore are needed to determine if myostatin inhibition will be an effective treatment option for MD patients. Here we present the structure of myostatin in complex with two different protein antagonists, follistatin and follistatin-like 3. We also compare the structure of myostatin to other TGFβ family members to define unique ligand properties needed for signaling, degradation and interaction with antagonists. We also present modifications to follistatin that make it a more attractive to therapy.

4:20-4:40 p.m.: Michael Lawlor, M.D., Ph.D., Children's Hospital of Boston

Weakness and Responses to Treatment Are Dependent on Fiber Type and Mutation in Murine Models of Myotubularin Deficiency.

Michael W. Lawlor1, Robert W. Grange2, Jeffrey J. Widrick3, Marissa G. Viola1, Rachel V. Edelstein1, Christopher R. Pierson4, Anna Buj-Bello5, Jennifer L. Lachey6, Jasbir S. Seehra6, Alan H. Beggs1

X-linked myotubular myopathy (XLMTM) is a congenital myopathy caused by deficiency of the lipid phosphatase, myotubularin. XLMTM patients often present with severe perinatal weakness that requires mechanical ventilation to prevent death from respiratory failure. Muscle biopsies from XLMTM patients display small myofibers with central nuclei and central aggregations of organelles in many cells. We postulated that therapeutically increasing muscle fiber size would cause symptomatic improvement in myotubularin deficiency. Recent studies have elucidated an important role for the activin receptor type IIB (ActRIIB) in the regulation of muscle growth, and have shown that ActRIIB inhibition results in significant muscle hypertrophy. We recently reported that treatment with the extracellular domain of the activin type IIB receptor fused to a murine Fc region (ActRIIB-mFC) produced a 17% extension of lifespan in severely symptomatic (Mtm1Δ) myotubularin-deficient mice, with transient increases in body mass, forelimb grip strength, and myofiber size. Additionally, pathological analysis of Mtm1Δ mice during treatment revealed that ActRIIB-mFC produced marked hypertrophy restricted to type 2b myofibers,. Our subsequent studies have focused on the potential of ActRIIB-mFC as a treatment for moderately symptomatic (Mtm1C205T) myotubularin-deficient mice, and this preclinical trial is currently in progress. Our preliminary studies suggest that the fiber type composition of muscles in Mtm1C205T mice differs dramatically from that seen in Mtm1δ mice, with a greater number of oxidative fibers seen in Mtm1C205T mice. Contractile studies of untreated Mtm1δ and Mtm1C205T mice also revealed marked differences in the observed physiological deficits in these mice. While ex vivo field stimulation of soleus and extensor digitorum longus (EDL) muscles of Mtm1δ mice showed marked decreases in isometric force in comparison to WT counterparts, Mtm1C205T mice showed marked decreases in isometric force in the EDL and higher than normal strength in the soleus. Preliminary contractile studies performed on ActRIIB-mFC treated Mtm1C205T mice revealed a treatment-dependent increase in the contractile strength of the soleus muscles, with no effect on the EDL muscles. These findings suggest that the effects of myotubularin deficiency are fiber type specific, and that the different mutations in these two models may determine whether oxidative fibers are capable of responding to ActRIIB-mFC treatment.

[1] Division of Genetics and Program in Genomics, Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts

[2] Virginia Polytechnic Institute and State University, Department of Human Nutrition, Foods, and Exercise, Blacksburg, Virginia

[3] Dept. of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital and Harvard Medical School, Boston, Massachusetts

[4] Research Institute, Nationwide Children's Hospital and Department of Pathology, Ohio State University College of Medicine, Columbus, Ohio

[5] GENETHON (Human Genome Research Centre), Evry, France

[6] Acceleron Pharma Inc. Cambridge, Massachusetts

4:40-5:00 p.m.: Thien Nguyen, M.D., Ph.D., Johns Hopkins School of Medicine
Axonal protective effects of netrin-1

Osefame Ewaleifoh1, John W. Griffin1,2,3, Thien Nguyen1
Departments of 1Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, 2Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21287; 3Department of Pharmacology, The Johns Hopkins University, Baltimore, Maryland 21205.

Axonal degeneration is an inevitable feature of CMT type 1 and is the primary determinant of irreversible clinical deficits seen in these patients. Prevention of the axonal degeneration thus likely improves clinical outcomes for patients with CMT 1. The same principal should also be applicable to other types of CMT, such as CMT type 2. Together, these inherited peripheral nerve disorders reinforce an important message. Mutations in genes that are primarily expressed in myelinating Schwann cells may instruct axons to degenerate through certain specific signaling molecules. Myelin-associated glycoprotein (MAG) is one of these molecules. We have shown that MAG signals to the axon promote stability of axonal microtubules and axonal survival in the face of insults, such as vincristine, acrylamide and inflammatory mediators. Another candidate molecule with similar characteristics is netrin-1, which is also present at the Schwann cell-axon interface of all myelinated internodes. This report establishes for the first time that netrin-1 also promotes resistance to axonal injury and prevents axonal degeneration in post-natal neuronal cell culture. This effect on axonal stability depends on the interaction with UNC5, DSCAM, and/or b-1-integrin receptors with netrin-1. Unlike MAG where its expression remains unchanged in trembler-J (TrJ) mice (a mouse model of human CMT1 disease), our preliminary data show that netrin-1 expression is markedly reduced by 3-4 folds in TrJ mice. This suggests that netrin-1 may play an important role in axonal survival in CMT and thus may serve as a potential target for therapy. Exploiting this pathway may lead to important neuroprotective strategies to treat CMT and other neurological diseases characterized by axonal loss.

GENE THERAPY SESSION:

CHAIRS: LOUIS M. KUNKEL, PH.D. AND BRIAN KASPAR, PH.D.
Wednesday, March 16th 8:15 a.m. – 12:30 p.m.

8:25-9:10 a.m.: James Wilson, M.D., Ph.D., University of Pennsylvania (Keynote Speaker)

Immunology and delivery – the key challenges for in vivo gene therapy

James Wilson, M.D., Ph.D.
University of Pennsylvania

An important challenge for gene therapy of neuromuscular diseases is delivery of the therapeutic gene to a wide range of tissues and cells. This likely requires systemic administration of vector which is inherently inefficient because of physical barriers of the microcirculation (contiguous endothelia and basal lamina) and blood brain barrier which interfere with transport of the vector from the vascular space to the relevant target cells such as muscle fibers and neurons. Systemic delivery of large quantities of vectors is complicated by a number of host defense responses including: pre-existing neutralizing antibodies which interfere with transduction and potentially confound toxicity, T cell responses to transduced cells via epitopes contained on the capsid or expressed by the transgene, and B cell responses to vector with diminish re-administration of vector or to the transgene product that may inhibit its function.

Vectors most commonly used for in vivo gene therapy are based on adeno-associated viruses (AAV). First generation applications of AAV gene therapy were based on the serotype 2 virus. We created vectors based on serotype 1 which were shown to have superior properties in terms of muscle gene transfer and are in phase II and III studies for a several recessive genetic diseases. More recently we discovered an expanded family of novel AAVs from primary isolates of monkeys and humans which are being widely used by the community in pre-clinical models of gene therapy for neuromuscular disease – the most popular are those based on AAV8 and AAV9. The interesting property of AAV9 is that it seems capable of overcoming the physical barriers of delivery noted above. We have collaborated extensively with Mavis McKenna from UF to define the three dimensional structures of many of these novel AAVs which has provided a basis for better understanding their biologies. We also have assimilated the largest experience of monitoring/evaluating immune responses to AAV gene therapy in large animals and humans. My talk today will review studies directed to an understanding of the limitations of AAV delivery and ways we may be able to overcome it. I will also discuss the potential for deleterious immune responses.

9:10-9:40 a.m.: Jeffrey Chamberlain, Ph.D., University of Washington
Gene therapy for the muscular dystrophies: overcoming the remaining challenges

9:40-10:00 a.m.: Joel Chamberlain, Ph.D., University of Washington

Validity of RNAi-based therapeutics as a treatment for FSHD as demonstrated in a mouse model of muscular dystrophy

Joel R. Chamberlain1, Sergia Bortolanza2,3, and Davide Gabellini2,3
1Department of Medicine, University of Washington, Seattle, WA, 98195
2Dulbecco Telethon Institute and 3Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

Facioscapulohumeral muscular dystrophy (FSHD) is a common dominant genetic disease for which no curative therapy exists. The predominant clinical disease feature is upper body muscle weakness resulting from progressive muscle wasting. Recent progress in understanding the underlying cause of FSHD has revealed that disease likely results from both chromatin relaxation, as a consequence of contraction of D4Z4 macrosatellite repeats (FSHD1) or from other influences (FSHD2), and a functional polyadenylation sequence that is permissive for DUX4 expression. DUX4 protein expression in a cultured muscle cell line causes an increased sensitivity to oxidative stress and causes cell death. Cellular toxicity of DUX4 in conjunction with detection of polyadenylated DUX4 full-length transcripts and protein in FSHD myoblasts, suggests a DUX4 gain-of-function mechanism for FSHD pathogenesis. For dominantly inherited diseases associated with gain-of-function mechanisms, a straightforward therapeutic scheme would be to eliminate the genetic product that governs all pathogenic processes by using RNA interference (RNAi). The in vivo model chosen to test our RNAi therapeutic approach is the FRG1 mouse that displays a variety of typical physical and molecular features of FSHD patients and is the first animal model of an FSHD-like disease. Using the FRG1 mouse model we can harness the endogenous cellular RNAi pathway to degrade mRNA from genes expressing a toxic product in a targeted fashion. We have packaged modified short hairpin RNA (shRNA) expression cassettes targeting FRG1 mRNA into muscle-tropic adeno-associated virus serotype 6 (AAV6) shuttles for systemic delivery in vivo. Intravenous tail vein injection of the AAV6 FRG1 shRNAs into dystrophic mice resulted in significant improvements in histological features, including a reduced fiber size, central nucleation, adipose accumulation, and fibrosis. We also observed a significant improvement in muscle function as measured by treadmill running relative to saline-injected controls. Our data indicate that RNAi-mediated mRNA knockdown is a feasible approach to disease therapy and can be applied after onset of symptoms of an FSHD-like muscular dystrophy in mice to reverse the course of the disease. Application of this method to target relevant components involved in the complex cascade of events leading to disease offers a route to clinical application of RNAi-based therapeutics for treatment of FSHD.

10:00-10:20 a.m.: Dawn Delfin, Ph.D., Ohio State University

A novel treatment strategy for muscular dystrophy-based cardiomyopathy and heart failure: gene therapy using the tight junction protein claudin-5.

Dawn A. Delfín1, Kevin E. Schill1, Ying Xu2, Benjamin Canan2, Jeffrey S. Chamberlain3, Paul M.L. Janssen2 and Jill A. Rafael-Fortney1, Departments of 1Molecular & Cellular Biochemistry and 2Physiology & Cell Biology, College of Medicine, The Ohio State University, Columbus, Ohio 43210; 3Dept. of Neurology, University of Washington, Seattle, WA 98195.

We have previously demonstrated heart-specific reductions of the tight junction protein claudin-5 in a mouse model of severe muscular dystrophy and heart failure (dystrophin/utrophin-deficient [dko] mice). We have also shown the broad clinical relevance of these reductions. Over 60% of human cardiac explant samples, from patients with heart failure due to a variety of primary etiologies, show significantly reduced claudin-5 levels. The loss of claudin-5 occurs coincidently with the onset of early indicators of heart failure in dko mice around 8 weeks-of-age when these mice show depressed active cardiac contractile force development, an impaired force-frequency relationship, and a severely blunted β-adrenergic response. We hypothesized that sustained expression of claudin-5 in dko hearts would prevent heart failure progression. We therefore treated 4 week-old dko mice via a single intravenous injection with recombinant adeno-associated virus serotype 6 containing a claudin-5 cDNA expression cassette (AAV6-C5), to confer striated muscle-specific expression of claudin-5. We then analyzed hearts from AAV6-C5 treated dko mice compared to phosphate-buffered saline (PBS) injected dko controls at 8 weeks-of-age. Claudin-5 was expressed in cardiac tissue of AAV6-C5-injected dko mice to levels higher than in PBS dko controls. Active developed contractile force doubled (37°C, 4 Hz; $p<0.05$), the β-adrenergic response was increased nearly two-fold, and the force-frequency relationship was improved in isolated multicellular cardiac preparations from AAV6-C5 treated dko mice. Cardiac damage—assessed by the presence of degenerating cardiomyocytes and fibrotic scars—was also substantially reduced by AAV6-C5 treatment. Together, these data support that claudin-5 prevents cardiac dysfunction and represents a completely novel treatment target for heart failure. Supported by the Muscular Dystrophy Association and NIH T32 HL098039.

10:50-11:20 a.m.: Brian Kaspar, Ph.D., Nationwide Children's Hospital
Gene Delivery for Motor Neuron Disease: Pathway to the Clinic

11:20-11:40 a.m.: Zejing Wang, M.D., Fred Hutchinson Cancer Research Center

Long term transgene expression and amelioration of muscle function following large scale rAAV-micro-dystrophin treatment in dogs with Duchenne muscular dystrophy

Zejing Wang, MD, PhD1*, Rainer Storb, MD1, Donghoon Lee, PhD2, Christine Halbert, PhD3, Martin K Childers, D.O., PhD4, Glen Banks PhD5, Martin Kushmeric, MD, PhD2, Dusty Miller, PhD3, Jeffrey S Chamberlain, PhD5 and Stephen J Tapscott, MD, PhD1,3,5.
1 Transplantation Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; 2 Radiology, University of Washington, Seattle, WA; 3 Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; 4 Neurology and Regenerative Medicine, Wake Forest University, NC; 5 Neurology, University of Washington, Seattle, WA.

Duchenne Muscular Dystrophy (DMD) in both humans and dogs (cxmd) is a fatal, X-linked, recessive muscle disease caused by mutations in a single gene, dystrophin. Adeno-associated viral (AAV) vectors mediated gene therapy holds great promise for treating DMD. While the approach has been successful in the mouse model of the disease, mdx, many barriers remain to be addressed before translation into human trials. By using a clinically relevant dog model of DMD, we previously showed that intramuscular injections of different AAV serotypes resulted in robust T-cell responses to viral capsid proteins. Others have shown that cellular immunity to AAV capsid proteins coincided with liver toxicity and elimination of transgene expression in a trial of human hemophilia B. We then demonstrated that the inflammatory responses can be monitored by ELISpot assay and non-invasive magnetic resonance imaging (MRI) systemically and locally, respectively. We further demonstrated that immune response to AAV vectors in DMD dogs could be averted by a brief course of immunosuppression with anti-thymocyte globulin (ATG), cyclosporine (CSP), and mycophenolate mofetil (MMF), which resulted in long-term and robust expression of transgenes in the skeletal muscle. Here, our studies demonstrated that long term expression (2 years) of a species – specific, functional canine-μ-dys can be achieved in large scale in skeletal muscles of DMD dogs with the application of ATG/CSP/MMF. More over, for the first time, the amelioration of muscle histology and function as a result of sustained expression of the therapeutical gene was achieved and demonstrated by MRI and by kinametic gait analysis, respectively. The results demonstrated the feasibility of translating these strategies to a human trial of DMD.

11:40-12:00 p.m.: Louise Rodino-Klapac, Ph.D., Nationwide Children's Hospital and Research Institute

rAAV5 Mediated Delivery of Dysferlin as a Therapeutic Strategy for LGMD2B and Miyoshi Myopathy

Louise R. Rodino-Klapac, K. Reed Clark, Danielle Griffin, Kimberly M. Shontz, Chrystal L. Montgomery, Vinod Malik, Paul M.L. Janssen, Robert H. Brown Jr., Jerry R. Mendell

The dysferlinopathies comprise a group of untreatable muscle disorders including limb girdle muscular dystrophy type 2B, Miyoshi myopathy, distal anterior compartment syndrome, and rigid spine syndrome. The size of the DYSF cDNA (6.5 kb) negates packaging into traditional AAV serotypes known to express well in muscle (i.e. rAAV1, 2, 6, 8, 9). Potential advantages of a full cDNA versus a mini-gene include: maintaining structural-functional protein domains, evading protein misfolding, and avoiding novel epitopes that could be immunogenic. Findings demonstrating that rAAV5 capsid packages up to 8.9 kb [Alloca, M et al. (2008)], prompted us to generate a vector containing full-length DYSF. Additional studies revealed homologous recombination of the transgene cassette from two virions as the hypothesized mechanism for full-length transgene expression. Herein we describe our in vivo work delivering AAV5.DYSF to skeletal limb muscle and diaphragm of dysferlin deficient (Dysf-/-) mice by both intramuscular and vascular approaches. We generated a cassette containing the DYSF cDNA driven by the muscle specific MHCK7 promoter and packaged it into an AAV2/5 vector. Physiological characterization of three dysferlin deficient mouse strains (AJ, SJL, and 129-Dysf tm1Kcam/J) revealed a significant functional deficit in the diaphragm but not skeletal muscle. To test the efficacy of AAV5 mediated dysferlin gene replacement, we performed intramuscular injections in skeletal muscle and diaphragm of 4-6 week old Dysf -/- mice (all three species) with rAAV5.MHCK7.DYSF. The number of transduced muscle fibers reached 67.3 ± 13.1% with Bioquant image analysis and western blots demonstrating restoration of expression to wild-type levels. This was accompanied by functional improvement in both force generation and resistance to fatigue. Molecular characterization of transduced muscle revealed full-length DYSF genomes and transcript supporting restoration through homologous recombination. Intravascular regional gene transfer through the femoral artery achieved comparable levels of transduction and enabled targeting of specific muscle groups affected by the dysferlinopathies setting the stage for potential translation to clinical trials. These experiments support the use of AAV5 for delivery of the DYSF gene in patients with dysferlinopathies.

Poster Presentations

1. MOHAMMED AKAABOUNE, UNIVERSITY OF MICHIGAN

Molecular dynamics of nicotinic acetylcholine receptors at the neuromuscular junction deficient in alpha syntrophin in Vivo

Isabel Martinez1, Marcelo De Olievra1, Chakib Mouslim1, Marvin Adams3, Stanley Froehner3 and Mohammed Akaaboune1,2
1: Department of Molecular, Cellular and Developmental Biology and 2: Program in Neuroscience, University of Michigan, Ann Arbor, Michigan; 3: Department of Physiology and Biophysics, University of Washington, Seattle

The Dystrophin glycoprotein complex (DGC) is critical for both integrity of the muscle cells and stability of neuromuscular synapses. Using mice deficient in alpha syntrophin, a cytoplasmic component of the DGC, we found that rates of AChRs turnover are increased significantly compared to wild type synapses. Most of removed receptors from syn-/- synapses are targeted to degradation as few internalized AChRs are able to recycle into the postsynaptic membrane. Analysis of syn-/- NMJs during synaptic development shows that alpha syntrophin is dispensable for maturation as synapses form normally but mature aberrantly containing few receptors. Surprisingly, the low number and density of AChRs in mature syn-/- NMJs is not a consequence of a deficiency in AChR transcription as transcript levels of AChRs and chromatin decondensation and histone H3 phophos-acetylation are undistinguishable from wild type synapses . These results demonstrate the involvement of alpha syntrophin in the maintenance of the synapse and establish the role of this protein as a key control point for regulation of receptor trafficking and stability.

2. HUGH ALLEN, NATIONWIDE CHILDREN'S HOSPITAL

Natural history of cardiomyopathy in Duchenne muscular dystrophy and the effects of angiotensin-converting enzyme inhibitor and/or β-blocker

Philip T. Thrush MD, Laurence Viollet PhD, Kevin M. Flanigan MD,
Jerry R. Mendell MD, Hugh D. Allen MD
Nationwide Children's Hospital Heart Center and Center for Gene Therapy, Columbus, OH

Introduction: Cardiomyopathy (CM) is an invariable consequence in Duchenne muscular dystrophy (DMD). Several published reports suggest treating with angiotensin-converting enzyme inhibitors (ACEI) and/or β-blockers (BB), but few large series have been reported. The purpose of this study is to present a large experience with 65 DMD patients with CM treated with either ACEI or ACEI and BB. The natural history of CM in DMD prior to therapy and their responses to therapy are shown.

Methods: Serial echocardiograms were prospectively performed and therapy arbitrarily started when EF <55%. Twenty patients were excluded from treatment analysis due to prior therapy, short follow-up (<6 mo), or inadequate echocardiographic images. Acceptable EF was obtained in 45 patients at the initiation of therapy. Initial lisinopril dosage was either 2.5mg or 5 mg depending on weight. Dosages increased if continued decrease was noted in EF. BB therapy was initiated when average heart rate on Holter monitor >100 beats/min. Data were analyzed using paired t-test and ANOVA.

Results: Natural history data were available for 24 patients with a downward trend and eventual progression to cardiomyopathy (R2=0.22). The mean age at initiation of ACEI was 14.7 years (SD 4.4 years). Mean EF at initiation of ACEI was 44.3% (SD 8.3%). BB therapy was used in 25/45 patients. Both ACEI only group and ACEI + BB group demonstrated significant improvement compared to the natural history control group ($p<0.0001$ and $p< 0.001$, respectively). There was no significant difference in degree of improvement between the treatment groups, $p= 0.947$. Patients treated with ACEI and BB demonstrated progressive deterioration in EF after 18 months.

Conclusions: All patients with DMD enrolled in this study showed a gradual decline in myocardial function. Treatment with ACEI or ACEI and BB resulted in significant improvement during the first 12 months compared

to natural history. There is no significant difference in EF improvement between either treatment group. Effectiveness of treatment with either ACEI or ACEI and BB is unsustained as the disease progresses.

3. ELLIOT ANDROPHY, INDIANA UNIVERSITY

Characterization and Optimization of New Chemical Leads for the Treatment of SMA

Jonathan Cherry*1, Matthew Evans1, Sungwoon Choi2, Gregory Cuny2, Marcie A.Glicksman2, Erik Osman3, Christian Lorson3, and Elliot Androphy1
1 Indiana University School of Medicine, Indianapolis, IN; 2 Laboratory for Drug Discovery in Neurodegeneration, Harvard Neuro
Discovery Center, Brigham and Women's Hospital, Cambridge, MA; 3 University of Missouri, Columbia, MO

From a reporter cell-based high throughput screen of a chemical diversity library for entities that increase SMN protein from the SMN2 gene, we selected two scaffolds to characterize and optimize. We have synthesized and tested over 100 analogues and selected compounds for increased activity and improved drug-like characteristics. At nanomolar concentrations, these two lead compounds increased total SMN protein level up to 2-fold in 3813 SMA-derived fibroblasts and increase SMN gem counts up to 3-fold. One of these compounds increased SMN levels > 2-fold in brain tissue from the SMNΔ7 mouse following intraperitoneal administration. We are using our reporter assay to drive medicinal chemistry and explore the structure activity relationships (SAR) for these two leads. We have identified compounds with increased potency and a nearly 3 time longer half-life in the in vitro mouse metabolic stability assays. As we select new active analogues, changes in SMN mRNA and protein levels will be evaluated in our reporter assay and in primary SMA-derived human fibroblasts, and active, pharmacologically improved compounds will be tested in SMA model mice.

4. LILI ANGLISTER, HEBREW UNIVERSITY

Endocrine regulation of serum cholinesterases in normal and dystrophin-deficient mutant mice

Andrea R. Durrant and Lili Anglister
Department of Medical Neurobiology, Institute for Medical Research (IMRIC), Hebrew University Medical School, Jerusalem 91120, Israel

Cholinesterases (ChEs) include acetylcholinesterase (AChE) and butyrylcholinesterase (pseudocholinesterase, BChE) and are abundant in the nervous system and other tissues. While the role of AChE in terminating cholinergic transmission is well established, the role of BChE is not yet clear. Recent evidence suggests that both enzymes may function in normal development of the nervous system and participate in neurodegenerative diseases. Moreover, as ChEs levels change during development, they might reflect (or influence) muscle growth and differentiation and relate to the pathologies in muscular dystrophies. Because AChE activity in sera of dystrophin-deficient mutant (mdx) mice, an animal model for Duchenne muscular dystrophy, remains constant, whereas in normal mice it decreases towards puberty, we investigated whether the ChEs are subjected to hormonal regulation, which may be impaired in mdx mutants. First, we found that there was more BChE than AChE in mice sera. But while AChE increased in mdx-sera, BChE was a third lower than in wt, resulting in a total ChE activity decrease in mdx sera compared to control. Second, we found that testosterone (T) was a negative modulator of BChE but not of AChE in mouse serum: orchidectomy elevated BChE activities in both strains, and T-replacement (via T-releasing implant) reversed the effect. Removal and replacement of T had no effect on AChE. These findings are consistent with the involvement of the hypothalamic–hypophysial axis in the impairment of BChE activity in mdx-sera. Third, mdx BChE activities were significantly elevated after orchidectomy, reaching the gonadal baseline level of wt BChE, but not the agonadal BChE baseline level of orchidectomized wt. Thus, it could be that circulating T-levels and function (in regulating BChE) were normal in mdx; yet, the circulating growth hormone (GH) levels, which can also act as negative modulator of serum ChE, were high in mdx. Consistent with this notion of differential roles for T and GH, the appearance of dimorphism in body weight of mdx mice was delayed, although they were reproductively competent. Our studies are being

extended by examining the endocrine regulation of ChE (specifically by T) in normal and dystrophic muscles and in sera of other mammalian systems (bovine, primate) closer to humans. Determining the regulation of circulating ChEs in mammals may reveal dystrophin-dependent factors that cause the abnormalities in serum ChEs, as well as in other properties of dystrophic animals (or humans), and consequently help develop therapeutic means to repair the abnormalities. (Supported by MDA)

5. ANTHONY ANTONELLIS, UNIVERSITY OF MICHIGAN

High-throughput mutation analysis of the human aminoacyl-trna synthetase genes: in search of additional loci responsible for inherited peripheral neuropathies

Anthony Antonellis (1,2), Heather McLaughlin (1), NISC Comparative Sequencing Program (3,4), Victor Ionasescu (5), James R. Lupski (6,7,8,9), Garth Nicholson (10,11), Kevin Talbot (12), Jeff Vance (13), and Stephan Zuchner (13).
1) Departments of Human Genetics, and (2) Neurology, University of Michigan Medical School, Ann Arbor, MI, USA; (3) Genome Technology Branch, and (4) NIH Intramural Sequencing Center (NISC), National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; (5) Division of Medical Genetics, Department of Pediatrics, University of Iowa, Iowa City, IA, USA; (6) Departments of Molecular and Human Genetics, (7) Neurology, and (8) Pediatrics, Baylor College of Medicine, Houston, TX, USA; (9) Texas Children's Hospital, Houston, TX, USA; (10) Northcott Neuroscience Laboratory, ANZAC Research Institute and Molecular Medicine Laboratory, Concord Hospital, Concord, New South Wales, Australia; (11) Faculty of Medicine, University of Sydney, Camperdown, New South Wales, Australia; (12) Department of Clinical Neurology, University of Oxford, Oxford, UK; and (13) Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA.

Aminoacyl-tRNA synthetases are a ubiquitously expressed, essential class of enzymes responsible for charging tRNA molecules with cognate amino acids. The human nuclear genome encodes all 37 ARSs, which include 17 enzymes responsible for charging tRNA in the mitochondria, 17 enzymes responsible for charging tRNA in the cytoplasm, and 3 bi-functional enzymes; the latter charge tRNA molecules in both locations. Mutations in five genes encoding aminoacyl-tRNA synthetases have been implicated in inherited neurological diseases in human and mouse, and two of these (those encoding glycyl- and tyrosyl-tRNA synthetase) are associated with discreet human peripheral neuropathies characterized by either a primary axonopathy or an intermediate form of Charcot-Marie-Tooth disease. These data strongly suggest that all ARS genes should be considered excellent candidates for harboring mutations in patients with peripheral neuropathy and no known mutation. To address this, we have compiled a cohort of 364 such patients, the majority of which have been diagnosed with axonal peripheral neuropathy by a collaborating physician. Subsequently, we screened this cohort for mutations in the 37 human ARS genes using a high-throughput PCR- and DNA sequencing-based approach. These efforts have revealed 146 novel predicted missense variants in 25 different ARS genes. Importantly, missense mutations are a hallmark of disease-associated glycyl- and tyrosyl-tRNA synthetase mutations, deeming this data set relevant for follow up analyses. A major effort is now underway to evaluate these variants for disease association and functional consequences. The patient cohort and sequencing strategy will be presented along with interesting vignettes from our genetic and functional characterization studies.

6. ANDREA ARNETT, UNIVERSITY OF WASHINGTON

rAAV6-mediated expression of Dp116 in mdx:utrn-/- mice does not protect myofibers from dystrophic degeneration

Arnett, AL1, 2, Odom, GL1, Boyle, K1, Judge, LM1, 2, Finn, E1, and Chamberlain, JS1
1University of Washington, Department of Neurology 2Medical Scientist Training Program, University of Washington

Mice deficient in both dystrophin and utrophin (mdx:utrn-/-) exhibit a phenotype similar to that seen in DMD patients, including severe muscle wasting, skeletal deformities, joint contractures, and premature death. In these mice, the absence of both dystrophin and utrophin leads to extremely low expression of members of the dystrophin-glycoprotein complex (DGC). Using transgenic mice expressing the Dp116 isoform of dystrophin in skeletal muscle, we have shown that expression of Dp116 on the mdx:utrn-/- background leads to a dramatic increase in lifespan and muscle mass. In contrast, Dp116 expression has minimal effects on mdx mice. Dp116 is a non-muscle isoform of dystrophin that lacks the actin-binding domains and the majority of the rod domain found in the full-length dystrophin isoform. Our studies suggest that Dp116 does not have a mechanically strong link to the actin cytoskeleton, but can still assemble and stabilize the DGC at the sarcolemma. To decipher the mechanism through which Dp116 is contributing to phenotypic improvement in mdx:utrn-/- mice, we systemically introduced Dp116 into young mdx:utrn-/- mice via recombinant adeno-associated viral vectors (rAAV6). Interestingly, Dp116-positive fibers were not protected from dystrophic turnover, and transduced fibers were gradually lost from the muscle before any beneficial effects on the mice were observed. These results are suggestive of a non-mechanical mechanism for Dp116-mediated rescue. Further analysis of wt and mdx mice injected with rAAV carrying a reporter construct, revealed that rAAV6 vectors do not efficiently transduce muscle satellite cells in vivo. Thus, the loss of rAAV-delivered Dp116 appears to result from an inability of Dp116 to prevent myofiber necrosis, which is then compounded by regeneration of muscle by satellite cell progeny that do not carry Dp116 genomes. In contrast, muscle regeneration in Dp116 transgenic mdx:utrn-/- mice results in continuous expression of Dp116 in regenerated myofibers which leads to the a dramatic phenotypic amelioration. In addition, we have generated a series of staggered deletions in the Dp116 cDNA within the dystroglycan binding domain (DgBD), including the WW and ZZ domains. Each deletion construct demonstrates a distinctive intracellular distribution when expressed in mdx muscle, and none are protective. Furthermore, analysis of other DGC members suggests that DGC assembly and expression is uniquely altered with each deleted domain. These unique localization and expression patterns indicate that subdomains within the DgBD are not functionally equivalent.

7. WILLIAM ATCHISON, MICHIGAN STATE UNIVERSITY

A mutation in the Ca2+ channel β subunit at the NMJ decreases end-plate area and slows ACh release.

Desiree Rolon-Dones, Elizabeth Molina-Campos and William D. Atchison.,
Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824-1317

Voltage gated calcium channels (VGCCs) are formed by several subunits. One of them is the β subunit, which makes up the selective pore for Ca2+ and determines most of the subtype-specific attributes of VGCCs. This subunit contains binding sites for various pharmacological agents as well as the gating regions of the channel. The α subunit strongly influences the physiological features, regulates the assembly and membrane localization of the α1 subunits., The α1A subunit is associated with the P/Q-type VGCC, the principal form of VGCC found at mammalian neuromuscular junction. The α1A subunit is the target of several neurological disorders including Lambert Eaton Syndrome . The α1A subunit normally coassociates with α4 at adult mammalian neuromuscular junctions (NMJ). A mutation in the α4 subunit of the P/Q (Cav2.1) VGCC present in lethargic (lh) homozygous mice causes ataxia and lethargic behavior at 15 days of age. Given its' interaction with the α1A subunit, disruption of this subunit in lh mice might result in aberrant neuromuscular transmission. lh mice have decreased release of acetylcholine (ACh). Since there is a correlation between the synapse size and the neurotransmitter release level, we wanted to determine whether this mutation, affected the size of the postsynaptic receptor field. Reduced ACh levels could result from slowed release of ACh vesicles. We stained the ACh receptors were stained with fluorescently labeled α-bungarotoxin and NMJ area and muscle fiber width measured. The ratio of NMJ area to muscle fiber width was determined. It is significantly decreased in lh mice compared to wt littermates. lh mice have a decrease of 13% in the muscle fiber width and a reduction of 47% in the NMJ area. To determine the vesicle dynamic process, we used the FM1-43 staining method. It is severely affected in lh mice. KCl (40 mM) depolarization of wt mice we caused almost complete destaining, reflecting a depletion of fluorescently labeled vesicles. In lh mice there is still 38% of stain remaining. Thus the vesicle

release process is significantly slower in lh mice. Therefore, in the lh mouse, the severe neurological phenotype might be due in part to altered vesicle dynamics and/or the postsynaptic ratio of NMJ surface area/muscle fiber width. Supported by NIH grants R01 NS051833 and R25NS056777.

8. GLEN BANKS, UNIVERSITY OF WASHINGTON

Influence of muscle structure on the pathogenesis of DMD

Glen B. Banks1, F. Steven Korte2, Andrea O'Neil3, Robert J. Bloch3, Michael Regnier2, Jeffrey S. Chamberlain1
1Department of Neurology and 2Department of Bioengineering, University of Washington, WA 98195, USA.
3Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201, USA.

To determine the influence of muscle structure on the pathogenesis of DMD we generated mdx:desmin double knockout (dko) mice. We found that utrophin expression was increased in the dko heart, and in type 1, 2a and 2d skeletal muscle fibers compared to mdx mice. Utrophin did not fully compensate for the lack of dystrophin in the dko heart, resulting in large fibrotic lesions and increased heart mass:body mass ratio that was significantly worse than desmin knockout controls. This resulted in a severe dilated cardiomyopathy with reduced cardiac output and death at approximately 11 weeks of age. In contrast, utrophin expression was sufficient to protect the sarcolemma of type 1, 2a and 2d skeletal muscle fibers from an in situ contraction-induced injury assay. These fiber types had a low level of central nuclei (~9%), probably owing to the lack of desmin and insufficient restoration of the NODS (nNOS/dystrobrevin/syntrophin) complex. The fast type 2b fibers of dko mice had negligible utrophin expression on the sarcolemma in extra-synaptic regions, and little evidence for a mechanical connection between the peripheral sarcomeres and the sarcolemma. The lack of lateral force transmission led to muscle fiber necrosis and membrane tears in distinct isolated type 2b muscle fibers, not in patches of multiple fibers like that found in mdx controls. The fast type 2b fiber areas were selectively smaller and the gastrocnemius peak force was significantly reduced in dko mice compared to wild-type, desmin knockout and mdx controls. Taken together, the influence of muscle structure on the pathogenesis of DMD varies considerably between cardiac and skeletal muscle, and between different skeletal muscle fiber types.

9. JAMIE BARNUM, OHIO STATE UNIVERSITY

Claudin-5 reductions are an early step in the pathogenesis of muscular dystrophy associated cardiomyopathy.

Jamie A. Barnum1, Kevin E. Schill1, Kara E. Zang1, Dawn A. Delfín1, Dan Bogan2, Joe Kornegay2, Jill A. Rafael-Fortney1,
1Department of Molecular & Cellular Biochemistry, College of Medicine, The Ohio State University, Columbus, OH 43210; 2Department of Pathology, University of North Carolina, Chapel Hill, NC 27599.

We originally identified cardiac-specific claudin-5 reductions in the utrophin/dystrophin-deficient mouse model of Duchenne muscular dystrophy and cardiomyopathy. We then showed that over 60% of human cardiac explant samples from patients with heart failure resulting from diverse etiologies display claudin-5 reductions. These data support the hypothesis that reduction of claudin-5 may be a common mechanistic step and represents a novel therapeutic target in cardiomyopathy. We now show that claudin-5 levels are normal prior to signs of cardiac damage in utrophin/dystrophin-deficient mice, but are reduced coincident with cardiac muscle contractile force deficits and blunting of the b-adrenergic response. Claudin-5 is reduced prior to the onset of dysregulation of matrix metalloproteinase (MMP) activity leading to ventricular remodeling, fibrosis, and subsequent whole heart dysfunction. Claudin-5 is a membrane protein that localizes to the cell-matrix junction at the lateral membranes of cardiomyocytes. We now show preliminary evidence that the presence of claudin-5 increases cellular affinity for matrix proteins and therefore its reduction could represent an initial step in the cardiomyocyte slippage necessary for ventricular remodeling. We are currently studying several other models of muscular dystrophy that differ mechanistically in the cause of cardiomyopathy. Preliminary data

suggests that claudin-5 levels are similarly reduced in the cardiac tissue of dystrophin-deficient golden retriever model of Duchenne muscular dystrophy and in the dysferlin-deficient mouse model of limb-girdle muscular dystrophy type 2B. These data support a consistent role of claudin-5 in the progression of cardiomyopathy associated with multiple types of muscular dystrophy.

10 LISA BAUMBACH-REARDON, UNIVERSITY OF MIAMI

From discovery of UBE-1 Mutations in X-linked Lethal Infantile SMA (XL-SMA) to further understanding infantile lower motor neuron diseases: Investigations of functional effects on ubiquitination and new gene discoveries.

L. Baumbach-Reardon1, M. E. Ahearn1, J. Ramser2, K. O. Yariz1, Z. Nawaz1, S. Zuchner1, G. D'Urso1, A. Meindl2
1) Miller School of Medicine, University of Miami, Miami, FL; 2) Gynaekologische Tumorgenetik Ismaningerstr, Munich, Germany;

Infantile lower motor neuron diseases (ILMD) are a clinically, genetically heterogeneous collection of disorders. They are most often lethal, either in late gestation, and/or the newborn period. Tremendous phenotypic overlap occurs within this group, and extends to other possibly related diseases, such as lethal congenital contracture syndromes and arthrogryposis (ARGY). Many disease genes remain to be identified. Our research group has focused on one of these disorders, X-linked lethal infantile spinal muscular atrophy (XL-SMA; MIM 301830). It is a rare lethal infantile neurodegenerative disorder, similar to Type I SMA, but with additional features of ARGY. Our long-term efforts identified the first XL-SMA disease-associated mutations in a known gene, UBE-1 (Ramser et al, 2008), which catalyzes the initiating step in the protein ubiquitination pathway (UPP) - activation of ubiquitin. "Activated" ubiquitin can be transferred from UBE1 to a member of the family of ubiquitin carrier protein enzymes (E2s) by a thioester linkage (E2-S-Ub). In order to evaluate possible functional effects of UBE1 mutations on the UPP, we are completing a series of in vitro and in vivo experiments which will be described. We are also evaluating overall ubiquitination activity in several XL-SMA patient cell lines (as compared to controls) as another measure of in vivo effects. The latest results of these combined studies will be presented.

More recently, we have identified and collected 20 new cases of putative XL-SMA, and are completing UBE-1 mutation screening. Despite numerous phenotypic similarities of this patient cohort with our previously reported UBE-1 mutation-positive XL-SMA cases, no new UBE-1 mutations have been detected. This raises strong suspicion of yet- to- be identified disease genes/mutations. We are pursuing a new strategy to identify unknown interacting and modifying genes for human UBE-1 using a combination of complementary approaches (gene expression profiling, exome sequencing, yeast synthetic lethality screen). It is likely that defects in these genes will account for previously undiagnosed cases of infantile lower motor neuron disease. The long-term study results should allow for continued understanding of the genetic and biological basis of selected infantile lower motor neuron disorders, as well as their relationship to other forms of motor neuron disease.

11. MICHAEL BARRY, MAYO CLINIC

Enhancing Systemic Delivery of Recombinant Adeno-associated Viruses

Matthew L. Hillestad, Adam Guenzel, and Michael A. Barry
Mayo Clinic, Rochester, MN 55905

Previous work has demonstrated that Kupffer cells are a large pharmacological "sink" for intravenously injected gene therapy vectors like adenovirus. For this and many vectors, Kupffer cells inactivate as much as 98% of an injected dose of vector. To test the hypothesis that Kupffer cells may also deplete recombinant adeno-associated virus vectors (rAAV), we evaluated the effects of Kupffer cell depletion of intravenously-administered rAAV8 vectors in mice. Tail vein injection of rAAV8 produced co-localization of the vector with liver Kupffer cells within 4 hours of injection. Elimination of Kupffer cells by predosing with adenovirus before

AAV injection increased liver transduction by single-stranded rAAV8-luciferase vector up to 90-fold in male mice and up to 200-fold in female mice. Predosing with self-complimentary rAAV-Cre recombinase vector in cre-activated luciferase mice increased liver transduction 63-fold in males and 16-fold in female mice. These predosing effects suggested that liver Kupffer cells can deplete a significant fraction of rAAV8 after intravenous injection. Work is underway to determine if these effects are specific to Kupffer cell phagocytosis or may represent phagocytosis in combination with unique interactions between adenovirus and AAV. This suggests that manipulation of the host or vectors systems will improve vector pharmacology and allow lower doses of vector to be used for systemic muscle gene therapy.

12. SUZANNE BERRY, UNIVERSITY OF ILLINOIS.

Vessel-derived Stem Cell Therapy for Skeletal and Cardiac Muscle in Duchenne Muscular Dystrophy

Suzanne E. Berry1, Ju Lan Chun2, Lei Wang1, Robert O'Brien3, and Anant Kamath4
1 Department of Clinical Biosciences, University of Illinois, Champaign, IL (seberry@life.uiuc.edu)
2 Department of Animal Sciences, University of Illinois, Champaign, IL
3 Department of Veterinary Clinical Medicine, University of Illinois, Champaign, IL
4 Cellular Engineering Technologies, Coralville, IA

Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease affecting 1 in every 3500 boys born, and resulting in death of most patients before their third decade of life. Stem cell therapy offers a mechanism to repair muscle tissue in these patients, and restore functional dystrophin protein. We have isolated mesoangioblasts from aorta (ADM) that regenerate skeletal muscle, restoring dystrophin expression to approximately 50% of wild-type levels, protect muscle fibers from membrane damage, and differentiate into Schwann cells in peripheral nerve bundles in the mdx/utrn-/- phenotypic mouse model for DMD. We have begun research to determine whether ADM will restore dystrophin and/or improve function in cardiac muscle of mdx and mdx/utrn-/- mice as well. We have characterized the progression of cardiomyopathy in mdx/utrn-/- mice, demonstrating with echocardiography that the mice develop dilated ventricular cardiomyopathy similar to DMD patients within 15 weeks of age. Following intracardiac injection of ADM, some donor cells express cardiac specific markers, and dystrophin expression is also present. Preliminary studies using echocardiography of mdx/utrn-/- heart before and after stem cell injection indicate an increase in functional parameters including ejection fraction (EF) and fractional shortening (FS) after ADM transplantation. ADM may therefore be good candidates for treatment of both skeletal and cardiac muscle in DMD patients.

Summary: Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease affecting 1 in every 3500 boys born, and resulting in death of most patients before their third decade of life. We have isolated vessel-derived stem cells and tested them for repair and regeneration of both skeletal and cardiac muscle in animal models of DMD. The cells contribute to

13. CARMEN BERTONI, UNIVERSITY OF CALIFORNIA, LOS ANGELES

Restoration of dystrophin expression in the mdx mouse model for Duchenne Muscular Dystrophy (DMD) mediated by nonaminolycoside read-through compounds 13 and 14

Refik Kayali1, Liutao Du3, Jin-Mo Ku2, Gladys Completo2, Olga Prikhodko1,Yosuf Subat1, Hailiang Hu3, Michael Jung2, Richard A. Gatti3, 4 and Carmen Bertoni1*
Department of Neurology, David Geffen School of Medicine, University of California Los Angeles, CA, 2. Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA; 3. Department of Pathology and Laboratory Medicine, David Geffen School of Medicine University of California Los Angeles, CA; 4. Department of Human Genetics, David Geffen School of Medicine University of California Los Angeles, CA.

Molecules that induce ribosomal read-through of nonsense mutations in mRNA and allow production of a full-length functional protein hold great therapeutic potential for the treatment of many genetic disorders.

Two such read-trough compounds, RTC13 and RTC14, were recently identified by a luciferase-independent high-throughput screening assay and were shown to have potential therapeutic functions in the treatment of nonsense mutations in the ATM and the dystrophin genes. We have tested the ability of RTC13 and RTC14 to restore dystrophin expression into skeletal muscles of the mdx mouse model for Duchenne muscular dystrophy (DMD). Intramuscular injections of RTC13, promoted read-through of the mdx UAA stop codon more efficiently than gentamicin, PTC124 or RTC14 making it our lead drug candidate. When administered systemically, RTC13 was shown to restore dystrophin protein in different muscle groups, including diaphragm and heart. Improved muscle strength was detected in all treated animals and was accompanied by a significant decrease in creatine kinase (CK) levels demonstrating that the compound was able to slow down muscle degeneration and turnover. The levels of direct bilirubin (DBIL), blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were significantly decreased in mdx mice that received RTC13 as compared to untreated mdx mice or mdx mice that received vehicle alone demonstrating that systemic administration of RTC13 had no toxic effects on liver and kidney functions in mice, and confirmed that restoration of dystrophin expression in muscles was able to ameliorate some of the secondary pathologies associated with the disease in mice. These results advance the development of RTC13 as an effective drug candidate for DMD. They also offer hope for the treatment of numerous other genetic disorders due to nonsense mutations and premature termination of protein synthesis.

14. LAURENT BOGDANIK, THE JACKSON LABORATORY

Altering motoneuron activity with Ache or Glyt2 mutations differentially affects disease progression in an Agrin-mutant mouse model of congenital myasthenic syndrome

Laurent Bogdanik, Robert W Burgess
The Jackson Laboratory, Bar Harbor, 04609 Maine, USA

Altering motoneuron activity with Ache or Glyt2 mutations differentially affects disease progression in an Agrin-mutant mouse model of congenital myasthenic syndrome. Laurent Bogdanik, Robert W Burgess. Animal models of heritable neuromuscular diseases provide the opportunity to study disease mechanisms, disease progression, and the positive or negative outcomes of therapeutic strategies. We have identified a new mouse mutation in Agrin (Agrn), caused by a single amino-acid change that results in a postnatal disaggregation of the neuromuscular junctions through a partial loss of AGRIN function. AGRIN signals through MuSK, DOK7 and RAPSYN during the embryonic formation of NMJs. Mutations in the genes encoding any of these proteins cause congenital myasthenic syndromes (CMS) in humans, thus making this new Agrin mutant a valuable model for CMS resulting from mutations in this pathway. We show that the Agrin mutation specifically reduces the secretion of AGRIN. This indicates that, even after NMJs have formed, AGRIN signaling is required for their maintenance. In the mutant mice, the time course of the denervation is different among different muscles, with slow-twitch muscles being affected later than fast-twitch muscles, and among individual muscle fibers in a given muscle, with slow fibers being more resistant to denervation. Therapeutic approaches in CMS include acetylcholinesterase (ACHE) inhibitors and potassium channel blockers that respectively increase acetylcholine half-life in the synaptic cleft and increase the duration of the action potentials in the nerve resulting in increased acetylcholine release. Previous experiments in developing mouse embryos show that cholinergic release disrupts receptor aggregates when they are not stabilized by AGRIN. This raises the possibility that increasing synaptic acetylcholine in AGRIN-related CMS could be detrimental. To test this hypothesis, we generated double mutants for Agrin and Ache and for Agrin and the neuronal glycine transporter, Glyt2. The later mutation, identified in the course of the Agrin study, suppresses most of the inhibitory input onto motoneurons in the spinal cord, and leads to an increased activity. We found that the deletion of Ache dramatically hastens NMJ disruption in the Agrin mutants, whereas increased firing of motoneurons in Glyt2 mutants considerably delays it. In conclusion, we present a new CMS model in which ACHE inhibition has deleterious effects on NMJ maintenance, whereas increase of motoneuron activity slows the progression of the denervation. Supported by : Muscular Dystrophy Association, Myasthenia Gravis Foundation of America, and NIH R01 NS054154

15. BENJAMIN RIX BROOKS, CAROLINAS MEDICAL CENTER

Repurposing Drugs for Amyotrophic Lateral Sclerosis(ALS): Achievable Goals

Benjamin Rix Brooks, MD, Elena Bravver, MD, Urvi Desai, MD, Mohammed Sanjak, PT, PhD
Carolinas Neuromuscular/ALS-MDA Center, Department of Neurology, Carolinas Medical Center and University of North Carolina-Chapel Hill School of Medicine-Charlotte Campus

Background: Recent FDA approval in October 2010 of dextromethorphan-quinidine(NeuDextaTM) [DMq] for the symptomatic treatment of pseudobulbar affect in ALS has reopened the possibility that dextromethorphan, previously tested as a treatment to prevent progression of the disease, should be evaluated for its effect on other functions in ALS patients. In addition, 4-aminopyridine extended release or dalfampridine(AmpyraTM)[4APer]approved in January 2010 for multiple sclerosis will offer an opportunity to re-evaluate earlier clinical trials of potassium channel blockade in ALS patients. 4-aminopyridine, which blocks voltage-gated potassium(Kv)channels but not large-conductance calcium-activated potassium channels, enhances evoked end-plate potential(EPP)amplitude and could work in synergy with riluzole, which activates calcium-activated potassium channels. Objective: Define protocols to evaluate clinically relevant outcome measures to assess the symptomatic effect of DMq and 4APer in ALS. Methods: Speech rate assessments of alternate motion rates for labial and lingual musculature and swallowing rate assessments are assessed pre- and post-DMq and 4APer at two different ascending dose regimens in a single case N-of-1 design. Patients are randomized to one dose level and continued on treatment for 7 days, stopped for 7 days then re-randomized to one dose level for 7 days and stopped for 7 days. This cycle is repeated once for an additional 28 days. Patients may be randomized to low or high levels or to low followed by high or high followed by low levels. Placebo drug is provided at the low drug level to blind patients and examiners. Slow vital capacity, negative inspiratory force, 25-foot walk velocity, 6-minute walk distance, and ALS Functional Rating Scale-Revised are performed at 7 day intervals for safety and tolerability. Changes in speech rate and swallowing rate will be compared across dose levels and between DMq and 4APer dose levels as the primary outcome measures. Safety and tolerability will be assessed by changes in functional measurements and adverse events. Results: Preliminary studies with DMq are under analysis. Conclusion: Repurposing of available drugs in ALS require controlled clinical therapeutic trials to assess feasibility, safety, tolerability and treatment effect size for more definitive controlled clinical trials to establish efficacy. Randomized dose-ranging challenge-dechallenge-rechallenge N-of-1 single case design is sufficiently powerful to provide information for planning future clinical trials.

16. ELENA BRAVVER, CAROLINAS MEDICAL CENTER

Molecularly Characterized Muscular Dystrophy Patients in Carolinas Medical Center Adult and Pediatric Neuromuscular MDA Clinics.

Elena Bravver(1), Susan E Sparks(2,3), Colleen A Wunderlich(4), William L Bockenek(4) Urvi G Desai(1), Nicole M Williams(1), Mindy S Nichols(1), Ann L Martin(2), Jaime L Shue(1), Nicole P Smith(1), Priscilla C Russo(1), Amy D Harper(1,3), James D Dollar(5), William E Ballinger Jr(5), John W Longshore(5), Elizabeth Keramaris-Vrantsis(65), Qui L Lu(6), Benjamin R Brooks(1)
1- Carolinas Medical Center, Department of Neurology
2- Carolinas Medical Center, Department of Pediatrics
3- Levine Children's Hospital and Jeff Gordon's Children's Hospital
4- Carolinas Rehabilitation
5- Carolinas Medical Center, Carolinas Pathology Group
6- Carolinas Medical Center, Cannon Research Center McColl-Lockwood Laboratory for Muscular Dystrophy Research

Background: Muscular dystrophies (MD) are inherited primary diseases of muscle which include a great variety of genetically distinct conditions that vary in their clinical features, muscle histology, molecular genetics, and protein defects. Duchenne-Becker's MD(DMD/BMD) is the most common and severe form, with

limb-girdle MD(LGMD), and facioscapulohumeral MD(FSHMD) comprising the next most common forms at the Carolinas Medical Center adult and pediatric MDA Neuromuscular Clinics. Two therapeutic approaches advanced in clinical trials for DMD/BMD include antisense-mediated exon skipping and forced read-through of premature stop codons. Future clinical trials of gene therapies will require MD patient populations with well characterized molecular defects. Therefore, it is important to prepare accurate registries of molecularly characterized patients to facilitate their recruitment into future therapeutic trials. Objectives: This clinical data review of our MDA Neuromuscular Clinics.was performed to estimate the number of patients with different MD types, the means of establishing their diagnosis (DNA test, muscle biopsy, family history, clinical phenotype), and to identify potential candidates for exon skipping therapy of DMD/BMD. We identified patients registered with the 359.1 ICD-9 diagnostic codes for DMD/BMD, FSHMD, and LGMD. Results: Prior to initiating a concerted effort for confirmed molecular diagnosis, 72/104(69%) DMD/BMD patients had any molecular diagnostic testing; only 68% were informative. With awareness of the importance of molecular testing, MPLA tests positive for large duplication or deletion mutation within dystrophin gene were present in 85/104(81.7%) males with dystrophinopathy and 4/104(3.8%) patients had DNA sequencing test proven point mutation. However, 15/104(14.4%) patients had clinical diagnosis based on features along with family history and/or muscle biopsy alone. DNA test confirmed a common 4q35 deletion in 19/30(63.3%) patients with FSHMD; 11/30(36.7%) patients had their diagnosis based on clinical phenotype, family history, and/or muscle biopsy alone. In patients with LGMD, DNA tests confirmed FKRP deficiency 5/40(12.1%); calpainopathy 3/40(7.3%); dysferlinopathy 2/40(4.9%), alpha-sarcoglycanopathy 1/40(2.4%); and beta-sarcoglycanopathy 1/40(2.4%). One additional patient (2.4%) had diagnosis of calpainopathy based on muscle biopsy immunohistochemistry analysis alone while 27/40(65.9%) LGMD patients had a clinical diagnosis, supported by biopsy and/or family history alone. Conclusion: Adult and pediatric Neuromuscular MDA Clinics will need to identify the molecular genetics of each patient and establish accurate registries of molecularly characterized patients to facilitate their recruitment into future therapeutic trials. This review helps to benchmark the clinic readiness for conducting clinical trials in MD patient populations revealing that 14.4%(DMD/BMD), 36.7%(FSHMD) and 65.9%(LGMD) of patients do not have molecularly confirmed diagnoses.

17. SUSAN BROWN, ROYAL VETERINARY COLLEGE

Generation of a new mouse model for therapeutic testing in the dystroglycanopathies.

Mark R.Ackroyd1, Charlotte Whitmore2, Attia Ashraf3, Francesco Muntoni4, Susan C.Brown5.
6Department of Veterinary Basic Science, Royal Veterinary College, London.
7The Institute of Child Health, University College London.

Mutations in fukutin related protein (FKRP) are responsible for a common group of muscular dystrophies ranging from adult onset limb girdle muscular dystrophies to severe congenital forms with associated structural brain involvement, including Muscle Eye Brain Disease. A common feature of these disorders is a variable reduction in the glycosylation of skeletal muscle a-dystroglycan (ADG). Glycosylated ADG is an essential component of the dystrophin-related glycoprotein complex (DGC) whose known ECM ligands include laminin alpha 2, perlecan, agrin and neurexin. The glycans that decorate ADG mediate these interactions and their loss, specifically those identified by the antibody IIH6, are associated with a group of neuromuscular disorders now collectively known as the dystroglycanopathies. We have previously generated a mouse with a knock-down in Fkrp expression levels (FKRPKD) due to insertion of a floxed neomycin cassette in intron 2 of the mouse Fkrp gene. Since this mouse dies at birth due to central nervous involvement we have now replaced FKRP activity in the developing neural tube by crossing this line with one expressing Cre recombinase under the Sox-1 promoter. This has resulted in a near normal lifespan and a muscle phenotype by 12 weeks of age. This mouse should prove invaluable in the design and testing of future therapeutic strategies in the dystroglycanopathies.

18. DEAN BURKIN, UNIVERSITY OF NEVADA, RENO

Integrin-based therapeutics for the treatment of Duchenne muscular dystrophy

Ryan D. Wuebbles Praveen Gurpur, Jachinta E. Rooney, Dean J. Burkin
Department of Pharmacology, University of Nevada School of Medicine, Reno, NV 89557.

Duchenne muscular dystrophy (DMD) is a lethal degenerative muscle disease which results from mutations in the gene encoding the dystrophin. Dystrophin is a critical sarcolemmal protein which acts as a scaffold linking the cell cytoskeleton to the extracellular matrix providing structural and functional integrity to muscle. This lack of sarcolemmal integrity leads to breaks in the plasma membrane and a constant need for muscle repair which eventually exhausts and depletes satellite cells. One potential therapeutic approach for DMD is to circumvent the loss of dystrophin and stabilize the muscle membrane is by increased levels of alpha7beta1 integrin, a transmembrane protein which also links the extracellular matrix and the cell cytoskeleton. To identify therapeutics which increase the alpha7beta1 integrin in muscle, we developed a novel muscle cell-based assay to report alpha7 integrin promoter activity. Using this assay we screened Prestwick Chemical, Microsource Spectrum and DIVERset compound libraries produced nine strong hits which passed subsequent counter screens and increased alpha7 integrin expression in primary myogenic cells from DMD patients. These hits include Laminin-111 and Valproic acid which we have recently shown to be effective in preventing muscle disease onset in mouse models of DMD. Analyses of the additional alpha7 integrin enhancers, which include two FDA approved drugs, are currently being evaluated in dystrophic mouse models. The identification of alpha7 integrin enhancing compounds may lead to a novel therapeutic approach for the treatment of this devastating neuromuscular disorder. Supported by NIH grants R21NS58429 and R21AR060769.

19. LOUIS CHICOINE, NATIONWIDE CHILDREN'S HOSPITAL

Pre-existing antibodies to AAV8 attenuates gene expression following targeted vascular delivery

Louis G Chicoine1,2, Louise R Rodino-Klapac1,2, K Reed Clark1,2, Chrystal L Montgomery1,2, William G Bremer1,3, Katherine J Campbell1,3, Zarife Sahenk1,2,4, Paul Martin1,2, Christopher M Walker1,3, and Jerry R Mendell1,2,4
1 Pediatrics, The Ohio State University/Nationwide Children's Hospital, Columbus, Ohio
2 Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio
3 Center for Vaccines and Immunity, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio
4 Neurology, The Ohio State University/Nationwide Children's Hospital, Columbus, Ohio

Gene therapy for muscle disease has taken several positive steps forward. We recently reported sustained AAV mediated alpha-sarcoglycan (SGCA) gene expression for as long as 6 months in 5 of 6 limb girdle muscular dystrophy (LGMD2D) treated patients (Mendell et al. 2010). The one patient that did not express SGCA was found to have high AAV neutralizing antibodies prior to gene transfer. With vascular gene therapy to treat multiple muscles as the next logical step clinically, impediments may lie with the immune response and the presence of pre-existing antibodies to various AAV serotypes. We tested the hypothesis that pre-existing antibodies to AAV8 would attenuate transgene expression following vascular delivery of two potentially therapeutic transgenes AAV8.MCK.micro-dystrophin.Flag and AAV8.MCK.Galgt2 in a large study of 72 non-human primates. Prior to gene transfer, all animals were pre-screened for binding antibodies to AAV8. Those animals with a titer of <1:100 were considered negative, while others (>1:100) were considered positive. Animals were stratified into positive and negative groups with scheduled sacrifice times at 3 and 6 months (n=9 per group). Vascular delivery to the gastrocnemius muscle of an isolated limb (Rodino-Klapac et al. 2010) was performed on each animal where 2 x 1012 vg/kg of the appropriate vector was given. None of the animals suffered noticeable edema or adverse effects from the procedure. Three or six months after transfer, the macaques were euthanized, and the gastrocnemius muscle was harvested. Samples of the proximal, central and distal muscle were stained with anti-FLAG or CT2 antibody. The contralateral gastrocnemius served as a negative control. Gene expression was visualized in all subjects and subjects without pre-existing AAV8 antibodies

demonstrated significantly higher transgene expression than subjects with pre-existing antibodies (62% vs 33% muscle fibers transduced, $P \leq 0.001$). Regression analysis confirmed a direct inverse correlation between the AAV8 antibody titer and the percent gene expression. From these data we conclude that the presence of pre-existing antibodies to the vector will attenuate transgene expression. Plasmapheresis studies appeared to optimize transgene expression and this procedure would potentially be used to increase the number of patients amenable to gene therapy and make some patients candidates for retreatment when necessary.

20. MARTIN CHILDERS, WAKE FOREST UNIVERSITY

Preclinical Benchmarks in a Large Animal Model of X-Linked Myotubular Myopathy

Xuan Guan1, Melanie Holder1, Christopher J Tegeler1, Anna Buj Bello2, Michael W Lawlor3, Alan H Beggs3, Robert W Grange4, and Martin K Childers1,5
1Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, 2Genethon, Evry, France, 3Children's Hospital and Harvard Medical School, Boston, MA, 4Virginia Polytechnic and State University, Blacksburg, VA, 5Department of Neurology, Wake Forest University Health Sciences

Purpose: The overall goal is to bring to clinical trial gene replacement therapy for patients with X-linked myotubular myopathy (XLMTM), a devastating congenital muscle disease caused by mutations in the gene encoding the muscle protein myotubularin. Affected boys are born as floppy infants with profound muscle weakness. Many patients succumb to respiratory weakness and die within the first year of life. No effective treatment for the disorder exists currently. Based on a previous gene replacement study in myotubularin knockout mice, we hypothesize this approach will similarly ameliorate the severe muscle pathology in XLMTM human patients. To bridge the translational gap between small rodent and human studies, we plan to conduct a gene replacement therapy trial in a canine XLMTM model. Due to the dog's large body size, and similarities between canine and human XLMTM including genetics, muscle physiology, pathogenesis, and clinical course, a canine preclinical trial represents a critical step towards first-in-human studies. We recently established the first experimental colony of XLMTM dogs, and here we present our initial physiological baseline findings.

Methods: We followed ACUC protocols at all institutions. We studied F1 and F2 litters from the founding XLMTM carrier. For dogs, a custom in vivo muscle physiology rig measured dynamic contractile characteristics and mechanics in the hindlimb. Muscles were stimulated percutaneously and resulting torque recorded. Measurements were performed at 9,11,13, and 18 weeks-of-age.

Results: The clinical phenotype in XLMTM dogs appeared analogous to XLMTM patients. Dogs showed a typical onset of weakness, gait abnormality and rapid clinical deterioration after 4 months of age. In vivo muscle strength and responses to repeated contractions in the first two litters of XLMTM dogs (n=15; 2 affected, 5 carriers, 8 normal) revealed that affected dogs were weaker than normal at all ages. Following repeated eccentric contractions, affected dogs exhibited a lower initial torque, but mounted a progressive increase with each successive contraction. However, the maximum torque was always less than that of the normal controls or carriers. Affected dogs also demonstrated a rightward shift in the force-frequency relationship, and a lower-than-normal ratio of twitch to tetany torque. Together, these data point to impairment in E-C coupling as a cause of muscle weakness in XLMTM dogs, and by extension, in patients with XLMTM.

Conclusions: These physiological benchmarks establish a baseline for preclinical trials in the XLMTM dog.

21. RAJ CHIMANJI, OHIO STATE UNIVERSITY

Identification of optimal timing and effects of current clinical cardiomyopathy drug treatment using muscular dystrophy mouse models.

Raj Chimanji1, Ying Xu2, Kevin E. Schill1, Tam Tran3, Ben Canan2, Dawn A. Delfin1, Paul M.L. Janssen2, Subha V. Raman3, Jill A. Rafael-Fortney1, Departments of 1Molecular & Cellular Biochemistry, 2Physiology & Cell Biology, and 3Division of Cardiology, Internal Medicine, College of Medicine, The Ohio State University, Columbus, OH 43210.

Duchenne and Becker Muscular Dystrophy (DMD and BMD) patients display cardiomyopathy that can lead to heart failure. Current standard-of-care treatment for cardiomyopathy patients with DMD and BMD uses the angiotensin converting enzyme inhibitor (ACEi) lisinopril, while the aldosterone analog spironolactone is used in conjunction with ACEi in other cardiomyopathy populations. This combination of treatments has indicated a reduced progression of cardiac damage in clinical studies; however, this drug combination has not been applied directly to dystrophin-associated cardiomyopathy models. To test the effectiveness of this treatment strategy, we have used a mouse model deficient for dystrophin and haploinsufficient for its partially compensating homolog utrophin (mdx;utrn+/-), which displays a progression of cardiomyopathy similar to patients. Three groups of mice were included in the study: 1) untreated controls; 2) a group starting lisinopril + spironolactone treatment at 8 weeks-of-age concurrent with initial signs of cardiac damage; and 3) a group starting treatment at 4 weeks-of-age prior to signs of cardiac damage. The physiological and histological effects of this combinatorial treatment strategy were investigated, as well as whether the strategy was effective as a prophylactic treatment. We observed substantial improvement in both functional and histological indicators of cardiomyopathy in dystrophic mice starting treatment at 4 weeks-of-age compared to untreated dystrophic mice. Earlier treatment resulted in a more profound improvement in cardiac muscle function compared to starting treatment at a later time-point. A similar prevention of cardiac damage and sustained cardiac muscle function in DMD and BMD patients could have profound effects on longevity and quality of life.

22. WEI-CHUN CHIN, UNIVERSITY OF CALIFORNIA, MERCED

Silk-Carbon Nanotube Scaffolds for Neuronal Repairs

Chi-Shuo Chen1, Eric Y-T Chen1, Cheng-En Lue2, Tseng-Fu Lu2, Chao-Sung Lai2, Wei-Chun Chin1
1Bioengineering, University of California, Merced, CA, USA 2Institute of Electronic Engineering, Chang Gung University, Taiwan

Many muscular dystrophy diseases, such as Lou Gehrig's disease (ALS), spinal muscular atrophy, Charcot-Marie-Tooth disease or Lambert-Eaton syndrome, result from degeneration of motor neurons, peripheral nerves or neuromuscular junctions. The fact that our bodies are not capable of regenerating axons and re-innervating target cells makes stem cell-based transplant therapy a feasible and promising approach. Human embryonic stem cells (hESCs) can differentiate into specific neuronal lineages, holding great promise for many muscular dystrophy diseases. Building a microenvironment that can promote preferential cell differentiation in the harsh injury site during transplantation has been a challenge. Our study focused on the improvement of neuronal scaffolds, based on the modification of material properties and surface topography. Carbon nanotubes (CNTs) have been demonstrated to be biocompatible and can be used to support neuron growth and differentiation. CNT scaffolds have also been shown to guide neuronal regeneration across injury sites. However, due to their hydrophobicity, CNTs experience difficulty for acceptable dispersion in scaffold matrices. Although various functionalization strategies have been developed to modify CNTs with hydrophilic chemical groups, the poor dispersion dilemma has not been adequately resolved. Silk (fibroin) from commercial silk worms (Bombyx mori) has been used as building material for scaffolds for many biomedical applications. In addition, the amphophilic fibroin can effectively serve as a dispersant for CNTs. Our results indicate that silk-CNT based composite materials can create homogeneous matrices and acquire better neuronal differentiation efficiency from hESCs. The critical role of surface topography in hESC differentiation has been shown in many studies; structures in nanometer ranges can influence stem cell differentiation. However, high cost and complex fabrication have limited the applications of nanometer structures. With economical standard photolithographic techniques, we built micrometer scale surface grooves with different spacing. The preliminary results indicated that smaller spacing favor neuronal differentiation and promote axon elongation. The hESCs grown on larger-spacing structures preferentially differentiated toward oligodendrocytes. Our data suggest a "tunable" surface can selectively differentiate certain desired type of neuronal cells from hESCs. With the integration of soft-lithography, those specific patterns can be transferred to various scaffolds surfaces. Both surface groove modification and silk-CNT material improvements will be incorporated in our scaffold development. The targeted

scaffolds can serve as efficient supporting matrices for stem cell derived neuronal transplants to offer promising therapies for muscular dystrophy patients. *this study is supported by a MDA grant (#154888).

23. PAULA CLEMENS, UNIVERSITY OF PITTSBURGH

Addition of PTD-NEMO binding domain inhibitory peptide to AAV serotype 9 microdystrophin gene transfer for treatment of muscular dystrophy in mdx mice

Daniel P Reay1, Gabriela Niizawa1, Jon F. Watchko2, Molly Daood2, Eugene Raggi1, Paula R. Clemens1,3
1Department of Neurology, University of Pittsburgh, Pittsburgh, PA; 2Department of Pediatrics, Magee-Women's Research Institute, University of Pittsburgh, Pittsburgh, PA; 3Neurology Service, Department of Veterans Affairs Medical Center, Pittsburgh, PA

Dystrophin deficiency, which causes the single gene disorder Duchenne muscular dystrophy (DMD), initiates a cascade of pathologic effects in skeletal muscle that result in progressive weakness. One possible therapeutic approach is replacement of the missing dystrophin protein by gene or cell therapy. Additionally, strategies that modulate downstream signaling of dystrophin deficiency in affected muscle tissue offer novel means to treat diseased muscle and ameliorate the disease process. The nuclear factor of kappa B (NF-κB) is a critical transcription factor that is pathologically activated in dystrophic muscle. Activation of NF-κB enhances inflammation and impairs tissue regeneration in diseased muscle. In the mdx mouse model of DMD we demonstrated that systemic delivery of a NEMO binding domain (NBD) peptide fused to a protein transduction domain (PTD) decreased pathologic activation of NF-κB in muscle tissue. Furthermore, we observed decreased necrosis and increased regeneration of muscle fibers of the hind limb and diaphrgam. Ex vivo functional capacity of costal diaphragm was improved. The results suggested that decreased pathological NF-kB activation in muscle improves the tissue milieu and thereby provides a treatment effect. To extend these studies, we tested combined treatment with an AAV9 carrying a truncated dystrophin cDNA and PTD-NBD peptide, both delivered systemically to the mdx mouse. The aim was to test the hypothesis that the addition of PTD-NBD peptide treatment could enhance the therapeutic benefits of replacement gene delivery by diminishing inflammation and necrosis that persist with partial restoration of normal dystrophin expression. In mdx mice that were treated with 5 weeks of PTD-NBD peptide in addition to AAV gene delivery the quadriceps muscle demonstrated increased levels of recombinant dystrophin expression suggesting that PTD-NBD treatment promoted an environment in muscle tissue conducive to higher levels of recombinant dystrophin expression. Indices of necrosis and regeneration were diminished with either AAV gene delivery alone or in combination with PTD-NBD treatment. In diaphragm muscle, transgene expression was sufficiently high (90-100% of muscle fibers expression recombinant dystrophin) that little difference was observed with the addition of PTD-NBD treatment. The data support potential benefit from PTD-NBD treatment to complement gene transfer therapy for DMD in muscle tissue that receives incomplete levels of transduction by gene transfer.

24. KAY DAVIES, UNIVERSITY OF OXFORD

Utrophin Upregulation in DMD Therapy: Current Status and New Tools for the Future

Rebecca. J. Fairclough1, Akshay Bareja2, Dave Powell1, Allyson Potter2, Sarah Squire2 and Kay E. Davies2
1Department of Physiology, Anatomy and Genetics; University of Oxford; Parks Road; Oxford, OX1 3PT; UK
2MRC Functional Genomics Unit; University of Oxford; Parks Road; Oxford, OX1 3PT; UK

Duchenne muscular dystrophy (DMD) is a devastating X-linked muscle wasting disease with a high new mutation rate caused by the lack of the cytoskeletal protein dystrophin. Although several therapeutic strategies are being developed, there is currently no effective treatment. Our work is focused on the development of a drug which would increase the levels of the dystrophin-related protein, utrophin which we have shown can functionally compensate for the lack of dystrophin. Importantly, this approach is applicable to all patients, irrespective of their mutation. BMN-195 (SMT C1100) - the lead compound identified from our recent small compound screening programme in collaboration with Summit plc has recently become the first

drug for utrophin upregulation to enter Phase I trials in humans. This is no longer in clinical trials because of poor pharmokinetics and it the drug is being re-formulated. We are currently investigating the mechanism of action of this drug at the utrophin promoter. Our future screens will be based on screening the complete endogenous utrophin promoter in its genomic context, using an immortalised myoblast cell line developed from the new utrophin luciferase (LUmdx) knock-in mouse model. This will mimic the in vivo situation and enable us to identify compounds which transcriptionally upregulate utrophin through key elements which have recently been identified outside of the 10 kb promoter A fragment that formed the basis of our previous screen. The new LUmdx model will circumvent the problems caused by large variations in background utrophin, encountered when using the mdx model in preclinical screens for utrophin upregulation. The luciferase reporter enables quantification of the level of luminescence being emitted from this model in vivo during a drug trial, enabling assessment of drug efficacy without having to sacrifice the animal and terminate the trial. The ability to prolong the length of promising trials and prematurely terminate those where efficacy is low should dramatically improve the throughput of in vivo preclinical drug trials for utrophin up-regulation in the mdx mouse.

25. PRITI DEKA, THE SCRIPPS RESEARCH INSTITUTE

Structure and RNA binding by Muscleblind protein: Splicing activity and role in Myotonic Dystrophy.

Pritilekha Deka, Maria Martinez-Yamout, H. Jane Dyson and Peter E. Wright
Mailcode: MB2, Molecular Biology Department, The Scripps Research Institute, La Jolla, CA-92037

Muscleblind is a CCCH zinc finger protein that acts as a developmentally programmed regulator of alternative splicing within the cardiac troponin (cTNT) pre-mRNA. Misregulation of MBNL activity leads to Myotonic Dystrophy (DM) in humans. In myotonic dystrophy, expanded CUG repeats accumulate in nuclear RNA foci and recruit muscleblind proteins through a mechanism not completely identified yet. This sequestering of muscleblind proteins interferes with MBNL regulated pre-mRNA splicing activity and disrupts normal cellular developmental programs. MBNL1 is a 384 residue protein containing 4 zinc fingers, with a 90 residue linker between zinc fingers 2 and 3. We have determined the high resolution structure of MBNL-zf12 by using NMR. We have also shown that one pair of zinc fingers (zinc fingers 1 and 2, or, zinc fingers 3 and 4) is sufficient to impart binding activity to the single stranded fragment of cTNT pre-mRNA as well as the pathogenic CUG repeat RNAs. Through electrophoretic mobility shift assays and NMR titration experiments we have established that each pair of zinc fingers in MBNL binds to a consensus CGCU(G/U) motif within the 3′ end of intron 4 of human cTNT RNA.

26. DENNIS DISCHER, UNIVERSITY OF PENNSYLVANIA

Muscle-like matrix elasticity directs myogenesis - evidence from Mass Spectrometry and effects of Prednisolone

(with T Chaudhuri and H Lee Sweeney)

Human mesenchymal stem cells (hMSCs) express markers of different lineages when grown on matrices that mimic the elasticity of mesenchyme-derived tissues (1), but the proteomic changes are understudied as are the coupled effects of soluble factors. Matrix programming is combined here with a single glucocorticoid prednisolone (PDN) that is widely used in the clinic for muscular dystrophies, and we demonstrate a similar clinical profile: PDN proves pro-myogenic, pro-adipogenic, and anti-osteogenic with most responses depending on matrix elasticity. Cross-linked gels coated with collagen-I are used to mimic either a muscle-like elastic microenvironment or the stiffer matrix typical of fibrosis and nascent bone, but only the muscle-like substrate is seen to induce both satellite cell and myoblast markers. A novel mass Spectrometry method is developed to characterize expression of multiple transcription factors in a single run, with Westerns and full genome Microarrays providing much additional insight into kinetics of matrix elasticity induction.

Engler, Sen, Sweeney, Discher. Cell (2006).

27. JINGER DOE, UNIVERSITY OF NEVADA, RENO

Transgenic overexpression of alpha7 integrin in the dyW mouse reduces muscle pathology, maintains muscle strength and increases viability.

Jinger A. Doe1, Ryan D. Wuebbles1, Erika T. Allred1, Jachinta E. Rooney1 and Dean J. Burkin1,2 1Department of Pharmacology, University of Nevada School of Medicine, Reno, NV 89557. Telephone: (775) 784-6288; Fax: (775) 784-1620.
2Nevada Transgenic Center, University of Nevada School of Medicine, Reno, NV 89557. Telephone: (775) 784-6935; Fax: (775) 784-1620

Merosin-deficient congenital muscular dystrophy (MDC1A) is a devastating neuromuscular disease caused by a mutation in the lama2 gene. This gene encodes laminin alpha2 protein which is essential for the assembly of laminins 211 and 221. Laminin 211 is a key component of mature skeletal muscle extracellular matrix and loss of this key protein leads to severe muscle wasting. Patients with MDC1A suffer from hypotonia and delayed motor milestones. Severely affected patients fail to achieve independent ambulation and have decreased life expectancies. Patients with MDC1A also have reduced expression of the alpha7beta1 integrin complex. The role that this secondary down-regulation of alpha7beta1 integrin plays in MDC1A pathology has not been examined. Similar to MDC1A patients the dyW mouse model of MDC1A shows reduced alpha7 integrin. In order to evaluate the role of the alpha7 integrin in MDC1A we overexpressed alpha7 integrin in the skeletal muscle of the dyW mouse model. Overexpression restored alpha7beta1 integrin to the sarcolemma in dyW mice. Transgenic mice had a reduced myopathic phenotype, maintained muscle strength and an increased life expectancy. The mechanism of this improvement is via augmentation of several extracellular matrix proteins. Therefore, the alpha7 integrin may represent a novel therapeutic target for this MDC1A. Supported by an NIH grant R01AR053697

28. DONGSHENG DUAN, UNIVERSITY OF MISSOURI

Dystrophin-deficient dogs, the missing link for Duchenne muscular dystrophy gene therapy?

Dongsheng Duan
Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO, USA

A milestone of molecular medicine is the identification of dystrophin gene mutation as the cause of Duchenne muscular dystrophy (DMD). Over the last two decades, major advances in dystrophin biology and gene delivery technology have created an opportunity to treat DMD with gene therapy. Remarkable success has been achieved in treating dystrophic mice. Several gene therapy strategies, including plasmid transfer, exon skipping and adeno-associated virus (AAV)-mediated micro-dystrophin therapy, have entered clinical trials. However, therapeutic benefit has not been realized in DMD patients. Bridging the gap between mice and humans is no doubt the most pressing issue facing DMD gene therapy now. In contrast to mice, dystrophin-deficient dogs are genetically and phenotypically similar to human patients. Preliminary gene therapy studies in the canine model offer critical insights that cannot be obtained from murine studies. It is clear that the canine DMD model may represent an important link between mice and humans. Unfortunately, our current knowledge of dystrophic dogs is limited and the full-picture of disease progression remains to be clearly defined. We also lack rigorous outcome measures in particular physiologic assays, to monitor therapeutic efficacy in dystrophic dogs. Undoubtedly, maintaining a dystrophic dog colony is technically demanding and the cost of dog studies cannot be underestimated. A carefully coordinated effort from the entire DMD community is needed to make the best use of the precious dog resource. Successful DMD gene therapy depends on valid translational studies in dystrophin-deficient dogs.

29. HEATHER DURHAM, MCGILL UNIVERSITY

Evaluating candidate therapeutic agents for motor neuron diseases against specific biomarkers of disease pathogenesis in primary culture models

Heather D. Durham, Miranda L. Tradewell, Benoit J. Gentil.
Montreal Neurological Institute, McGill University, Montreal QC Canada

Dysregulation of intracellular Ca2+, mitochondrial dysfunction, inhibition of intracellular transport, protein aggregation and cytoskeletal disruption are documented abnormalities in motor neuron disorders and reflect aspects of the physiology of motor neurons contributing to preferential vulnerability. We are investigating how these abnormalities relate to one another in a pathogenic cascade, the commonalities and differences among various motor neuron disorders, and sensitivity to various interventions by incorporating vital imaging of organellar function into primary culture models of familial ALS (fALS) and Charcot-Marie-Tooth disease (CMT).

In motor neurons expressing the fALS1-associated SOD1 mutant, G93A, toxicity manifested as follows: The earliest abnormalities identified were increased mitochondrial Ca2+ and loss of mitochondrial membrane potential, followed by increased endoplasmic reticular Ca2+, and dramatic rounding of mitochondria concomitant with impaired mitochondrial fission/fusion and axonal transport; increase in cytosolic Ca2+, which was significantly higher in neurons containing inclusions of mutant protein; fragmentation and nuclear condensation of nuclear DNA in neurons with inclusions, and finally motor neuron death. These abnormalities are generally consistent with observations in motor neurons of transgenic mice and fall into the general categories of calcium dysregulation, disruption of mitochondrial dynamics, and aggregation into inclusions. The effect of chaperone-based therapy was evaluated, given the evidence for protein misfolding underlying the toxicity conferred by dominantly inherited mutations. HSP inducers reduced impairment mitochondrial dynamics induced by SOD1G93A (fusion/fission and rounding) as well as formation of cytoplasmic inclusions, but did not impact on calcium dysregulation.

In terms of CMT, disruption of mitochondrial dynamics was as expected with mutations in the mitochondrial fusion protein, mitofusin 2 (Mfn2); however, similar disruption of mitochondrial fusion/fission and mitochondrial rounding occurred with expression of CMT2E-causing NFL mutants, prior to disruption of the neurofilament network; mitochondrial axonal transport was disrupted subsequently. The Q333P and P8R NFL mutants behaved differently in terms of the mechanism by which they affected mitochondria and the sensitivity to different types of HSP (Hsp70 or Hsp25).

Despite commonalities in general physiological effects of different mutant proteins and different mutations within them, genetic or pharmacological upregulation of individual or networks of heat shock proteins (HSP) differentially impacted on toxicity. Microfluorometric imaging of biomarkers of toxicity in these primary culture models is useful for preclinical screening of individual and combinations of therapeutic candidates.

30. GALINA FLIPPOVA, FRED HUTCHINSON CANCER RESEARCH CENTER

Role of CTCF in Developmentally Regulated Silencing of D4Z4

Natalia A. Rabaia, Yvonne D. Krom, James M. Moore, Judit Balog, Jessica C. de Greef, Leslie E. Smith, Rabi Tawil, Stephen J. Tapscott, Daniel G. Miller, Silvere van der Maarel, Galina N. Filippova*
Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA Leiden University Medical Center, Leiden, 2333 ZA, The Netherlands University of Washington, Seattle, 98195, USA

Recent studies have suggested that loss of repressive chromatin marks and DNA methylation at the 4qD4Z4 region in FSHD may result in de-repression of DUX4, the candidate FSHD gene imbedded in the D4Z4 repeat unit. The chromatin insulator protein, CTCF, has been shown to protect genes from epigenetic silencing. CTCF binding is generally inhibited by DNA methylation and CTCF prevents spreading of DNA methylation. We have identified and characterized multiple CTCF binding sites in the D4Z4 repeats and surrounding sequences, and demonstrated both enhanced binding of CTCF to the disease-associated pathogenic D4Z4 alleles and decreased

repressive chromatin marks and DNA methylation at these CTCF binding sites in FSHD cells. siRNA-mediated depletion of CTCF in primary myoblasts resulted in reduction of the DUX4 transcripts levels, suggesting that aberrant CTCF binding at D4Z4 in FSHD may be involved in maintaining the open chromatin structure and active transcription in the region. Finally, the enhanced CTCF binding to D4Z4 was also observed in pluripotent cells and was lost upon differentiation into embryoid bodies. Using induced pluripotent stem cells from controls and FSHD patients, in collaboration with Dan Miller we showed that D4Z4 undergoes epigenetic silencing in control cells during differentiation into embryoid bodies whereas in FSHD cells it retains its open chromatin structure. We propose that inappropriate CTCF binding to D4Z4 in FSHD might interfere with the setting of the repressive chromatin marks at the D4Z4 repeat array in early development. Funding: MDA Research Grant 69798 (GNF), NIH P01 NSO69539 (GNF, SVDM, DGM, SJT).

31. SEAN FORBES, UNIVERSITY OF FLORIDA

Effects of myostatin inhibition in skeletal muscle of Golden Retriever muscular dystrophy dogs.

SC Forbes1, D Wilson, S Wickham1, K Vandenborne1, W Mai2, LT Bish2, KJ Morine2, GA Walter1, MM Sleeper2, HL Sweeney2
University, of Florida, Gainesville, Florida1 and University of Pennsylvania, Philadelphia, Pennyslvania2

Background: The purpose of this study was to evaluate the effects of systemic myostatin inhibition on skeletal muscle size and magnetic resonance proton transverse relaxation time (T2) in Golden Retriever muscular dystrophy (GRMD) dogs. Methods: Myostatin inhibition by liver-targeted gene transfer was performed in four GRMD dogs (9-10 months old). 3D-gradient echo and spin echo axial images were acquired of the lower hindlimbs using a 1.5T GE scanner at monthly intervals for 6 months and at an additional time point at 21-25 months of age. Also, images were acquired on three untreated GRMD dogs (23-25 months old). Muscle volume was measured in the anterior compartment (AC) and maximum cross sectional area (maxCSA) and T2 in the flexor digitorum superficialis (FDS), tibialis cranialis (TC), extensor digitorum longus (EDL), and medial (MG) and lateral gastrocnemius (LG). Results: Following treatment, muscle volume in the AC increased ($p<0.05$) over 6 months (17+/-11%), with similar increases in maxCSA of the FDS, TA, EDL, MG, and LG. The AC volume was greater ($p<0.05$) in treated GRMD dogs (27.2+/-4.7cm3) than untreated GRMD dogs (19.1+/-3.4cm3) at 21-25 months of age. In the treated GRMD dogs no changes were observed in muscle T2 pre and post treatment and between treated and untreated dogs at 21-25 months of age. Conclusions: The findings of this study indicate that systemic myostatin inhibition increases skeletal muscle mass in GRMD dogs. Furthermore, the maintenance of T2 with age suggests that the increase in muscle size in the treated GRMD dogs was not accompanied by a disproportionate elevation in fatty tissue infiltration.

32. KEITH FOSTER, UNIVERSITY OF LONDON

Antisense-induced myostatin exon skipping leads to muscle hypertrophy in mice following octa-guanidine morpholino oligomer treatment

Jagjeet K Kang, Alberto Malerba, Linda Popplewell, Keith Foster, George Dickson
School of Biological Sciences, Royal Holloway - University of London, Egham, Surrey, TW20 0EX, UK

Myostatin is a negative regulator of muscle mass, and several strategies are being developed to knockdown its expression to improve muscle-wasting conditions. Strategies using antimyostatin-blocking antibodies, inhibitory-binding partners, signal transduction blockers, and RNA interference system (RNAi)-based knockdown have yielded promising results and increased muscle mass in experimental animals. These approaches have, however, a number of disadvantages such as transient effects or adverse immune complications. We report here the use of antisense oligonucleotides (AO) to manipulate myostatin pre-mRNA splicing and knockdown myostatin expression. Both 2'O-methyl phosphorothioate RNA (2'OMePS) and phosphorodiamidate morpholino oligomers (PMO) led to efficient exon skipping both in vitro and in vivo leading to a knockdown of myostatin at the transcript level. The substantial myostatin exon skipping observed after systemic injection of Vivo-PMO into

normal mice led to a significant increase in soleus muscle mass as compared to the controls injected with normal saline suggesting that this approach could be feasible to ameliorate muscle-wasting pathologies.

33. KEITH FOSTER, UNIVERSITY OF LONDON

Chronic systemic therapy with low dose morpholino oligomers profoundly ameliorates the pathology and normalises behaviour in mdx mice

Alberto Malerba1, Paul S. Sharp2, Ian R. Graham1, Virginia Arechavala-Gomeza3, Keith Foster1, Francesco Muntoni3, Dominic J. Wells2, 4, George Dickson1
1: School of Biological Sciences, Royal Holloway - University of London, Egham, Surrey, TW20 0EX, UK
2: Department of Cellular and Molecular Neuroscience, Division of Neuroscience and Mental Health, Imperial College London, Hammersmith Hospital Campus, London W12 0NN, UK
3: The Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London WC1N 1EH, UK
4. Current address: Department of Veterinary Basic Sciences, The Royal Veterinary College, London NW1 0TU, UK

Duchenne's muscular dystrophy (DMD) is a severe muscle wasting disorder affecting 1/3500 male births. Lack of dystrophin in skeletal muscle compromises the integrity of the muscle membrane and thus muscle fibres are prone to contraction induced injury. Further rounds of muscle degeneration and regeneration leads to the replacement of muscle fibres with non contractile fatty/fibrotic tissue. These alterations lead to progressive muscle wasting, weakness and death in late adolescence. The administration of antisense oligonucleotides (AO) to skip one or more exons in mutated forms of the DMD gene and so restore the reading frame of the transcript is one of the most promising approaches for the treatment of DMD. Due to the transient effect of this treatment, regular administration of AO would be necessary throughout the patients' lifetime. However at present, preclinical studies demonstrating the efficacy and safety of a long term AO administration have not been conducted. Furthermore, it is essential to determine the minimal effective dose and frequency of administration. In this study, two different low doses of phosphorodiamidate morpholino oligomer (PMO) designed to skip the mutated exon 23 in the mdx dystrophic mouse were administered for up to 12 months. In mice treated for 5 months the muscles showed histological improvement, increased muscle strength and resistance to eccentric exercise. Mice treated for 12 months showed a substantial dose-related amelioration of the pathology, particularly in the diaphragm, where the degenerative process was significantly delayed. Moreover, the generalised physical behavioural activity was profoundly enhanced compared to untreated mdx mice showing that widespread, albeit partial, dystrophin expression can restore the normal activity and movement behaviour in mdx mice. Our results show for the first time that a chronic long-term administration of very low doses of unmodified PMO is safe, significantly ameliorates the muscular dystrophic phenotype and improves the activity of dystrophin-deficient mice, thus encouraging the further clinical translation of this approach in humans.

34. DIEGO FRAIDENRAICH, UMDNJ

Induced pluripotent stem cell corrections in Duchenne muscular dystrophy mice via blastocyst injection

Amanda J. Beck1*, Joseph M. Vitale1*, Qingshi Zhao1, Joel S. Schneider1, Corey Chang1, Aneela Altaf1, Jennifer Michaels2, Mantu Bhaumik3, Robert Grange4 and Diego Fraidenraich1#
1Departments of Cell Biology and Molecular Medicine and 2Neurology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ, 07107
3Department of Pediatrics, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ, 80901
4Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24060
*These authors contributed equally to the work

We injected WT mouse induced pluripotent stem cells (iPSCs) into mdx and mdx:utrophin mutant blastocysts, which are predisposed to develop DMD with an increasing degree of severity (mdx <<< mdx:utrophin). In mdx chimeras, iPSC-dystrophin was supplied to the muscle sarcolemma to effect corrections at morphological and functional levels. In the mdx:utrophin mutant chimeras, although iPSC-dystrophin was also supplied to the muscle sarcolemma, mice still displayed poor skeletal muscle histopathology. Not only dystrophin-expressing tissues are affected by iPSCs. Mdx and mdx:utrophin mice have reduced fat/body weight ratio, but iPSC injection normalized this parameter in both mdx and mdx:utrophin chimeras, despite the fact that utrophin was compromised in the mdx:utrophin chimeric fat. The results suggest that the presence of utrophin is required for the iPSC-corrections in skeletal muscle. Furthermore, the results highlight a potential (utrophin-independent) non-cell autonomous role for iPSC-dystrophin in the corrections of non-muscle tissue like fat, which is intimately related to the muscle.

35. RANJIT GANGULY, OHIO STATE UNIVERSITY

Functional Improvement of Dystrophic Skeletal Muscles Resulting from a Combinatorial Drug Treatment

R. Ganguly1, R. Chimanji1, K.E. Schill1, C. Martin2, J. Stangland2, J. Murray2, T.A. Mays, S.V. Raman3, P.M.L. Janssen2, and J.A. Rafael-Fortney1
Departments of
1Molecular & Cellular Biochemistry, 2Physiology & Cell Biology, and 3Division of Cardiology, Internal Medicine, College of Medicine, The Ohio State University, Columbus, OH 43210.

Duchenne muscular dystrophy (DMD) is caused by the absence of the dystrophin protein and results in degeneration of both skeletal and cardiac muscles and deposition of fibrotic tissue. The angiotensin converting enzyme inhibitor (ACEi) lisinopril is currently used by clinicians to treat heart failure progression in DMD patients, and the aldosterone analog spironolactone is often used in combination with ACEi to treat other forms of cardiomyopathy and has an anti-fibrotic effect. With the original goal of testing whether a combination of lisinopril and spironolactone is able to prevent dystrophic cardiomyopathy, mice deficient for dystrophin and haploinsufficient for utrophin (mdx; utrn+/-) were given this drug combination beginning at either four weeks or eight weeks-of-age. Functional and histological analyses of treated dystrophic mice were conducted at 20 weeks-of-age and compared to untreated genotypic controls, which show large amounts of fibrotic collagen deposition in both skeletal muscles and heart. To determine whether this drug treatment has any effect on fibrosis in striated muscles besides the heart, we first assessed function of both limb and respiratory muscles. Both extensor digitorum longus (EDL) and diaphragm muscles of untreated 20 week-old mdx; utrn+/- mice showed developed forces of approximately 40% that of age-matched wild-type controls. Diaphragm and EDL muscles from 20 week-old mdx; utrn+/- mice started on the drug treatment at 4 weeks-of-age showed a dramatic improvement of contractile force with active developed force in both muscle types equal to 80% of wild-type controls. Preliminary histological analyses reveal a reduction, but not a prevention of muscle damage in diaphragm and quadriceps muscles. Functional and histological improvements in skeletal muscles were less dramatic in the group starting the drug treatment at 8 weeks-of-age, suggesting a greater benefit of treatment started earlier in disease progression.

36. LINDA GENG, FRED HUTCHINSON CANCER RESEARCH CENTER

A new disease mechanism revealed by studies in FSHD

Linda N. Geng1, Lauren Snider1, Ashlee E Tyler1, Rabi Tawil3, Galina N Filippova1, Daniel G. Miller4, Silvere M. van der Maarel2, and Stephen J Tapscott1
1 Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America,
2 Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands,
3 Department of Neurology, University of Rochester, Rochester, New York, United States of America,
4 Department of Pediatrics, University of Washington, Seattle, Washington, United States of America

Double homeobox 4 (DUX4) is one of the leading candidate disease genes for facioscapulohumeral muscular dystrophy (FSHD), and it is a retrogene that resides within each unit of a macrosatellite repeat array. Pathological expression of DUX4 mRNA in FSHD muscle cells has been previously reported, but alternative splice forms and correlation of mRNA levels with DUX4 protein have not been rigorously investigated. Additionally, it was unclear whether DUX4 was endogenously expressed in any cell types of healthy individuals and could potentially serve a normal physiological role. Using a nested RT-PCR strategy, we found that FSHD muscle expresses a full-length form of DUX4 whereas control muscle expressed a short splice form. Furthermore, only the full-length form expressed by the FSHD muscle is toxic when introduced to cells in culture. By immunofluorescence and batched-cell RT-PCR studies, we found that the long DUX4 is produced at a relatively high abundance at the mRNA and protein level in a small fraction of FSHD muscle nuclei. While control skeletal muscle and most other somatic tissues do not express the full-length form of DUX4, both the full-length transcript and protein can be found in healthy human testis in the germ-cell lineage. Induced pluripotent stem (iPS) derived from both control and FSHD individuals express full-length DUX4, but only the control cells are able to suppress the expression of DUX4 when differentiated to embryoid bodies. Thus, FSHD represents the first human disease to be associated with incomplete silencing of a retrogene array normally expressed early in development.

37. MAHASWETA GIRGENRATH, BOSTON UNIVERSITY

Muscle Specific Expression of Insulin-Like Growth Factor 1 Improves Outcome in Lama2Dy-w mice, a model for Congenital Muscular Dystrophy Type 1A.

Ajay Kumar, Jenny Yamauchi, Tanya Girgenrath and Mahasweta Girgenrath.
Department of Health Sciences, Boston University, Commonwealth Avenue, Boston MA 02215.

MDC1A, the second most prevalent form of congenital muscular dystrophy, results from laminin-α2 chain deficiency. The disease is characterized by extensive muscle wasting that results in extremely weak skeletal muscles. A large percentage of children with MDC1A are faced with respiratory as well as ambulatory difficulties. We investigated the effects of overexpressing insulin-like growth factor-1 (IGF-1) as a potential therapeutic target for the disease in the Lama2Dy-w mouse, a model that closely resembles human MDC1A. IGF-1 transgenic Lama2Dy-w mice showed increased survivability, body weight, and muscle weight. In addition, they had greater hind limb muscle strength than their nontransgenic littermates. Lama2Dy-w have limited or no regenerative capacity; however, histology and immunohistochemistry analyses reveal increased regenerative capacity and proliferation in IGF-1 transgenic Lama2Dy-w muscles. Western blot analysis showed increased phophorylation of Akt and ERK1/2, both known to enhance myogenesis. Additionally, we saw increase in expression of regeneration markers such as MyoD, Myogenin and embryonic myosin isoform (MYH3). Therefore, we conclude that the increase in regeneration, identified by centrally nucleated fibers, was mainly improved through myogenesis as fibrosis and inflammation were not relieved with IGF-1 overexpression. Our results demonstrate that IGF-1 has a promising therapeutic potential in the treatment of MDC1A.

38. PAUL GREGOREVIC, BAKER IDI HEART & DIABETES INSTITUTE

Dystrophic mdx, and dystrophin:utrophin-null mice are differentially responsive to viral vector interventions designed to enhance muscle function

Lucy Cassar (1), Rachel Thomson (1), Andrea Arnett (2), Jeffrey Chamberlain (2), Paul Gregorevic (1,2,3,4) BakerIDI Heart & Diabetes Institute, Melbourne, Australia 2- Dept. of Neurology, The University of Washington School of Medicine, Seattle, USA 3- Dept of Biochemistry and Molecular Biology, Monash University, Australia 4- Dept. Of Physiology, The University of Melbourne, Australia

Because Duchenne muscular dystrophy is a condition characterized by the progressive loss of functional musculature, it may be necessary to develop interventions that increase the size and strength of patientss remaining muscle fibres, in parallel with means to restore expression of a dystrophin-based protein. We have

administered recombinant adeno-associated viral vectors containing an expression cassette for the 288 amino-acid isoform of human follistatin (rAAV6:Fst288) to the muscles of young-adult dystrophin-deficient mdx mice, and observed significant ($p<0.05$) increases in muscle mass and contractile performance as a consequence of follistatin-mediated muscle fibre hypertrophy. However, mdx mice do not demonstrate the progressive loss of muscle mass and strength experienced by patients, and therefore may not constitute the most appropriate model in which to evaluate the therapeutic potential of experimental interventions. We therefore chose to examine the effects of administering rAAV6:Fst288 to the muscles of dystrophin:utrophin-null mice (dko mice), that typically exhibit progressive loss of muscle mass and strength in a similar manner to that observed in patients with DMD. In contrast to outcomes observed in mdx mice, the muscles of dko mice did not demonstrate significant increases in muscle mass or muscle fibre size following rAAV6:Fst288 administration, despite achieving comparable levels of Fst288 expression. However, the hypertrophic response to rAAV6:Fst288 was restored in the muscles of dko mice when the vectors were co-delivered with rAAV6 vectors carrying a ~3.8kb "micro-dystrophin" expression cassette. These data indicate that components of the dystrophin-associated protein complex that are present in mdx mice, but are deficient in dko mice influence the cellular mechanisms associated with follistatin-mediated muscle hypertrophy. We present here, our current understanding of the mechanisms underlying the differential responsiveness of these two mouse strains, and the potential implications of these findings for the pre-clinical evaluation of interventions developed for DMD.

39. JEREMIAH HADWEN, CHILDREN'S HOSPITAL OF EASTERN ONTARIO RESEARCH INSTITUTE

FDA drug mining for novel therapeutics for Duchenne Muscular Dystrophy

Jeremiah Hadwen, Sean O'Reilly, Luke Witherspoon, Justin Lamb, Alex MacKenzie

Duchenne muscular dystrophy (DMD) is a severe recessive X-linked disease characterized by progressive muscle wasting eventually leading to death of patients often by their third decade from cardiac or respiratory failure. Mutation in the DMD gene, leading to a complete loss of functional Dystrophin protein in skeletal and cardiac muscle, is the cause of disease. One potential treatment strategy is upregulation of the Dystrophin homolog Utrophin in skeletal muscle which has been shown to rescue disease phenotype in mouse model of the disease. Working in collaboration with the Broad Institute in Massachusetts, we have screened and identified a number of potential utrophin upregulating FDA approved compounds. Eight compounds with utrophin enhancing ability have been identified. We have also demonstrated a significant increase in utrophin mRNA and protein levels in mouse C2C12 undifferentiated myoblasts when treated with low dose Celecoxib, a selective COX-2 inhibitor. We have also found that Celecoxib increases utrophin levels in diaphragm and gastrocnemius tissue samples of non-transgenic mice. Impact of this drug on mdx mouse (mice model for DMD) is currently under investigation. Currently, there are no effective treatments for DMD. This method of identifying potential pathways which regulate Utrophin gene and testing their potential as therapeutics for DMD may ultimately providing a better quality of life to the young patients who have this devastating disease.

40. CHADY HAKIM, UNIVERSITY OF MISSOURI

Absence of dystrophin compromises the passive properties of the extensor digitorum longus muscle in mice

Chady H. Hakim, Dongsheng Duan
Department of Molecular Microbiology and Immunology, University of Missouri

Absence of dystrophin, a cytoskeletal protein, causes Duchenne muscular dystrophy (DMD). DMD is a lethal muscle wasting disease that affects 1:3500 boys. In dystrophin deficient muscle, the sarcolemma gets damage by contraction force. Subsequent, the muscle undergoes a pathological change where muscle cell is replaced by fibrotic tissues. Little is known about the passive properties of the dystrophin-deficient muscle. Here, we hypothesize that the change in muscle pathology renders the muscle stiffer. Dystrophin is also highly

expressed at the muscle-tendon junction (MTJ). Thus, we further hypothesize that dystrophin deficiency compromises the MTJ strength. To test these hypotheses, we examined the stress-strain response in the extensor digitorum longus (EDL) muscle of mdx mouse, a mouse model for DMD. At the ages of 2, 6, 14 and 20 months, the mdx EDL muscles were significantly stiffer than those of age-matched normal controls. Further, the mdx EDL muscle showed a higher relaxation rate. In normal and ≤ 6-m-old mdx mice, muscle failure occurred within the muscle. Interestingly, in ≥14-month-old mdx, the muscle failed at the proximal MTJ. Electron microscopy revealed substantial MTJ degeneration in aged but not young mdx mice. In summary, our results suggest that the passive properties of the EDL muscle and MTJ strength are compromised in mdx. More importantly, the increase of muscle stiffness could contribute to the immobilization observed in DMD patient. Our findings open the door to investigate whether novel gene/cell/pharmacological therapies can halt the deterioration of the passive properties and improve mechanical function.

41. RENZHI HAN, LOYOLA UNIVERSITY MEDICAL CENTER

Role of the complement system in the muscle pathology of dysferlinopathy

Renzhi Han,1 Ellie M. Frett,2 Jennifer R. Levy,2 Erik P. Rader,2 John D. Lueck,2 Dimple Bansal,2 Steven A. Moore,3 Rainer Ng,4 Daniel Beltrán-Valero de Bernabé,2 John A. Faulkner,4,5 and Kevin P. Campbell2
1Department of Cell and Molecular Physiology, Loyola University Chicago, Maywood, Illinois, USA. 2Howard Hughes Medical Institute, Department of Molecular Physiology and Biophysics, Department of Neurology, Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, Iowa, USA. 3Department of Pathology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, Iowa, USA. 4Department of Molecular and Integrative Physiology and 5Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA.

Mutations in the dysferlin gene underlie a group of autosomal recessive muscle-wasting disorders termed as dysferlinopathies. Dysferlin has been shown to play an important role in muscle membrane repair with additional roles in muscle regeneration, cytokine release and ATP release, processes requiring vesicle-membrane fusion. However, the mechanism by which muscle becomes dystrophic in these disorders remains poorly understood. Although muscle inflammation is widely recognized in dysferlinopathy and dysferlin is expressed in immune cells, the contribution of the immune system to the pathology of dysferlinopathy remains to be fully explored. Here we show that the complement system is the major cause of muscle pathology in dysferlinopathy. Dysferlin deficiency leads to increased expression of complement factors in muscle, while muscle-specific transgenic expression of dysferlin normalizes the expression of complement factors and eliminates the dystrophic phenotype present in dysferlin-null mice. Furthermore, genetic disruption of the central component (C3) of the complement system ameliorates muscle pathology in dysferlin-deficient mice but has no significant beneficial effect on mdx mice. These results demonstrate that complement-mediated muscle injury is central to the pathogenesis of dysferlinopathy, and suggest that targeting the complement system might serve as a therapeutic approach for this disease.

42. MARIA DE HARO, BAYLOR COLLEGE OF MEDICINE

Novel modifiers of expanded CUG-induced pathogenesis.

Maria de Haro, Ismael Al-Ramahi and Juan Botas.
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Our aim is to identify genetic modifiers of Myotonic Dystrophy Type 1 (DM1), a neuromuscular disorder caused by a CTG expansion in the 3′ UTR of the DMPK gene. Pathogenesis in DM1 is triggered by a toxic gain of function of the expanded DMPK RNA. We used a genetic approach to identify novel genes and genetic pathways that are able of suppressing CUG-induced toxicity when their activities are altered. We generated a Drosophila DM1 model expressing a non-coding mRNA containing 480 interrupted CUG repeats. This (CUG)480 transcript accumulates in nuclear foci and its expression leads to muscle wasting and degeneration

in Drosophila. We found that altering the levels of two RNA-binding proteins known to be involved in DM1 pathogenesis, MBNL1 and CUGBP1, modify the (CUG)480 degenerative phenotypes. Expanded CUG-induced toxicity in Drosophila is suppressed by overexpression of human MBNL1. In contrast, increasing the levels of CUGBP1 worsens (CUG)480-induced degeneration. We carried out a genetic screen to find new genes involved in DM1 pathogenesis. We have tested a collection of mutants in genes encoding RNA-binding proteins (RNA-BPs) and discovered five novel RNA-BPs that are able to modulate CUG-induced toxicity in muscles. We have developed a molecular assay and a behavioral assay, to further test or validate putative modifiers.

43. STEVE HAUSCHKA, UNIVERSITY OF WASHINGTON

Design and Testing of Cell Type-Specific Regulatory Cassettes for Skeletal and Cardiac Muscle Gene Therapy.

Steve Hauschka, Quynh Nguyen, Connie Chen, Rob Welikson, John Angello, Maja Salva, Charis Himeda, Phil Tai, Deri Helterline, Eric Finn, James Allen, Guy Odom, and Jeff Chamberlain.
Depts. of Biochemistry & Neurology, University of Washington, Seattle WA, 98195.

A major technical component of optimal gene and cell therapy for neuromuscular diseases will require the expression of many different therapeutic proteins or RNAs at different levels in a variety of target cell types: satellite cells, satellite cell-derived replicating myoblasts, nascent myotubes, mature muscle fibers, cardiomyocytes, and in a variety of stem cell types that can be directed into myogenic lineages. Additionally, it may be necessary for expression that is beneficial in myoblasts or nascent myotubes to cease as fibers become mature; alternatively, it may be beneficial for expression levels to be externally regulated. Designing regulatory cassettes for these purposes entails the combination of multiple transcriptional control elements whose key binding factors are present and active in different myogenic and/or non-myogenic cell types. This, in turn, requires extensive basic knowledge about transcription factor expression profiles in different cell types, and transcription factor-DNA binding site interactions. An added complexity of gene expression in skeletal muscle is that fast and slow twitch muscle fibers, as well as different anatomical muscles, exhibit different expression levels of the same regulatory cassette. A second technical component for optimal regulatory cassettes is the requirement of their size compatibility with therapeutic cDNAs with respect to their packaging in viral vectors. This issue is particularly relevant in situations in which the vectors have packaging size constraints on the order of 5kb (e.g., AAV), since this can – in cases such as the expression of micro-dystrophin and micro-utrophin – constrain the size of regulatory cassettes to well less than 1 kb, thereby limiting the regulatory complexity that can be built into such cassettes. Strategies for the successful design of muscle type-specific regulatory cassettes to achieve these ends will be described, and comparative data from in vitro and in vivo studies will be presented. Importantly, several of the newest regulatory cassettes exhibit muscle specific expression levels that are equivalent to that of extremely powerful constitutively expressed cassettes such as CMV; and cassettes with graded expression levels are available for therapeutic situations in which lower levels of therapeutic products would be beneficial.

44. KENNETH HENSLEY, UNIVERSITY OF TOLEDO

Adaptation of Central Nervous System Lanthionine Metabolites for Treatment of Neurodegenerative Conditions

Kenneth Hensley, PhD
Associate Professor, and Research Director, Department of Pathology; Joint Appointment, Department of Neurosciences; University of Toledo Medical Center; 3000 Arlington Avenue MS 1090; Toledo, OH 43614 USA; Tel. 419-383-3442; Kenneth.hensley@utoledo.edu

Lanthionines are sulfur amino acid derived products that have long been recognized to exist at low concentrations in the mammalian central nervous system (CNS). Though these molecules historically have been considered waste products of promiscuous transsulfuration pathway enzymology, recent findings suggest that some

lanthionines are biofunctional. In particular, our laboratory recently discovered that the cyclic thioether lanthionine ketimine (LK) binds to the collapsin response mediator protein-2 (CRMP2) which has been implicated recently in diverse diseases including amyotrophic lateral sclerosis (ALS or Lou Gehrig's Disease) to Alzheimer's disease (AD) and schizophrenia. Cell-permeable and recently patented derivatives of LK are capable of promoting neurite growth in culture at low nanomolar concentrations, and protect neurons in culture from a variety of toxic insults. Moreover an LK-ethyl ester suppresses microglial activation in response to inflammatory cytokines, indicating anti-neuroinflammatory potential of these novel small molecules. LK-ethyl ester delivered to SOD1G93A mice, a model for ALS, slowed disease progression significantly. In other work from our laboratory, we have discovered that both LK and the ubiquitous redox-regulating tripeptide glutathione (GSH) bind a CNS protein, LanCL1 (lanthionine synthetase-like protein-1) that is homologous to a prokaryotic lanthionine-producing enzyme. These discoveries raise the intriguing possibility that lanthionine metabolites synthesized from novel GSH-utilizing pathways might be purposeful molecules within the CNS. Accordingly, we synthesized a glutathione-lanthionine chimeric molecule (gLan) which delayed paralysis and prolonged lifespan of the SOD1G93A mouse when administered intraperitoneally, beginning at symptomatic stage disease. These findings begin to suggest a type of "metabolite replacement" or "metabolite supplementation" therapy for particular neurodegenerative conditions, based on treatment with appropriate lanthionine derivatives or pro-drugs. This work was supported in part by a grant from the Judith and Jean Pape Adams Charitable Foundation (JJPAF).

45. BRAD HODGES, PROTHELIA

Laminin-111 as a Therapeutic in the mdx Mouse Model of Duchenne Muscular Dystrophy

Bradley L Hodges1, Kate A Hawkins1, Jachinta E Rooney2 and Dean J Burkin2
1Prothelia, Inc. and 2University of Nevada, Reno

Among the many isoforms of laminin the laminin-211 isoform predominates around normal skeletal and cardiac muscle cells and contributes to the structural integrity of muscle through engagement of the laminin receptors alpha dystroglycan and alpha7beta1 integrin. It was recently shown that systemic delivery of laminin-111, an isoform closely related to laminin-211, targets skeletal and cardiac muscles of the mdx mouse model of Duchenne muscular dystrophy (DMD). Laminin-111 stabilizes dystrophin-deficient muscles through increased synthesis of the alpha7beta1 integrin. The purpose of this study was to assess additional functional, histological and biochemical outcomes in mdx mice following acute and chronic dosing of mouse laminin-111. We have found that a single administration of laminin-111 to mdx mice resulted in reduced serum levels of ALT, AST and CK by 4 days post-injection and increased expression of the alpha7beta1 integrin. Chronic administration of laminin-111 to mdx mice improved forelimb grip strength, protected the quadriceps from the damaging effects of downhill treadmill exercise, and improved force retention in the diaphragm and EDL muscles following ex vivo eccentric contractions. Chronic dosing of laminin-111 also demonstrated low or nonexistent antibody responses, and kidney function was not compromised as assessed by serum BUN and creatinine. These data suggest that mouse laminin-111 is safe and effective in the mdx mice and provides justification for the continued development of human laminin-111 for treatment of DMD.

46. SACHIKO HOMMA, BOSTON BIOMEDICAL RESEARCH INSTITUTE

Motor nerve pathology in a laminin-alpha2-deficient mouse model of congenital muscular dystrophy includes altered Schwann cell differentiation and is ameliorated by doxycycline.

Sachiko Homma (1,2), Mary Lou Beermann (1), and Jeffrey Boone Miller (1,2,3)
(1) Neuromuscular Biology & Disease Group Boston Biomedical Research Institute Watertown, Massachusetts USA
(2) Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center Boston Biomedical Research Institute Watertown, Massachusetts USA
(3) Department of Neurology Harvard Medical School Boston, Massachusetts USA

The most common form of childhood congenital muscular dystrophy, Type 1A (MDC1A), is caused by mutations in the human LAMA2 gene that encodes the laminin-α2 subunit. In addition to skeletal muscle deficits, MDC1A patients typically show loss of motor nerve function. To identify mechanisms underlying this loss of motor nerve function, we have examined pathology and cell differentiation in sciatic nerves and ventral roots of the laminin-α2-deficient (Lama2-/-) mice, which are models for MDC1A. We found that, compared to wild-type, sciatic nerves of Lama2-/- mice had a significant increase in both proliferating (Ki67+) cells and premyelinating (Oct6+) Schwann cells, but also had a significant decrease in both immature/non-myelinating (GFAP+) and myelinating (Krox20+) Schwann cells. To extend our previous work in which we found that doxycycline, which has multiple effects on mammalian cells, improves motor behavior and more than doubles the median life-span in Lama2-/- mice, we also determined how nerve pathology was affected by doxycyline treatment. We found that myelinating (Krox20+) Schwann cells were significantly increased in doxycycline-treated compared to untreated sciatic nerves. In addition, doxycyline-treated motor nerves had significantly less pathology as measured by assays such as amount of unmyelinated or disorganized axons. This study thus identified aberrant proliferation and differentiation of Schwann cells as key components of pathogenesis in peripheral nerves and provided proof-of-concept that pharmaceutical therapy can be of potential benefit for peripheral nerve dysfunction in MDC1A. Supported by MDA, NICHD, NHLBI.

47. JAMES F. HOWARD, UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

James F. Howard, Jr, MD1,5, Zheng (Jane) Fan, MD1,5, Keith Kocis, MD2,3,5, Robert Valley, MD2,5, Manisha Chopra, MD1,5, Hongyu An4, PhD, Weili Lin4, PhD, Joseph Muenzer, MD, PhD3,5,6 and William Powers, MD1,5. Departments of 1Neurology, 2Anesthesiology, 3Pediatrics, 4Radiology, 5Wellstone Muscular Dystrophy Cooperative Research Center and 6Gene Therapy Center, The University of North Carolina School of Medicine, Chapel Hill, NC; United States, 27599.

Objectives: Assess the safety and feasibility of the transvenous limb perfusion gene delivery method in human muscular dystrophy Background: High-pressure retrograde transvenous limb perfusion has been successfully used to deliver gene therapies into skeletal muscles in experimental animals. Translating this promising technique to humans with muscular dystrophy requires addressing multiple safety and logistical aspects separate from the therapeutic agent. Design/Methods: A dose escalation study of transvenous single limb perfusion with 0.9% saline in adults with Becker or limb-girdle muscular dystrophies starting with 5% of limb volume. Cardiac, vascular, renal, muscle and nerve function were monitored. Anesthesia was provided with fentanyl, midazolam and propofol. An 18 or 20 g intravenous catheter was inserted into the distal great saphenous vein. A single cuff tourniquet was placed just above the knee with pressure of 310 mmHg. Infusion of normal saline was carried out with a Belmont FMS 2000 Rapid Infuser at a maximum line pressure of 300 mmHg with a goal infusion rate of 80ml/min. Results: Infusion volume was escalated stepwise to 20% limb volume in six subjects. No subject complained of any post procedure pain other than due to needle punctures. Safety warning boundaries were exceeded only for transient depression of limb tissue oximetry and transient elevation of muscle compartment pressures; these were not associated with nerve, muscle or vascular damage within 72 hours post procedure. Muscle MRI demonstrated fluid accumulation in muscles of the perfused leg at 20% limb volume. Conclusions: High-pressure retrograde transvenous limb perfusion with saline up to 20% of limb volume at these infusion parameters is safe and feasible in human muscular dystrophy. These studies will serve as a basis for future gene therapy clinical trials.

48. DENA JACOB, THOMAS JEFFERSON UNIVERSITY

Expression of the multi-drug resistant efflux transporter P-glycoprotein increases in spinal cord astrocytes throughout disease progression in the ALS mouse

Jacob DA, Jablonski MR, Bogush A, Goffredo M, Pasinelli P, Trotti D
All authors are affiliated with:
Weinberg Unit for ALS Research, Thomas Jefferson University, 900 Walnut Street, Philadelphia, PA 19107

Despite numerous animal drug trials targeting different pathogenic pathways, pharmacological approaches to cure the mouse that models amyotrophic lateral sclerosis (ALS) have so far failed. This suggests either yet undefined crucial pathogenic mechanisms or a frank pharmacoresistance to treatments in these mice. Drug transporters influence pharmacokinetics and pharmacodynamics; among different drug transporter systems, the multi-drug resistance efflux transporter P-glycoprotein (P-gp; mdr1) extrudes a broad range of xenobiotics from cells and confers chemoresistance and a poor clinical outcome. In addition to normal expression in several peripheral tissues, P-gp is expressed in the central nervous system with predominant localization to capillary endothelial cells and, to a lesser extent, parenchymal and perivascular astrocytes of the blood brain/blood cerebrospinal fluid barriers (BBB/BCSF). P-gp is up-regulated under certain neuropathological conditions such as intractable epilepsy and in rodent models of temporal lobe epilepsy, and this appears to be regulated by the excessive glutamate release occurring in these seizure episodes. Kainate-triggered seizures induce neuronal P-gp expression and up-regulate P-gp in BBB endothelial cells and astrocytes, but P-gp expression, localization, and distribution under pathological conditions of the spinal cord are virtually unexplored. Although glutamate excitotoxicity and neuroinflammation are also components of ALS, drugs that increase glutamate clearance in the SOD1-G93A mutant mouse that models ALS have been largely unsuccessful. We previously reported consistent yet transient effects of nordihydroguiaiaretic acid (NDGA), a glutamate uptake enhancer, as well as a disease-driven up-regulation of spinal cord P-gp in SOD1-G93A mice, suggesting that this and other pharmacotherapeutic failures may result from acquired, P-gp-mediated pharmacoresistance. Here we investigate P-gp cellular localization and expression in the spinal cord of SOD1-G93A mice throughout ALS disease progression. We find that P-gp is expressed in motoneurons and that P-gp expression increases in ventral spinal cord astrocytes throughout disease. We also examined P-gp expression in spinal cord homogenate from humans and report that P-gp is elevated in ALS patients. Thus, P-gp expression in spinal cord parenchyma may impart overt pharmacoresistance to ALS affected cells and account for the poor therapeutic effect observed in multiple ALS clinical drug trials. Additionally, up-regulation of P-gp in diseased CNS parenchyma may provide a cellular compensatory response to extrude increased levels of endogenous toxins resulting from the disease.

49. VANESSA JAHNKE, CHILDREN'S NATIONAL MEDICAL CENTER

Metabolic remodeling (AMPK and PPAR delta agonists) agents have beneficial effect in MDX mouse model of dystrophy

Vanessa E Jahnke, Jack Van Der Meulen, Helen Johnston, Eric P Hoffman and Kanneboyina Nagaraju. Research Center for Genetic Medicine, Children's National Medical Center, Washington DC.

Background: Mitochondria can sense signals linked to variations in energy demand to regulate nuclear gene expression. This retrograde signaling pathway is presumed to be involved in the regulation of myoblast proliferation and differentiation. Duchenne muscular dystrophy is a genetic disease inducing a severe muscle wasting characterized by rounds of degeneration regeneration cycles. Enhancing mitochondria activity, in healthy mice, is known to prevent muscle from muscle wasting and increase muscle function. The aim of the study was to use metabolic remodeling agents to determine whether an increase in mitochondrial biogenesis and/or metabolism contributes to improved muscle function in dystrophin deficient mdx mice. Methods/Results: Twelve-weeks-old MDX mice were treated with two different metabolic remodeling agents (GW501516 and AICAR) separately or as a combination for 4 weeks starting histological, and biochemical measures to assess the drug(s) effect in these mice. We found a gain in body weight and muscle weight in treated mice compared to the vehicle. The histology analysis of the EDL muscle demonstrates a decrease of the inflammation, a decrease of the number of fibers with central nuclei and an increase of the peripheral nuclei. Indeed, MDX mice treated with these drugs have an increase of their overall activity and more importantly a significant gain forelimb and the hindlimb strength. Further, treated mice have a significant decrease of the activated satellite cells, a reduction of the regenerated fibers and an inhibition FoXO signaling, indicating a better regulation of myogenesis and inhibition of muscle wasting. Over all combination treatment showed significant improvements in disease phenotype. Conclusion: Our findings suggest that treatment with metabolic remodeling agents showed beneficial

effects in mdx mice. Triggering mitochondrial biogenesis and metabolism may therefore represent a new approach to improve quality of life for DMD patients.

50. PAUL JANSSEN, OHIO STATE UNIVERSITY

Improvement of cardiac contractile function by peptide-based inhibition of NF-κB in the utrophin/ dystrophin-deficient murine model of muscular dystrophy.

Dawn A. Delfín1, Ying Xu2, Jennifer M. Peterson3, Denis C. Guttridge3, Jill A. Rafael-Fortney1, and Paul M.L. Janssen2
1Department of Molecular and Cellular Biochemistry, 2Department of Physiology and Cell Biology, 3Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University.

Duchenne muscular dystrophy (DMD) is an inherited and progressive disease causing striated muscle deterioration. Patients in their twenties generally die from either respiratory or cardiac failure. In order to improve the lifespan and quality of life of DMD patients, it is important to prevent or reverse the progressive loss of contractile function of the diaphragm and heart. Recent studies by our labs have shown that the peptide NBD (Nemo Binding Domain), targeted at blunting NF-κB signaling, reduces inflammation, enhances myofiber regeneration, and improves contractile deficits in the diaphragm in dystrophin-deficient mdx mice. To assess whether cardiac function can also be improved, we investigated contractile function in papillary muscles isolated from mice deficient for dystrophin and its homolog utrophin (double knockout = dko) treated with NBD peptide. The dko model, untreated, shows classic pathophysiological hallmarks of heart failure, including an impaired force-frequency response and a severely blunted β-adrenergic response. In addition, we studied histopathological and inflammatory markers in these mice. At baseline conditions, active force development in muscles from NBD treated dko mice was more than double that of vehicle-treated dko mice (12.5 ± 1.8 vs. 5.2 ± 1.8 mN/mm2, $p<0.05$). NBD treatment also significantly improved frequency-dependent behavior of the muscles; in vehicle treated mice a shift from 4 to 10 Hz stimulation frequency resulted in a 46 ± 6% loss of force ($p<0.05$, negative force-frequency typical of failing myocardium), whereas in NBD-treated mice change in force was not significant (flat force frequency, typical of healthy murine myocardium). The increase in force in NBD-treated dko muscles to β-adrenergic stimulation was robustly restored compared to vehicle-treated mice (10.0 ± 3.2 mN/mm2 vs. 2.8 ± 1.5 mN/mm2, $p<0.05$). Histological features, including collagen content and inflammatory markers were not significantly different between NBD-treated and vehicle-treated dko mice. We conclude that NBD can significantly improve cardiac contractile dysfunction in the dko mouse model of DMD and may thus provide a novel therapeutic treatment of heart failure.

51. GENRI KAWAHARA, CHILDREN'S HOSPITAL BOSTON

Drug screening using the model fish of Duchenne Muscular Dystrophy

Genri Kawahara1-2, 4 Jeremy Karpf1-2, 4, Jennifer Myers1-4, Matthew Alexander1-4, Jeffrey R. Guyon 5 and Louis M.Kunkel1-4*
1Division of Genetics, Program in Genomics, Children's Hospital Boston, 2Department of Genetics, Harvard Medical School, 3Howard Hughes Medical Institute, and 4the Manton Center for Orphan Disease Research, Children's Hospital Boston, MA, USA. 5Alaska Fisheries Science Center.

Two known zebrafish dystrophin mutants, sapje and sapje-like (sapc/100) are known as excellent small animal models of Duchenne Muscular Dystrophy. These model fish have disorganized skeletal muscle structure. We have screened 1120 chemicals from the Prestwick chemical library in order to identify small molecules that modulate the muscle phenotype in these fish. With a quick and easy birefringence assay, we have identified seven small molecules that influence muscle pathology in two dystrophin-null zebrafish without restoration of dystrophin

expression. Three of the seven candidate chemicals restored normal birefringence in skeletal muscle and increased survival of dystrophin-null fish. One chemical of aforementioned three candidate chemicals, aminophylline, which is known to be a non-selective phosphodiesterase (PDE) inhibitor, had the greatest ability to restore normal muscle structure compared to those of non-treated, dystrophin-null fish and to up-regulate the cAMP-dependent protein kinase (PKA) pathway in treated dystrophin-null fish. Moreover, other PDE inhibitors also reduced the percentage of affected sapje fish. These compounds, which moderate the muscle phenotype in these dystrophin-null zebrafish may lead to potential therapeutic interventions in human muscular dystrophy. Further screening of other chemical libraries may lead to the discovery of new potential therapeutic drugs to treat Duchenne Muscular Dystrophy.

52. CATHERINE KRULL, UNIVERSITY OF MICHIGAN

The role of SMN is motor axon growth and fasciculation and in keeping motor neuron cell bodes in the ventral neural tube.

Catherine E. Krull, Fengyun Su, Mustafa Sahin
Dept of Biologic and Materials Sciences at the University of Michigan,
Dept of Neurology at Harvard University and Childrens' Hospital.

SMN retains motor neurons in the ventral neural tube. SMN is the gene responsible for Spinal Muscular Atrophy (SMA). We have taken a loss of function approach in chick using shRNAs against SMN in motor neurons. Using this approach combined with immunocytochemistry, we have determined that MMC(m) motor neurons leave the neural tube and are mislocalized and appear to "migrate" on the spinal nerve. SMN is responsible, as applying human SMN rescues the mislocalization/"migration" of motor neurons on the spinal nerve. Indeed, most MMC(m) motor neurons extend their axons and then escape the ventral neural tube. Using confocal microscopy, we have focused on understanding how motor neurons escape from the ventral neural tube. At high magnification, GFP-positive/SMN shRNAs-treated motor neurons seem to use the process of translocation to escape the ventral neural tube. Although the basal lamina of the neural tube is altered, it is currently unknown if that is a direct consequence or indirectly related to SMN knockdown. Experiments are in progress to conduct time-lapse imaging to determine if mislocalization or "migration" on the spinal nerve is as active or passive process.

53. SHIHUAN KUANG, PURDUE UNIVERSITY

Dlk1 is necessary for proper skeletal muscle development and regeneration

Jolena N Waddell1, Peijing Zhang1, Yefei Wen1, Sanjay K Gupta2, Aleksey Yevtodiyenko3, Jennifer V Schmidt4, Christopher A Bidwell1, Ashok Kumar2, Shihuan Kuang1,5*
1Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
2Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40202
3Division of Oncology, School of Medicine, Stanford University, Stanford, CA 94305, USA
4Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA
5Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA
*Presenting author: Shihuan Kuang, Ph. D., Department of Animal Sciences,

Delta-like 1homolog (Dlk1) is an imprinted gene encoding a transmembrane protein whose increased expression has been associated with muscle hypertrophy in animal models. However, the mechanisms by which Dlk1 regulates skeletal muscle plasticity remain unknown. Here we combine conditional gene knockout and over-expression analyses to investigate the role of Dlk1 in mouse muscle development, regeneration and myogenic stem cells (satellite cells). Genetic ablation of Dlk1 in the myogenic lineage resulted in reduced body weight and skeletal muscle mass due to reductions in myofiber numbers and myosin heavy chain IIB gene expression. In addition, muscle-specific Dlk1 ablation led to postnatal growth retardation and impaired muscle regeneration, associated with augmented myogenic inhibitory signaling mediated by NF-kB and inflammatory cytokines. To examine the role of Dlk1 in satellite cells, we analyzed the proliferation, self-renewal and differentiation of satellite cells cultured on their native host myofibers. We showed

that ablation of Dlk1 inhibits the expression of the myogenic regulatory transcription factor MyoD, and facilitates the self-renewal of activated satellite cells. Conversely, Dlk1 over-expression inhibited the proliferation and enhanced differentiation of cultured myoblasts. As Dlk1 is expressed at low levels in satellite cells but its expression rapidly increases upon myogenic differentiation in vitro and in regenerating muscles in vivo, our results suggest a model in which Dlk1 expressed by nascent or regenerating myofibers non-cell autonomously promotes the differentiation of their neighbor satellite cells and therefore leads to muscle hypertrophy.

54. JENNIFER LACHEY, ACCELERON PHARMA

The effect of combining activin receptor type IIB inhibition and prednisolone treatment in mdx mice

J.L. Lachey, V. Wong, J. Seehra
Acceleron Pharma Cambridge, MA USA

Activin receptor type IIB (ActRIIB) is a signaling receptor for ligands involved in suppressing muscle growth. Blocking ActRIIB signaling increases muscle mass and function. RAP-031, a soluble fusion protein comprised of a form of ActRIIB extracellular domain fused to a murine Fc, also increases muscle mass and improves muscle weakness in mdx mice. There is currently no cure for Duchenne muscular dystrophy (DMD); however, glucocorticoids, such as prednisolone, slow disease progression and are commonly used for the management of disease. To study the effect of combined glucocorticoid/RAP-031 regime in a dystrophy model, mdx mice received either: vehicle/vehicle (VEH/VEH), prednisolone /vehicle (PRED/VEH) or prednisolone/RAP-031 (PRED/RAP). Prednisolone was dosed 5 mg/kg, 5X/week, po and RAP-031 was dosed once weekly at 10 mg/kg sc. At 2 weeks, PRED/VEH gained 8.5% less body weight ($p<0.01$) than VEH/VEH. In contrast, PRED/RAP gained body weight at a similar rate to VEH/VEH. NMR analysis established the body weight effect was due to decreased lean mass gain in PRED/VEH ($p<0.01$) compared to VEH/VEH whereas PRED/RAP gained lean mass similarly to VEH/VEH. At study day 9, VEH/VEH and PRED/VEH had comparable absolute forelimb grip strength, but given the PRED/VEH cohort had reduced lean mass, their grip strength per unit of muscle is greater ($p<0.05$). PRED/RAP mice had significantly greater absolute grip strength ($p<0.05$ compared to VEH/VEH and PRED/VEH). Importantly, the normalized grip strength improvement is retained in PRED/RAP (VEH/VEH=$p<0.05$, PRED/VEH=ns). Therefore, RAP-031 improved mdx muscle function in mice by increasing overall lean mass or by preventing lean mass loss, as is the case when given with prednisolone. In contrast, prednisolone improved muscle function independent from a lean mass increase. These data support the use of ACE-031, the human version of RAP-031, as a potential distinct therapeutic for the treatment of DMD.

55. DALE LANGE, HOSPITAL FOR SPECIAL SURGERY/WEILL MEDICAL COLLEGE OF CORNELL UNIVERSITY

Does pyrimethamine decrease sod1 levels in patients with Familial ALS?

Dale J. Lange, M.D.*, Peter M. Andersen, M.D. PhD#, Stephan L. Marklund, MD, PhD#, Rahul Remanan, MBBS+, Daniel Benjamin PhD-
1Weill Medical College of Cornell University, New York, NY
2Umea University, Umea Sweden,
3Hospital For Special Surgery, New York, NY
4Cenoxsys Corporation, Princeton, NJ

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease causing relentless progressive weakness of the arms, legs and respiratory muscles that is uniformly fatal. Inserting the SOD1 mutant gene into mice causes them to develop a disease closely resembling ALS. Inhibiting expression of the SOD1 gene prevents animals from developing the disease. Increasing or decreasing the number of mutated genes proportionately speeds or slows the progression of the disease. Therefore, reducing SOD1 levels in patients with SOD1 associated FALS may be a promising therapeutic approach. Through an extensive in vitro screening program for medications having the ability to reduce SOD1 levels, several molecules that reduce SOD1 protein levels are known. One of the most potent

molecules is pyrimethamine, an FDA approved medication used for the treatment of malaria and toxoplasmosis. Pyrimethamine reduces SOD1 levels in mice and our preliminary studies show similar findings in humans. Our study's primary objective is to determine if familial ALS patients taking pyrimethamine will show a decline in SOD1 levels in peripheral blood and CSF. Safety and tolerability of pyrimethamine in patients with FALS was also monitored. We studied 16 patients with definite ALS and known SOD1 mutations. The mutations included A4V (3), G93S (3), D90A heterozygous (2), D90A homozygous (1), E100G (2), L144F (2), D109Y, Frameshift codon 125. Two patients withdrew because of adverse effects. Of the remaining 14 patients, all patients showed lowering of SOD1 levels though significant variability was observed during observational points during the study. Two patients had cerebrospinal fluid analysis before and after treatment and showed sustained reduction of SOD1 content and activity for the duration of treatment. Patients showed a variable ability to tolerate the medication but all were able to tolerate 50mg per day or above. Men were better able to reach 100 compared to women. We therefore have shown that pyrimethamine was able to lower SOD1 levels in the peripheral blood lymphocytes and SOD1 levels and activity are reduced in the CSF in patients with FALS. Measuring the biologic effect of a therapeutic intervention with secondary measurement of clinical change may be an alternative way to investigate potential therapies in FALS.

56. DEJIA LI, UNIVERSITY OF MISSOURI

Implication of nNOSμ delocalization on the muscle force in dystrophin deficient and δ-sarcoglycan knockout mice

Dejia Li , Yongping Yue , Yi Lai , Chady H. Hakim , Dongsheng Duan
Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO, USA

The mechanism of force reduction is not completely understood in Duchenne muscular dystrophy (DMD), a dystrophin-deficient lethal disease. Nitric oxide regulates muscle force. Interestingly, neuronal nitric oxide synthase μ (nNOSμ), a major source of muscle nitric oxide, is lost from the sarcolemma in DMD muscle. We hypothesize that nNOSμ delocalization contributes to force reduction in DMD. To test this hypothesis, we generated dystrophin/nNOSμ double knockout mice. Genetic elimination of nNOSμ significantly enhanced force in dystrophin-null mice. Pharmacological inhibition of nNOS yielded similar results. To further test our hypothesis, we studied δ-sarcoglycan-null mice, a model of limb-girdle muscular dystrophy. These mice had minimal sarcolemmal nNOSμ delocalization and muscle force was less compromised. Annihilation of nNOSμ did not improve their force either. To determine whether nNOSμ delocalization itself inhibited force, we corrected muscle disease in dystrophin-null mice with micro-dystrophins that either restored or did not restore sarcolemmal nNOSμ. Similar muscle force was obtained irrespective of nNOSμ localization. Additional studies suggest that nNOSμ delocalization selectively inhibits muscle force in dystrophin-null mice via nitrosative stress. In summary, we have demonstrated for the first time that nitrosative stress elicited by nNOSμ delocalization is an important mechanism underlying force loss in DMD.

57. ARTURO LOPEZ CASTEL, VALENTIA BIOPHARMA

An innovative in vivo platform for drug discovery in myotonic dystrophy

López Castel, A.1, Garijo R.1, García-Alcover I.2, Tomás A.1, Tormo J.R.1, Álvarez-Abril M.C.1, Artero R.2 & Pérez-Alonso M.1,2
1Valentia BioPharma SL, Parc Cientific UV, Catedrático José Beltrán, 2. 46990 Paterna, España
2Dept.Genética, Universitat de València, Dr Moliner, 50. 46100 Burjassot, España

One of the main limitations of drug discovery programs using cell culture or biochemical testing is the lack of a whole organism evaluation. The therapeutic efficiency of a given compound does not only depend on its ability to interact with a given target, but also on its behavior with regards to adsorption, distribution, metabolism, excretion and/or toxicity in a living organism (ADME-Tox).

The use of Drosophila melanogaster in functional assays aiming at discovering new chemical entities offers a differential advantage over traditional approaches because, in addition to assessing activity against a

given target, and with the same investment, ADME-Tox restrictions are simultaneously evaluated. Moreover, Drosophila testing presents short turnarounds from dosing to results, low variability with highly reduced costs, and the availability of a large number of genetic tools and tricks, improved over a whole century.

Valentia Biopharma SL (established as a spin-off from the University of Valencia, Spain). offers a unique platform, completely automated, of high throughput screening (HTS) for screening libraries of candidate compounds for treating human diseases in in vivo conditions using Drosophila melanogaster as experimental model. Currently the HTS platform is being used in combination with a transgenic Drosophila model for myotonic dystrophy (DM1) in order to find lead compounds to treat this rare disease.

This project involved generating "humanized" transgenic flies carrying constructs designed to detect mis-splicing events, similar to those observed in DM1 patients in muscles, simultaneously expressing minigenes and 480 non-coding CTG repeats (DM1 flies). These transgenes fused a known human minigene to the reporter so that normal or aberrant splicing could be read as bioluminescence.

Our HTS platform can now be adapted to several other screening applications and therapeutic targets to quickly evaluate in Drosophila up to thousands of compounds within a few months. Examples of results of our current DM1 campaign will be provided at the meeting.

58. LINDA LOWES, NATIONWIDE CHILDREN'S HOSPITAL

Defining Outcome Measures in Sporadic IBM for a Follistatin Gene Transfer Clinical Trial

Linda Pax Lowes, PT PhD; Lindsay Alfano, PT, DPT, PCS; Laurence Viollet, PhD; Xiomara Rosales, MD; K Reed Clark, PhD, Kevin M Flanigan, MD; Zarife Sahenk, MD,PhD, Brian Kaspar PhD, Jerry R Mendell, MD

Introduction: Sporadic inclusion body myositis (sIBM) presents with stereotyped quadriceps muscle weakness resulting in falls and loss of ambulation. Direct intramuscular injection of the alternatively spliced follistatin gene (FS344) mediated by AAV1 transduction results in the synthesis and secretion of the follistatin 315 isoform leading to increased muscle size and strength in the mdx mouse and nonhuman primates. Translating this to clinical trial requires outcome measures designed to test quadriceps strength and function in sIBM patients. PURPOSE: Evaluate the relationship between quadriceps strength and functional tests. Questions persist as to whether the strength of the quadriceps muscle will serve as a surrogate for functional improvement assessed by the timed walking tests(distance over time) and rising from a chair. DESIGN: Measure quadriceps strength of 51 subjects with sIBM (39 men; mean age 65 years; range 44-88) using maximum voluntary isometric contraction. Functional tests included Timed Up and Go (TUG), distance walked for two (2MWT) and six (6MWT) minutes. RESULTS: Quadriceps strength ranged from 1 to 90% (Mean=18%) of age-expected values. Quadriceps strength shows a moderate correlation with the 2MWT (0.587, p=0.00) and 6MWT (0.555 p=0.00) and a mild correlation to TUG (-0.390 p=0.004). Analysis of variance revealed that quadriceps strength accounted for 35 % of the variability in 6MWT distances (r2 = 0.345 p=0.000). Thirty five participants completed the TUG (Range 6 to 41 seconds mean = 12). Subjects that exceed 14 seconds or are unable to complete the test (53% of sample) are considered to be at a high risk for falling (Bohannon, 2006). Distances walked in 2 and 6 minutes were highly correlated (0.949 p=0.00) with quadriceps strength, suggesting that 2 minutes might be sufficient for testing function. Conclusions: Preserved quadriceps muscle strength predicts superior functional performance for TUG and distance covered in the 2 MWT and 6MWT suggesting that targeting this muscle will improve function. However, the variability in quadriceps strength and complex patterns of weakness emphasizes the need for careful patient selection to avoid the pitfalls of a false-negative effect of FS344 gene therapy. For some patients, improvement may require targeting multiple muscles to achieve clinically meaningful outcomes.

59. QI LU, CAROLINAS MEDICAL CENTER

Rescue of Dystrophin expression and muscle function by Long Term treatment of morpholino antisense oligomer

Bo Wu, Bin Xiao Peijuan Lu, Juan Li,, Xiao Xiao & Qi Long Lu
McColl-Lockwood Laboratory for Muscular Dystrophy Laboratory, Neuromuscular/ALS Center, Carolinas Medical

Center 2. Division of Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA

Phosphorodiamidate morpholino oligomers (PMO) has been proved successful to direct alternative splicing pathways to skip targeted dystrophin exon with systemical production of truncated but functional dystrophin protein in animal models. Furthermore, clinical trials have demonstrated efficacy of morpholino as antisense drug targeting exon 51 by local and systemic administration. Early result from the on going Phase 1b/2 clinical trial with systemic injections of PMO also detected expression of targeted dystrophin. However, long-term efficacy and its associated potential toxicity remains to be determined. We have now examined long-term (one year) systemic effect of PMO treatment targeting mutated exon 23 in mdx mice. Our results demonstrated that PMO improved disease phenotype in a dose-dependant manner. A biweekly administration was able to maintain therapeutic levels of dystrophin in most muscle fibers with significant improvement in muscle functions. No detectable toxicity was observed. The potential of PMO as a safe and effective antisense drug for long-term-treatment of Duchenne Muscular Dystrophy will be discussed. The effective dosage established in the mouse model could be used as a reference for determining therapeutic regimen of individual new antisense drug when its efficacy in targeted exon skipping and the truncated dystrophin protein is taken into consideration.

60. DAVID LYNCH, CHILDREN'S HOSPITAL OF PHILADELPHIA

Measures of Progression in Friedreich Ataxia

David Lynch, Ph.D.

Background/Hypothesis: The Collaborative Clinical Research Network in Friedreich Ataxia is conducting a longitudinal natural history study defining the neurologic rate of progression and its relationship to specific disease factors. The present work summarizes ongoing results from this cohort. Methods: Over 500 subjects with FRDA were examined at 11 sites in the United States and Australia for up to 7 years. Annual medical histories, Friedreich ataxia rating scale (FARS), performance measures and their composites (Z2,Z3) were collected and analyzed.. Results: The cohort had an age at onset of 13.9, an Age at Baseline of 25.2, and a mean GAA repeat length of 632. The baseline FARS was 63.5+21.2. We then examined change in neurological measures. Every measure worsened over time. Initially rates of change for all measures were almost linear before floor/ceiling effects were noted 3-6 years after baseline visit. All measures showed evidence of floor/ceiling effects except Low contrast letter acuity. The ratio of yearly change/SD change decreased over the first 3 years of follow-up for the FARS and Z2 measures (from 2-2.5 at year 1, decreasing to approximately 1 by year 3) with slightly lower values being found for the performance composite Z2 than the FARS exam. Over longer times evaluation of this ratio was confounded by floor effects and by bias in the group returning for follow-up. Conclusions: The present data show that the FARS and performance measure composites both capture neurologic progression in FRDA. Using the present data, the composite performance measure Z2 would require the lowest samples size in clinical trials of 1-2 years duration. However, such trials would still require >50 subjects per arm presuming a 50% retardation in progression rate. Based on the lower susceptibility of composites to placebo effects in recent trials, such composites may prove optimal for neurological assessment in clinical trials.

61. GORDON LYNCH, UNIVERSITY OF MELBOURNE

Modulating IGF:IGFBP Signaling to Improve Muscle Function in Muscular Dystrophy

Gordon S. Lynch
Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, Victoria 3010

Muscle wasting and weakness are symptoms of many neuromuscular disorders, including Duchenne muscular dystrophy (DMD). Although considerable efforts are being directed to developing gene and cell therapies for DMD, these are far from perfected. In the interim, alternative and complementary therapies must be developed to preserve functional muscle fibers, enhance muscle regeneration, and promote muscle

growth. Strategies are needed to ameliorate the dystrophic pathology and enhance quality of life, enabling patients to take advantage of other therapies when they eventually become available. Growth factors, like insulin-like growth factor-I (IGF-I), have potential for improving muscle function in muscular dystrophy. The biological actions of IGF-I in vivo are strongly modulated by a family of six IGF binding proteins (IGFBPs) that bind ~99% of IGF-I in the circulation. This large reservoir of bound, biologically inactive IGF-I has a prolonged half-life and is protected from degradation. IGFBPs are believed to primarily transport IGF-I to target tissues, modulate IGF-I's action, and prevent hypoglycemia. Although IGFBPs have been thought to generally inhibit the actions of IGF-I, their role in skeletal muscle is not well understood. For example, we still lack a definitive answer to the logical question "Why do we need six IGFBPs?" Recent evidence suggests that the IGFBPs can have both enhancing and inhibiting effects on IGFs and that the IGFBPs may exert independent effects. While each of the IGFBPs is likely to have a unique function, evidence from transgenic mice and mice selected for body weight, indicates that IGFBP-2 plays a critical role in modulating muscle growth. Better understanding the role of IGFBPs in skeletal muscle, their possible functional redundancies, and their role in the pathophysiology of muscular dystrophies, has practical importance for the application of IGFBPs as therapeutic agents, as well as for therapies targeting IGF-I. While an examination of the functional roles of all six IGFBPs is a long-term goal, the purpose of this research is to focus on one of the most important IGFBPs in skeletal muscle, IGFBP-2, particularly its effects on the pathophysiology of muscular dystrophy. This research will provide entirely new information about the role of IGFBP-2 in muscular dystrophy and its role in IGF signaling in normal and diseased muscles. Supported by the Muscular Dystrophy Association (175821)

62. ALEX MACKENZIE, CHILDREN'S HOSPITAL OF EASTERN ONTARIO RESEARCH INSTITUTE

Faraz Farooq1, 2, Francisco Abadía Molina3, Jeremiah Hadwen1, 2, Duncan MacKenzie1,2, Martin Holcik1, 2 and Alex MacKenzie1, 2

A potential treatment strategy for SMA is to upregulate levels of SMN protein originating from the SMN2 gene compensating in part for the absence of SMN1 gene. STAT5 has been shown to activate SMN2 expression, in SMA-like mouse embryonic fibroblasts and human SMN2-transfected NSC34 cells. Given that Prolactin is also known to activate the STAT5 signalling pathway, we elected to assess its impact on SMN levels. In this manner we have demonstrated a significant induction in SMN mRNA and protein levels in NT2 and MN-1 cells upon treatment with Prolactin. We have shown that activation of STAT5 pathway by Prolactin results in the transcriptional upregulation of SMN gene. We have also found that Prolactin treatment induces SMN expression in brain and spinal cord samples of wild type mice. Prolactin treatment increased SMN levels, improved motor function and enhanced survival in a severe SMA mouse model (extending median survival from 13 to 21 days). This study thus provides a new mechanistic insight of how SMN protein is regulated through Prolactin via STAT5 pathway and its effect on the phenotype of the disease. Our results confirm earlier work suggesting STAT5 pathway activators as potential therapeutic compounds for the treatment of SMA and identify prolactin as one such promising agent.

63. KRISHNA MALLELA, UNIVERSITY OF COLORADO, DENVER

Missense mutations in the N-terminal actin binding domain of dystrophin that trigger muscular dystrophy decrease protein stability and lead to cross-beta aggregates

Surinder M. Singh, Narsimulu Kongari, Javier Cabello-Villegas, and Krishna M.G. Mallela
Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Anschutz Medical Campus, 12700 E 19th Ave, C238-P15, Aurora, CO 80045, USA.
Email: krishna.mallela@ucdenver.edu

A deficiency of functional dystrophin protein in muscle cells causes muscular dystrophy (MD). More than 50% of missense mutations that trigger the disease occur in the N-terminal actin binding domain (N-ABD

or ABD1). We examined the effect of four disease-causing mutations - L54R, A168D, A171P, and Y231N - on the structural and biophysical properties of isolated N-ABD. Our results indicate that N-ABD is a monomeric, well-folded alpha-helical protein in solution, as is evident from its alpha-helical circular dichroism spectrum, blue shift of the native state tryptophan fluorescence, well-dispersed amide crosspeaks in 2D NMR 15N-1H HSQC fingerprint region, and its rotational correlation time calculated from NMR longitudinal (T1) and transverse (T2) relaxation experiments. Compared to WT, three mutants - L54R, A168D, and A171P - show a decreased alpha-helicity and do not show a cooperative sigmoidal melt with temperature, indicating that these mutations exist in a wide range of conformations or in a 'molten globule' state. In contrast, Y231N has an alpha-helical content similar to WT and shows a cooperative sigmoidal temperature melt but with a decreased stability. All four mutants experience serious misfolding and aggregation. FT-IR, circular dichroism, increase in thioflavin T fluorescence, and congo red absorption spectral shift and birefringence show that these aggregates contain intermolecular cross-beta structure similar to that found in amyloid diseases. These results indicate that disease-causing mutants affect N-ABD structure by decreasing its thermodynamic stability and increasing its misfolding, thereby decreasing the net functional dystrophin concentration. (Proc. Natl. Acad. Sci. USA, 107 (2010) 15069-15074)

64. ERIC MEADOWS, NATIONWIDE CHILDREN'S HOSPITAL

FSHD pathogenesis and RNAi-based therapy development for dominant muscular dystrophies

Eric Meadows, Lindsay Wallace, Kevin Flanigan, Jerry R. Mendell, Scott Q. Harper
Center for Gene Therapy, Nationwide Children[1]s Hospital, Columbus, Ohio

Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) was formally classified in 1954, and the primary genetic defect, D4Z4 contraction, was identified in 1992. However, the mechanisms underlying disease pathogenesis have only recently come into focus. Of late, an FSHD model shedding light on pathogenesis emerged through over-expression of the DUX4 gene, an open reading frame in the D4Z4 repeat that encodes a transcription factor. Incumbent in this model is the need for DUX4 to exhibit unequivocal myopathic potential. We therefore investigated the in vivo effects of DUX4 overexpression in skeletal muscle across multiple species. In both zebrafish and mice, we showed that DUX4 causes histological and functional deficits consistent with muscular dystrophy. In zebrafish, muscle-specific DUX4 expression, but not control GFP, caused body malformations, impaired mobility, somite defects, and myofiber degeneration, which are phenotypes seen in other fish models of muscular dystrophy. In neonatal mice, AAV6-delivered DUX4 caused muscle atrophy and adipose tissue replacement of muscle. In adult mice, AAV6.DUX4 produced massive muscle degeneration, apoptosis, histological evidence of muscle turnover, and gross muscle weakness. In addition, we show that DUX4-associated toxicity requires a DNA binding domain, whereas overexpression of a DUX4 DNA binding-domain-mutant (DUX.HOX1) produced no abnormalities in vitro or in vivo. These results support transactivation of a gene(s) downstream from DUX4 that are incompatible with normal muscle development and/or maintenance. Using real-time PCR arrays, we demonstrated activation of numerous genes in the p53 pathway in muscles overexpressing DUX4, but not mutant DUX4.HOX1, supporting that DUX4 transcriptional activity, directly or indirectly, activates apoptosis in vivo. Importantly, we showed that the myopathic effects of DUX4 are p53-pathway dependent, since muscles from p53 null mice were resistant to the DUX4-induced damage. These results are consistent with previous observations that some p53 pathway components are activated in muscles from FSHD patients. Together, our data support that DUX4 overexpression contributes to FSHD development. The translational extension of these studies is a strategy to reduce DUX4 as a potential treatment for FSHD. RNA interference (RNAi) has emerged as a candidate strategy for diseases with dominant gene abnormalities and we are now developing a gene therapy approach to knock down DUX4 using AAV vector-delivered microRNAs.

65. NICK MENHART, ILLINOIS INSTITUTE OF TECHNOLOGY

Stability of Exon Edited Dystrophin Rods

Neha Sahni, Khushdeep Mangat, and Nick Menhart.
Division of Biology, Illinois Institute of Technology, Chicago IL 60616

We are interested in the nature of rod region of dystrophin, in particular how the spectrin type repeat structures, STRs, interact with each other; as well as how these structures and the rod as a whole is affected by editing. These biophysical studies provide fundamental information about the nature of the rod that may be useful in understanding how to edit it while retaining maximal function, as well as providing the basis to understand the consequences of BMD type deletion defects. We have recently completed a full scan of all 24 STRs in isolation, as well as all 23 2STR motifs in tandem with their normal neighbors in the rod. This has identified regions of both low and high stability, as well as regions in which adjacent STRs from cooperative units enhancing their overall stability. One region in particular, for the 7th to the 9th STR is of very low stability, comparable to the putatively disordered hinge regions. Conversely the C-terminal region of the rod, past H3, from the 20th to the 24th STR, is of very high stability.

We have now extended this to studies of various edited rods, homologous the products of therapeutically envision exon skipping events (exon 51 and exon 44 regions) as well as large scale edits aimed at minidystrophin constructions (the large _Delta_ exon16-48 deletion, and variants thereof). In many cases, individual patient defects can be repaired by exon skipping in alternative fashions, by AON mediated skipping of different additional exons. This will produce differently edited rods, and our in vitro work has revealed quite different fundamental biophysical properties of these different edits – raising the possibility of different therapeutic potential. As well, the use of exon based edits to construct large scale rod edits for minidystrophin construction has been investigated. One o the most promising such edit is based upon the famous _Delta_ exon16-48 very mild BMD case; however examination of this edit in the context of the rod structure suggests that other edits such as _Delta_ exon17-47 or _Delta_ exon17-49 might also be useful. We have constructed these rods and applied our biophysical tests to them to determine if they exhibit different fundamental stabilities and other properties.

66. KANNEBOYINA NAGARAJU, CHILDREN'S NATIONAL MEDICAL CENTER

Novel membrane stabilizing and anti-inflammatory small molecule drugs as potential therapeutic option for DMD

Erica Reeves1, Andreas Baudy2, John McCall3, Sree Rayavarapu2, Arpana Sali2, Zuyi Wang2, Beryl Ampong2, Stephanie Duguez2, Debra DuBois4, Eric P. Hoffman2, and Kanneboyina Nagaraju2
1. Validus Biopharma, Inc. Rockville, MD. 2. Children's National Medical Center, Research Center for Genetic Medicine. Washington, DC. 20010. 3. PharMac LLC. Boca Grande, FL. 3392. 4. University of Buffalo, Dept. of Biological Sciences. Buffalo, NY. 14260

Background: Glucocorticoids (GC) are the current standard of care for DMD but their acceptance and long term use is limited due to significant adverse effects. As a result, many patients are unable to benefit from these drugs over the long term. Our hypothesis is that beneficial effects of glucocorticoids in DMD are due to non-genomic signalling activities (e.g., Inhibition of inflammation and cell membrane stabilization) whereas adverse effects are due to glucocorticoid receptor (GR) mediated transcriptional activity. We have developed a series of novel delta 9,11 steroids (VBP compounds) that lack transcriptional activity but retain beneficial signalling activities. Methods/Results: Using a series of in vitro experiments (GR binding, nuclear translocation, GRE dependent transcription and global gene expression profiling) we have confirmed that VBP compounds retained little or no transcriptional changes typical of GCs but showed significant inhibition of TNFβinduced NF-βB signalling as well as superior activity in plasma membrane stability assays compared to prednisone in skeletal muscle cells. To test for in vivo efficacy, we treated dystrophin-deficient mdx mice with both acute and chronic regimens. For the acute dosing experiment, daily oral administration (3 wks) of prednisone and VBP compounds showed significantly reduced skeletal muscle inflammation by live animal optical imaging (Cathepsin B activity) indicating that these compounds have anti-inflammatory activities that are similar to prednisone. To assess efficacy and adverse effect profiles, a four month chronic study was done with VBP compounds and prednisone. We found that VBP compounds lack typical side effects (e.g., Loss in body weight, spleen size and

steroid induced myopathy) that are obvious with chronic prednisone use. ADME, PK, safety studies on our lead candidate, VBP15, showed favourable new chemical entity properties. Conclusions: Taken together, these data suggests VBP compounds maintain efficacy profile of traditional glucocorticoids without the toxicity associated with chronic use suggesting that these drugs have a potential to replace current standard of care for DMD.

67. NIKOLAI NARYSHKIN, PTC THERAPEUTICS

Small molecule compounds that correct alternative splicing of the SMN2 gene and restore SMN protein expression and function

Nikolai Naryshkin, Jana Narasimhan, Amal Dakka, Vijayalakshmi Gabbeta, Maura Haley, Stephen Jung, James Crona, Priya Vazirani, Bansri Furia, Hongyan Qi, Matt Woll, Sam Lee, Soongyu Choi, Hiroo Koyama, Tianle Yang, Guangming Chen, Sophie Zhang, Nanjing Zhang, Song Huang, Chunshi Li, Anthony Turpoff, Jean Hedrick, Xin Zhao, Anna Mollin, Sergey Paushkin, Dione Kobayashi, Kathleen McCarthy, Mike Pleiss, Meg Winberg, Karen Chen, Gary Karp, Marla Weetall, Ellen Welch
PTC Therapeutics, 100 Corporate Court, South Plainfield, NJ 07080 and Spinal Muscular Atrophy Foundation, Suite 400, 888 Seventh Avenue, New York, NY 10019

Spinal muscular atrophy is caused by the reduced expression of the survival of motor neuron (SMN) protein due to the loss of functional SMN1 gene and exon 7 splicing in the SMN2 gene. PTC Therapeutics, Inc. is pursuing innovative drug discovery strategies aimed at restoring the expression of the SMN protein at the post-transcriptional level. Panels of cell based assays and animal models have been established and optimized to assess the effects of compounds on SMN mRNA and protein expression. Orally bioavailable, brain-penetrable small molecules have been identified that increase the inclusion of exon 7 into the SMN2 mRNA and the levels of SMN protein in cells from SMA patients. In addition, small molecules were found that increase the SMN-full length mRNA and protein in various tissues of SMA mouse models and result in improved survival and motor function. Lead compounds from this program are undergoing medicinal chemistry optimization of biological, pharmacological and pharmaceutical properties with the ultimate goal of identifying a molecule for preclinical and clinical development.

68. LI NIU, STATE UNIVERSITY OF NEW YORK AT ALBANY

Developing AMPA Receptor Aptamers as New Drug Candidates for ALS

Zheng Huang, Yan Han, Joe Wang and Li Niu
Department of Chemistry, and Center for Neuroscience Research, State University of New York (SUNY) at Albany, Albany, NY 12222, USA

In finding new treatment for amyotrophic lateral sclerosis (ALS), one of the important therapeutic strategies is to develop inhibitors of the βamino-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., GluA1-4), 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 GluA2, a key AMPA receptor subunit that mediates excitotoxicity: one class uniquely inhibits the open-channel whereas the other inhibits the closed-channel conformation. To turn

these aptamers into potentially useful drugs, we have now successfully generated a class of chemically modified aptamers that are biostable or resistant with ribonucleases so that these aptamers can be tested in vivo. Our results demonstrate the possibility of developing aptamers that have nanomolar affinity and are 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.

69. GUY ODOM, UNIVERSITY OF WASHINGTON

Therapeutic potential of systemic AAV/micro-utrophin delivery using muscle-specific vs ubiquitously active gene regulatory elements

Guy L. Odom1, Glen B. Banks1, Quynh Nguyen2, Jeffrey S. Chamberlain1,2, and Stephen D. Hauschka2, Department of Neurology1 and Biochemistry2, University of Washington, Seattle, Washington 98195, USA.

Duchenne muscular dystrophy (DMD is caused by mutations in the dystrophin gene and results in severe muscle wasting. Truncated (micro) dystrophin and utrophin coding sequences were previously shown to be therapeutic in small animal models of DMD when delivered via adeno-associated viral (AAV) vectors pseudotyped with the serotype 6 capsid. However, given the potential for a destructive cellular immune response against dystrophin transgene in some DMD patients, the capacity of utrophin to act as a therapeutic transgene is of high interest. To this end, we have evaluated microutrophin expression and functionality following AAV6 infusion in a highly dystrophic mouse model (mdx/utrn-/-). In these studies, micro-utrophin was expressed from either a ubiquitous (CMV) or a small and highly-active muscle specific promoter (MSP), that contains a modified version of the M-creatine kinase enhancer and promoter. Vectors expressing micro-utrophin from the MSP led to a halt in myofiber necrosis and significant functional alleviation of most pathophysiological abnormalities associated with muscular dystrophy in this model. Levels of correction or improvement were not different from those obtained when the CMV promoter was used to drive expression. However, the MSP demonstrated a clear lack of expression in non-muscle cell types both in vivo and in vitro. Treated mdx/utrn-/- muscles displayed a significant improvement in resistance to contraction induced injury, as well as myofiber degeneration/regeneration as indicated by the percentage of centrally-located nuclei in myofibers. Overall, this highly active muscle-specific microutrophin expression vector shows promise as a treatment vehicle for DMD given the tissue-specific expression pattern, proper intracellular localization of micro-utrophin, functional restoration of dystrophic muscles, and potential avoidance of immune responses against exogenous dystrophin.

70. ERIK OSMAN, UNIVERSITY OF MISSOURI

Delivery of Bifunctional RNAs that Target the Intronic Splicing Silencer N1 (ISSN1) and Increase SMN Levels in Δ7 Mouse Model of Spinal Muscular Atrophy

Erik Y. Osman1 , Travis D. Baughan1 and Christian L. Lorson1,2
1 Department of Molecular Microbiology and Immunology, Life Sciences Center, Room 404, University of Missouri, Columbia, MO 65211 2 Department of Veterinary Pathobiology, Life Sciences Center, Room 403, University of Missouri, Columbia, MO 65211

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by the loss of survival motor neuron-1 (SMN1). A nearly identical copy gene, SMN2, produces low levels of functional protein. SMN2 coding sequence has the potential to produce full-length SMN, however, 90% of SMN2-derived transcripts are alternatively spliced and encode a truncated protein. To optimize the efficiency of bifunctional RNAs previously we developed a set of RNAs that targeted a putative intronic repressor Element1 within intron 6 and modulated SMN2 splicing. There is a 2-fold mechanism of SMN induction: inhibition of the intronic repressor and recruitment of SR proteins via the SR recruitment sequence of the bifunctional RNA. Utilizing this two-pronged approach we used the same bifunctional RNAs synthesized as 2′-O-methyl RNAs to target the Intronic Splicing Silencer N1 (ISSN1) located in Intron 7. The bifunctional 2′-O-methyl RNAs were directly delivered in

the central nervous system of SMA mice via Intracerebroventricular (ICV) injections. Single-RNA injections were able to illicit a strong induction of SMN protein in the brain and throughout the spinal column of neonatal SMA mice. The mean life span as well as the percent weight gained after the injections was extended following the delivery of bifunctional RNAs. This technology has direct implications for the development of an SMA therapy, but also lends itself to a multitude of diseases caused by aberrant pre-mRNA splicing.

71. UDAI PANDEY, LOUISIANA STATE UNIVERSITY

A Drosophila model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43

Udai Pandey, PhD

Genetic interaction between FUS and TDP-43 influences the neurodegenerative phenotype in a Drosophila model of ALS Udai Bhan Pandey1, Astha Maltare1, Ji Han Kim2, Thomas Lloyd2 and Nicholas Lanson Jr1 1Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD FUS/TLS and TDP-43 are two DNA/RNA binding proteins found to be mutated in both sporadic and familial forms of ALS. To investigate the pathogenesis of ALS caused by FUS/TLS mutations, we established a series of transgenic Drosophila lines that ectopically express human wild type and mutant FUS/TLS. Targeted expression of mutant but not wild type FUS/TLS in Drosophila eyes causes the formation of ubiquitinated aggregates and an external eye degenerative phenotype. Interestingly, ectopic expression of mutant FUS/TLS in motor neurons resulted in larval paralysis, pupal lethality and axonal accumulation of synaptotagmin, whereas wild type FUS/TLS expression had minimal effect. Mutant FUS/TLS redistributes itself into cytoplasm whereas wild type FUS/TLS localizes predominantly in the nucleus suggesting that the cytoplasmic localization of FUS/TLS is required for causing the toxicity observed in human ALS patients. Furthermore, we found that deletion of nuclear export signals strongly suppressed toxicity associated with mutant FUS/TLS suggesting that cytoplasmic localization is a necessary step for ALS pathogenesis. We also observed that conditional expression of mutant FUS/TLS in the adult Drosophila nervous system causes a significant age-dependent and mutation-dependent reduction in life span and climbing ability. Cessation of FUS expression in symptomatic flies leads to an amelioration of the behavioral phenotypes suggesting the possibility that continuous expression of mutant is required for causing toxic effects. Of note, we observed that toxicity associated with mutant TDP-43 is strongly enhanced by co-expression of WT FUS/TLS in Drosophila. Together, these data suggest that TDP-43 and FUS/TLS genetically interact in a mutation-dependent manner. Studies are underway to identify the molecular and cellular pathways involved in the FUS/TLS-related ALS. Supported by the Robert Packard Center for ALS at Johns Hopkins (to UBP and TL).

72. MAURA PARKER, FRED HUTCHINSON CANCER RESEARCH INSTITUTE

Diprotin A enhances engraftment of donor cells to regenerating and dystrophic skeletal muscle

Maura H. Parker1, Carol Loretz1, Rainer Storb1,3, and Stephen J. Tapscott2,4
1Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, 2Human Biology Division, Fred Hutchinson Cancer Research Center, 3Department of Medicine, University of Washington, Seattle, WA, 4Department of Neurology, University of Washington

Muscle-derived cell transplantation has the potential to effectively treat many human diseases, including muscular dystrophy. However, immune rejection of donor cells prevented long-term engraftment in human trials of patients with Duchenne musclular dystrophy. We introduced immune tolerance in a canine model of muscular dystrophy (cxmd) using a clinically relevant regimen of bone marrow transplantation, thereby establishing a viable model for addressing muscle cell transplantation as a treatment for muscular dystrophy. A variety of cell populations engraft into skeletal muscle of mdx mice, effectively restore dystrophin expression and reconstitute the satellite cell pool. Yet, a direct and quantitative comparison of engraftment to determine the most effective cell population is lacking. We have developed a canine-to-mouse xenotransplantation model to rapidly and quantitatively compare canine muscle cell engraftment. Specifically, we demonstrate that canine

muscle derived cells engraft into regenerating mouse muscle, and engraftment is quantifiable and consistent. The canine-to-mouse model allows us to quantitatively compare cell populations and modulating factors, and establish priority for transplantation experiments using the immune tolerant cxmd model. We used the xenotransplant model to show that canine muscle derived cells sorted for expression of CXCR4 do not display a greater level of engraftment when compared to a mixed cell population. However, pre-treating a mixed cell population with diprotin A, a positive modulator of CXCR4-SDF-1 binding, significantly enhances engraftment of donor cells to the mouse satellite cell niche. Translating these results to the immune tolerant canine, we demonstrate that injection of diprotin treated donor cells results in a significantly increased number of muscle fibers expressing dystrophin as comapred to untreated cells. Temporal regulation of CXCR4/SDF-1 binding may be an important means of inducing donor cell migration and expanding the effective range of engraftment after transplantation. Further studies will establish the best protocols for muscle cell transplantation and lead to future human clinical trials of muscle cell transplants for the treatment muscular dystrophies.

73. GIULIO PASINETTI, MOUNT SINAI SCHOOL OF MEDICINE

Pharmacological induced ketonemic conditions attenuate ALS-type motor impairment through mechanisms involving promotion of mitochondrial bioenergetics in motor-neurons

Giulio Maria Pasinetti1,2
1Mount Sinai Medical Center, New York, NY, USA; 2Geriatric Research, Education and Clinical Center, James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA

Impaired energy homeostasis plays a causative role in motor neuron death in Amyotrophic Lateral Sclerosis (ALS). We recently found that human superoxide dismutase (SOD1) G93A transgenic mice administered with a ketonemic diet exhibited a significantly slower progression of ALS-type motor impairment, and a lower mortality rate. Further, we found a significant increase in ketone body levels and notably D-3-betahydroxybutyrate (BHB) in the blood of transgenic ALS mice fed the ketogenic diet. We hypothesize that the protective effects of the hyperketonemic diet are due to the increased utilization of ketone bodies in mitochondrial metabolism, which is defective in SOD1-G93A mice. Based on this consideration, we propose to develop and characterize a new experimental therapeutic approach to attenuate ALS-type motor impairment. This approach would promote ketogenic responses in SOD1-G93A mice following treatment with caprylic triglyceride. Caprylic triglyceride is a medium-chain triglyceride which is metabolized into ketone bodies, predominantly BHB and serves as an alternate energy substrate for neuronal metabolism. In preliminary studies using Seahorse XF24 Extracellular Flux analyzer technology, we explored the oxygen consumption rate (OCR) as a direct measure of cellular mitochondrial activity in response to caprylic triglyceride treatment. We found that differentiated NSC-34 like motor-neurons treated with caprylic triglyceride showed a significant elevation in both the basal and maximal OCR. We hypothesize that exposure to caprylic triglyceride may beneficially influence ALS- type motor impairment by the promotion of mitochondrial respiration at critical checkpoints in mitochondrial respiration activities in spinal cord motor neurons. In addition, we found caprylic triglyceride treatment significantly increased cellular contents of malate dehydrogenase (MDH), which is one of the key enzymes in the tricarboxylic acid cycle (TCA) cycle. Collectively, our feasibility study suggests that dietary medium-chain triglycerides may beneficially attenuate motor neuron degeneration by providing metabolic substrates (e.g., ketone bodies, FADH2) that promote the generation of ATP. And possibly by simultaneously increasing the metabolic capacity of the TCA cycle. Consistent with this evidence, in our ongoing feasibility we recently found that chronic treatment of G93A SOD-1 mutant ALS mice with caprylic triglyceride delivered in the food (Pasinetti, in preparation), attenuates ALS-type motor impairment relative to age-gender matched littermates. Our preclinical studies provide key information allowing for immediate therapeutic translational applications in ALS patients (supported by discretionary support to GMP).

74. STUART PELTZ, PTC THERAPEUTICS

Lessons Learned in the Discovery and Development of Ataluren for the Treatment of Nonsense Mutation Dystrophinopathy

Stuart Peltz1; John Babiak1; Ellen Welch1; Allen Reha1; Gary Elfring1; Manal Morsy1; Jay Barth1
1PTC Therapeutics, South Plainfield NJ, USA

The rigorous, multi-phased drug discovery and development process is designed to identify potential new treatments and establish their safety, activity, and efficacy. For diseases with urgent unmet medical need, regulatory authorities have established fast track approaches to rapidly bring new therapies to patients. Duchenne/Becker muscular dystrophy (DBMD) is a rare, X-linked, neuromuscular disorder with no treatment options that address the underlying cause of the disease. Ataluren is an investigational new drug for nonsense mutation DBMD (nmDBMD), which comprises ~13% of DBMD cases. The drug is designed to address the underlying cause of nmDBMD by coupling a patient's genetic diagnosis with a mutation-specific therapeutic approach. The goal of this presentation will be to describe 10 years of research leading to the conduct of a registration-directed trial of ataluren in patients with nmDBMD. The discovery process incorporates expertise from multiple functional areas including chemistry, biology, pharmacology, and toxicology. Once a novel therapeutic agent has been identified for clinical testing, additional clinical and regulatory challenges are posed, particularly in a rare genetic disorder such as DBMD with significant disease heterogeneity, limited natural history data, no existing regulatory pathway, and a lack of clinical outcome measures accepted by regulatory authorities. All of these issues must be addressed simultaneously during the expedited clinical trial path to registration. Lessons learned in the development of ataluren may support future drug discovery and clinical research facilitating registration of other investigational treatments for rare neuromuscular disorders.

75. JUSTIN PERCIVAL, UNIVERISTY OF WASHINGTON

Sildenafil Ameliorates Skeletal Muscle Pathology in the mdx Mouse Model of Duchenne Muscular Dystrophy

Justin M. Percival1, Nicholas P. Whitehead1, Michael Siegel3, Heidi N. Gray2, Candace M. Adamo2, Kendra N. Anderson1, David Marcinek3, Joseph A. Beavo2 and Stanley C. Froehner1
1Departments of Physiology & Biophysics, 2Pharmacology and Radiology3, University of Washington, Seattle, WA 98195, USA.

Duchenne Muscular Dystrophy (DMD) is a recessive X–linked neuromuscular disease that results from mutations in dystrophin. The majority of premature deaths of DMD patients result from respiratory failure due to progressive fibrosis that diminishes diaphragm function, while cardiac failure accounts for the remainder of deaths. The loss of functional dystrophin also leads to defective nitric oxide (NO) signaling in skeletal, cardiac and smooth muscle systems. We recently showed that systemic amplification of NO signaling with sildenafil (a phosphodiesterase 5 [PDE5] inhibitor) reversed cardiac dysfunction in the mdx mouse model of DMD. We now show that skeletal muscle cell lines and homogenates contain a pool of active PDE5 and that sildenafil significantly enhanced mdx respiratory muscle (diaphragm) health by reducing fibrosis and Evans Blue Dye uptake, while enhancing contractile force output. In contrast, sildenafil neither affected mdx hindlimb pathology or function nor impacted standard biomarkers of dystrophic disease. Nevertheless, sildenafil treatment appeared to enhance mitochondrial oxidative phosphorylation capacity in mdx hindlimb muscles in vivo, consistent with reported protective effects of enhanced NO-cGMP signaling on mitochondria. In summary, sildenafil may enhance skeletal muscle mitochondrial energetics and may be therapeutically useful in reducing fibrosis and enhancing respiratory muscle function in DMD patients. Funding sources: PPMD, MDA, NIH.

76. JENNIFER PETERSON, OHIO STATE UNIVERSITY

Peptide-based inhibition of NF-𝕂B rescues diaphragm muscle contractile dysfunction in a murine model of Duchenne muscular dystrophy.

Jennifer M. Peterson1, William Kline1, Benjamin D. Canan2*, Daniel J. Ricca3, Brian Kaspar4, Dawn A. Delfín5, Kelly DiRienzo1, Paula R. Clemens6,7, Paul D. Robbins6, Albert S. Baldwin3, Pat Flood3, Pravin Kaumaya8,

Michael Frietas1, Joe N. Kornegay9, Jerry R. Mendell4, Jill A. Rafael-Fortney5, Denis C. Guttridge1, and Paul M.L. Janssen2
1Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, 2Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, 3University of North Carolina at Chapel Hill and TheraLogics, Inc., Research Triangle Park, NC, 4NationwideChildren's Research Institute, Columbus, OH, 5Department of Molecular and Cellular Biochemistry, The Ohio State University, Columbus, OH, 6Department of Neurology, University of Pittsburgh, Pittsburgh, PA, 7Neurology Service, Department of Veterans Affairs Medical Center, Pittsburgh, PA, 8Department of Microbiology, The Ohio State University, OH, 9Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill.

Duchenne muscular dystrophy patients gradually lose contractile function of striated muscle. In the diaphragm, this ultimately leads to respiratory failure. Hence, to maximize patient survival and quality of life, it is critical to find a successful therapy that will restore diaphragm function. The NF-kB signaling pathway has been implicated as a contributing factor of dystrophic pathology, making it a potential therapeutic target. Previously, we demonstrated that pharmacological inhibition of NF-kB via a small NEMO Binding Domain (NBD) peptide provided benefit to mdx mice by reducing pathological features. Now, we stringently test the effectiveness and clinical potential of NBD by treating mdx mice with various formulations of NBD, using diaphragm function as our primary outcome criteria. We found that administering DMSO-soluble NBD rescued 78% of the contractile deficit between mdx and wild type diaphragm. Interestingly, synthesis of a GLP NBD peptide as an acetate salt permitted its solubility in water, but as a negative consequence, also greatly attenuated functional efficacy. However, replacing the acetic acid counterion of the NBD peptide with trifluoroacetic acid retained the peptide's water solubility and significantly restored mdx diaphragm contractile function. Together, these results support the feasibility of using a mass-produced, water-soluble NBD peptide for clinical use.

77. CHRISTOPHER PIERSON, NATIONWIDE CHILDREN'S HOSPITAL

Follistatin enhances muscle mass and strength in the Mtm1C205T mouse, a new model of myotubular myopathy with a milder phenotype

Christopher R. Pierson1,2, Ashley N. Dulin-Smith1, Ashley N. Durban1, Morgan L. Marshall1, Jordan T. Marshall1, Andrew D. Snyder1, Jordan T. Gladman1, Dawn S. Chandler1,3, Anna Buj-Bello4, Michael Lawlor4, James J. Dowling5, Brian Kaspar1,3, Alan H. Beggs4
1The Research Institute at Nationwide Children's Hospital1 and The Departments of Pathology2 and Pediatrics3, 2The Ohio State University College of Medicine. Columbus, OH. Genethon, Evry, France4. Division of Genomics, 3Children's Hospital Boston, and Harvard Medical School5. Boston, MA

MTM1 encodes myotubularin and mutations in this gene cause myotubular myopathy (MTM). The short life span (about 8 weeks) of the Mtm1 knockout mouse limits the scope of preclinical trials that can be performed. To address this issue we sought to generate a model of MTM with a milder phenotype. Based on data from human genotype-phenotype correlation studies we chose to model the C205T point mutation in exon 4, which is predicted to introduce the R69C missense change in the PH-GRAM domain of myotubularin and result in a hypofunctional protein. Mtm1C205T mice appear unaffected at birth, but at 2 months they develop amyotrophy and generate 70% of the grip strength force of wild type mice. The median survival period is 60 weeks. Histopathology shows many small myofibers with central nuclei. Myotubularin protein is not detectable because the C205T mutation induces skipping of exon 4 in most mRNAs, which alters the reading frame and introduces a premature stop codon that prevents the translation of functional myotubularin. However, some full-length mRNA bearing the C205T mutation is present, and even though myotubularin translated from this mRNA harbors the R69C change, it likely provides enough myotubularin function to account for the relatively mild phenotype. Exon 4 skipping was confirmed in muscle tissue from a C205T patient and, in four other human exon 4 missense mutations we modeled in a minigene system. Next, we tested the effects of follistatin, a myostatin inhibitor, in 3 week old Mtm1C205T mice. Each gastrocnemius was injected with half of the total dose of 1x10e11 AAV1-follistatin particles. AAV1-GFP was used in controls. Mice were followed for 7 months. Serum

follistatin increased 10-fold in treated animals versus controls. Gastrocnemius mass was 2.6 times greater in treated animals than controls, while the masses of non-injected muscles were not increased. This may account for how treated animals generated 30% more grip strength force than controls with their hindlimbs, but only 2% more force with their forelimbs. The median myofiber diameter in gastrocnemius was twice as large in treated animals suggesting that follistatin induced hypertrophy. Interestingly, treated animals had a significantly higher percentage of myofibers with central nuclei than control animals (19% vs. 12%). In conclusion, follistatin treatment increases muscle mass and strength locally and future work will aim to improve its systemic effects.

78. MARK PINES, VOLCANI CENTER

Halofuginone and muscular dystrophy

Mark Pines1 and Orna Halevy2
1Institute of Animal Sciences, the Volcani Center and 2Department of Animal Sciences, the Hebrew University of Jerusalem, Rehovot, Israel

Muscular dystrophies (MDs) are genetic disorders characterized by progressive muscle degeneration, impaired locomotion and often, premature death. Because of the hurdles that still prevent the application of gene- and cell-mediated therapies the development of complementary and supportive therapies that slow disease progression and improve patients' quality of life is critically important. In various MDs the progressive loss of muscle and of its ability to function, is associated with significant fibrosis. In Duchenne MD (DMD), the leading causes of death, respiratory and heart failure result from weakness in diaphragm and myocardium that are mostly affected by fibrosis. In congenital MDs (CMDs) the major pathophysiologic change results from muscle fibrosis. TGFβ network signaling has a profound influence on muscle differentiation, regeneration and fibrosis. We have discovered the clinical potential of halofuginone, an analog of a plant alkaloid, as an inhibitor of Smad3 phosphorylation down-stream of the TGFβ signaling. In the diaphragm and cardiac muscle of the mdx and the hind limbs of the dy2J/dy2J mice (models for DMD and CMD, respectively) halofuginone prevented the age-dependent increase in collagen synthesis that was associated with a decrease in the degenerated areas and number of central nuclei, with increased myofiber diameter and with a reduction in infiltrating fibroblasts located close to centrally nucleated myofibers. These changes were accompanied by decrease in Smad3 phosphorylation. The histopathology improvement resulted in enhanced motor coordination and balance and improvement in cardiac muscle function. In the mdx mice with established fibrosis, halofuginone reduced diaphragm fibrosis and significantly improved recovery from exercise and respiratory and cardiac function. Moreover, halofuginone improved muscle histopathology and function in mice representing dysferlinopathies with late onset of MD due to a complete or partial absence of dysferlin (dysf) in skeletal muscle. Halofuginone exhibited also a direct effect on muscle cells. In C2 muscle cell line and in primary myoblasts derived from mdx mice diaphragms halofuginone enhanced Akt, MAPK/ERK and p38 MAPK phosphorylation and inhibited Smad3 phosphorylation in myotubes resulted in enhanced myotube fusion. In a Phase I study, halofuginone was administered orally and therapeutically effective plasma levels were achieved at well-tolerated dosages making it a clinically attractive therapy for MD patients of various etiologies. In summary, halofuginone improves muscle histopathology and muscle functions in various MDs, via inhibition of muscle fibrosis on the one hand, and increased myotube fusion crucial for muscle function, on the other.

79. PIER LORENZO PURI, SANFORD-BURNHAM MEDICAL RESEARCH INSTITUTE

Epigenetic control of muscle stem cells - identification of target for pharmacological treatment of muscular dystrophy

Pier Lorenzo Puri
Sanford-Burnham Institute for Medical Research, La Jolla, CA. USA
Email: lpuri@sanfordburnham.org

Muscle regeneration relies on the activity of satellite cells (SCs), which proliferate and differentiate in response to cues released within their niche. The waves of proliferation of SCs exposed to regeneration cues define the size of the population that regenerates injured muscles or that returns to quiescence and reconstitutes the reserve pool. The elucidation of the intracellular signaling that controls the expression of genes, which regulate SC proliferation and differentiation in response to regeneration cues is crucial to identify the molecular basis of SC-mediated endogenous regeneration of diseased muscles and to understand the mechanism underlying the decline in SC-mediated regeneration that correlates with the functional declines obeserved at late stages of Duchenne Muscular Dystrophy –DMD.

We investigate the molecular basis by which regeneration signals are converted into chromatin modifications that control the expression of genes important for satellite cell proliferation and differentiation. We have recently identified a signaling, by which inflammatory cytokines (such as, TNF alpha) regulate the expression of Pax7 in satellite cells within regenerating muscles, via p38-dependent control of Polycomb Repressive Complex. The TNF-p38-PRC2 signaling to Pax7 controls SC decision to proliferate or differentiate. This study is beginning to shed light on the epigenetic control of SC activity in dystrophic muscles with the ultimate goal of identifying new targets for pharmacological manipulation of endogenous regeneration in the treatment of DMD.

In our future studies, we plan to monitor the integrity of the p38-PRC2 signaling to Pax7 during the progression of muscular dystrophy in the mouse model of DMD – the mdx mice - and in a mdx/mTRKO mutant mice, in which the proliferation ability of satellite cells is compromised by disruption of telomerase activity. We also plan to exploit new therapeutic strategies based on epigenetic drugs that selectively interfere with the p38-PRC2 signalling in satellite cells of mdx mice, in order to extend the activity of satellite cells toward endogenous therapeutic regeneration at late stages of the disease.

80. SREE RAYAVARA, INSTITUTE OF BIOMEDICAL SCIENCES, THE GEORGE WASHINGTON UNIVERSITY, CENTER FOR GENETIC MEDICINE, CHILDREN'S NATIONAL MEDICAL CENTER

Unbiased Proteomic Profiling of Low Molecular Weight Serum Proteins Identifies Unique Biomarkers for DMD

Sree Rayavarapu 1, 2, Brian Feild 3, William Coley 1, 2, Yetrib Hathout 1, 2, Eric P Hoffman 1, 2, and Kanneboyina Nagaraju 1, 2.
1 The George Washington University, Institute of Biomedical Sciences, Washington DC. 2 Center for Genetic Medicine, Children's National Medical Center, Washington DC. 3 Shimadzu Scientific Instruments, Columbia, MD.

Background and Rationale: Currently there are no reliable and reproducible biomarkers for Duchenne muscular dystrophy (DMD). The only serum biomarker used currently to monitor disease progression and response to therapy in DMD patients is creatine kinase (CK). However, CK is not a robust biomarker because of inherent variability in its levels due to a variety of confounding factors. Therefore, there is a need to identify a well-defined set of signature molecules that could be used as biomarkers to monitor disease progression and therapeutic response. Our hypothesis is that deficiency of dystrophin in the skeletal muscle membrane reduces the membrane integrity resulting in the release of muscle specific proteins into serum, and the levels of these proteins vary with the progression of the disease, as well as in response to therapy. Methods: Serum samples from DMD patients (n = 10) and normal subjects (n = 10) were processed using hydrogel nano-particles to enrich for the low molecular weight proteins and to eliminate the highly abundant serum proteins such as albumin. An unbiased global proteomic analysis using MALDI TOF-MS was performed to define the molecular signature in the sera of DMD patients. Results: Preliminary data indicated that the assay is reproducible and the nano-particles significantly reduced high molecular weight proteins and enriched the low molecular weight proteins. Unique low molecular weight proteins were detected in the DMD sera in comparison with age matched healthy controls indicating potential biomarkers. These unique proteins are currently being identified and validated in an independent set of samples using LTQ-Orbitrap based mass spectrometry analysis and immuno assays. Conclusions: Development of enrichment strategies for low molecular weight proteins in the serum would aid in identification of potential biomarkers for DMD.

81. FREDERIQUE RUF-ZAMOJSKI, CALIFORNIA INSTITUTE OF TECHNOLOGY

Muscle proteins and cellular dynamics during somitogenesis and early myogenesis in zebrafish embryos.

Frederique Ruf-Zamojski 1, Le Trinh 1, Sean Megason 2, Vikas Trivedi 1, Scott E. Fraser 1
1 California Institute of Technology, Department of Biology, Department of Biology, Pasadena CA 91125. USA.
2 Harvard Medical School, Systems Biology Department, Boston MA 02115, USA

Muscle proteins and cellular dynamics during somitogenesis and early myogenesis in zebrafish embryos. High-resolution cellular and molecular data from developing organisms has driven many of the advances in developmental biology. These same approaches, applied to muscle biology, should enable a better understanding of the key events of dynamics of muscles formation, maintenance, and degeneration. Applying high-resolution tools to muscular dystrophies cases will define the earliest differences between the development of muscle in normal animals and those with muscular dystrophies, offering both a better understanding and the development of new therapeutic strategies. Our studies strive to attain this goal by combining several approaches: 1) in vivo imaging of the cellular events of zebrafish somitogenesis and myogenesis, 2) a FlipTrap genetic screen to isolate lines with Citrine fusion protein expression in muscles, and 3) a new high-resolution in situ hybridization technique (ISH) known as Hybridization Chain Reaction (HCR) to decipher the co-expression of a variety of mRNA species in muscle and their corresponding proteins as the muscles are forming. Our work has analyzed the dynamics of several key molecules, at both the mRNA and the protein level, during somitogenesis and myogenesis in wildtype, mutant and morphant zebrafish embryos. We have established a dozen new transgenic lines, fusing fluorescent proteins into the normal protein encoding locus, enabling high resolution studies of endogeneous protein dynamics at normal levels of expression during muscle development in live animals. Our multiplex molecular techniques detect nascent dystrophin mRNA transcripts in the nuclei and follow their export and accumulation at the somite borders as the embryo is developing. These molecular and imaging studies are providing unprecedented details on the cellular dynamics and the expression of key players, such as dystrophin, during normal and dystrophic muscle development.

82. ZARIFE SAHENK, NATIONWIDE CHILDREN'S HOSPITAL AND RESEARCH INSTITUTE

NT-3 Gene Therapy Improves Compound Muscle Action Potential (CMAP) and Function in Mouse Model for Charcot-Marie-Tooth (CMT) Neuropathy

Zarife Sahenk, Gloria Galloway, Vinod Malik, Jerry R. Mendell, K. Reed Clark

CMT neuropathies represent the most common inherited neuromuscular conditions affecting 1/2500 people in the USA. Disabilities relate to degree of weakness and atrophy of distal extremity muscles. A primary Schwann cell (SC) disorder represents the most common form of CMT neuropathy. In previous studies we showed NT-3 improved the TremblerJ (TrJ) mouse phenotype and showed efficacy in patients with CMT1A (Sahenk et al. 2005). However, the short serum half-life of exogenously delivered NT-3 limits feasibility for clinical application. An improved paradigm permits muscle to act as secretory organ following AAV.NT-3 delivery enabling NT-3 peptide to reach remote sites through the circulation. Objective: To improve function in CMT neuropathy by AAV1.NT-3 gene therapy via intramuscular injection. This translational study in TrJ mice demonstrated the use of the CMAP as a reliable marker of functional improvement in CMT neuropathy. Methods: TrJ mice received injections of AAV1.NT-3 (1.2 X 1011 vg) into the quadriceps muscle. Sciatic nerve conduction was studied at baseline, 20 and 40 weeks post-gene delivery. Outcome measures included weekly tests of CMAPs, bilateral and ipsilateral hind limb grip strength and rotarod performance. Correlative morphological studies included muscle fiber size determinations in the gastrocnemius and tibialis anterior (TA) muscles. Results: Sciatic nerve conduction studies at 20 weeks produced 37% greater CMAP amplitude in the AAV1.NT-3 injected group

(n=14) compared to the control TrJ (n=14). CMAP amplitude increases correlated with hind limb grip strength corresponding to a 39% improvement [10.53 g (grip force) difference] in bilateral, and a 29 % improvement (6.4 g difference; p=0.0009) in ipsilateral values. At 40 weeks CMAP amplitude in the AAV1.NT-3 injected group showed 84% increase compared to the control TrJ. AAV1.NT-3 injected mice performed significantly better than TrJ controls in their rotarod performance recorded between 20 and 40 weeks post- gene delivery. In addition, nerve regeneration to both gastroc and TA muscles was demonstrated by increased muscle fiber diameter. Conclusion: TrJ mice receiving intramuscular AAV1.NT-3 show improvements in hind limb grip strength and CMAPs. CMAP can be used as a surrogate for clinical/functional improvement having direct relevance to future clinical trials as reliable outcome measure. These promising gene therapy results offer potential treatment for CMT1A neuropathy, and may be useful for other neuropathies as well.

83. JIN-HONG SHIN, UNIVERSITY OF MISSOURI

Age-matched comparison reveals early electrocardiography and echocardiography changes in dystrophin-deficient dogs

Deborah M Fine 1, Jin-Hong Shin 2, Yongping Yue 2, Dietrich Volkmann 1, Stacey B Leach 1, Bruce F. Smith 3, Mark McIntosh 2, Dongsheng Duan 2,*
1, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, The University of Missouri 2, Department of Molecular Microbiology and Immunology, School of Medicine, The University of Missouri 3, Scott-Ritchey Research Center and the Department of Pathobiology, College of Veterinary Medicine, Auburn University
Grant: NIH HL-91883 (DD), PPMD (DD and DMF), MDA (DD and BFS)

The absence of dystrophin in the heart leads to Duchenne cardiomyopathy. Dystrophin-deficient dogs represent a critical model to translate novel therapies developed in mice to humans. Unfortunately, little is known about cardiophysiology changes in these dogs. To establish the baseline, we performed electrocardiographic and echocardiographic examinations at 3, 6 and 12-months of age in four normal and three affected dogs obtained from the same litter. The genotype was diagnosed by PCR. Affected dogs showed characteristic growth retardation and serum creatine kinase elevation. Necropsy confirmed cardiac dystrophin deficiency and histopathology. Growth-associated changes were apparent in normal, but not affected dogs. Although most age-matched comparisons did not yield statistically significant differences between normal and affected, we have identified the reduction of the left ventricular internal diameter in diastole as the most consistent finding in affected dogs ≥ 6-month-old. Our results highlight the challenges in using the canine model to study Duchenne cardiomyopathy.

84. JINDRICH SOLTYS, SAINT LOUIS UNIVERSITY

Treatment with anti-C5 antibody in complement regulatory protein deficient mice attenuates the outcome of experimental autoimmune myasthenia gravis.

Jindrich Soltys DVM, PhD
Department of Neurology & Psychiatry, 1438 South Grand, Saint Louis University School of Medicine, Saint Louis, MO 63104

Background: Myasthenia gravis (MG) is the most common autoimmune disorder of neuromuscular transmission. To this point no specific therapeutic approach is directed to counter one of the most important effector mechanisms -complement mediated lysis of the neuromuscular junction (NMJ). The purpose of this study was to evaluate the effect of exaggerated complement activity on animal model of experimental autoimmune myasthenia gravis (EAMG) caused by single or combined deficiency in complement regulatory proteins. Ultimately the effect of complement inhibition on the outcome of EAMG was examined. Methods: Mouse EAMG was induced by subcutaneous administrations of purified acetylcholine receptor (AChR). The effect of complement inhibition with anti-C5 mab in complement regulator deficient (CD55KO, CD59KO, CD55/59KO) and wildtype C57BL/6 mice was assessed by measurement of weight loss and muscle grip strength. Complement activity was determined by hemolytic assay. ELISA assay was used to detect the level of AChR specific antibodies.

Release of Th1/Th2/Th17cytokines in blood serum was analyzed by CBA array. Splenic cells suspensions were analyzed for the proliferative ability and specific antigen recall responses. Results: Single (CD55KO, CD59KO) or double (CD55/CD59KO) deficiency in complement regulatory proteins did not affect the weight throughout EAMG. However, muscle grip strength loss correlated with disease progress and level of complement regulator deficiency. More profound weakness was detected in mice deficient in both regulatory proteins (CD55/CD59KO). Splenic cells isolated from complement regulatory deficient mice exhibited augmented proliferative ability when restimulated with AChR antigen. Specific recall responses to AChR showed that the frequency of splenic T-cells secreting IFN-γ was also increased. Repetitive continuous treatment with anti-C5 mab alleviated disease severity in complement regulators deficient EAMG (Day 49-63) but did not alter the number IFN-β secreting cells in CD55KO and CD59KO EAMG mice. Inhibition of complement activity reduced the level of AChR complement fixing antibodies (CD55KO, CD55/CD59KO). The level of proinflammatory cytokines released into the bloodstream of complement regulator deficient EAMG mice was significantly reduced. Conclusions: Complement regulator deficiency exaggerates complement activity and affects cellular immune responses. Inhibition of complement activity followed by anti-C5 treatment attenuates EAMG outcome. Targeted use of complement inhibitors in MG should be considered for the future therapeutic development.

85. CHRISTOPHER SPURNEY, CHILDREN'S NATIONAL MEDICAL CENTER

Effects of MyD88 deficiency on skeletal and cardiac muscle function in mdx mice

Spurney C, Cha HJ, Henrique-Pons A, Ampong B, van der Meulen J, Yu Q, Nagaraju K.
Center for Genetic Medicine Research Children's National Medical Center Washington, DC

Background: Inflammation and immune system activation are two key pathways involved in skeletal and cardiac muscle pathology of Duchenne muscular dystrophy (DMD). Toll-like receptors (TLR) are differentially expressed in DMD muscle. MyD88 is a protein essential for the signaling of TLR responsible for innate immune responses. The effects of Myd88 dependent TLR signaling in dystrophin deficient skeletal and heart muscle are not known at this time. Methods: MyD88 deficient and dystrophin deficient mdx mice were bred and compared to mdx mice up to 9 months of age. Skeletal muscle strength was assessed via non-invasive grip strength, Rotarod timing and in vitro force testing. Cardiac function was assessed using non-invasive high frequency echocardiography. Histology and fibrosis were assessed using hematoxylin/ eosin and picrosirius red staining. Results: There were not significant differences between groups in body weight, normalized forelimb and hindlimb grip strength, in vitro force contractions or Rotarod timing between Myd88 deficient and sufficient mdx mice at early stage in the disease. Cardiac function was significantly decreased in mdx mice compared to MyD88 deficient mdx mice at 9 months of age (Percent shortening fraction (mean±SEM) 30±2% vs. 41±2%; $p<0.001$). Histology showed significantly increased regenerating fibers in the gastrocnemius and significantly decreased fibrosis in the heart of MyD88 deficient mdx mice ($p<0.04$). Conclusions: MyD88 deficient mdx mice showed significantly improved skeletal muscle regeneration at an early age and significantly improved cardiac function with decreased fibrosis at 9 months of age. These findings suggest that TLR signaling plays a role in skeletal and cardiac muscle pathology in DMD and modulation of this pathway may have therapeutic benefits.

86. JONATHAN STIBER, DUKE UNIVERSITY MEDICAL CENTER

Effect of Oxidative Stress on Homer Proteins

Igor Nepliouev Ph.D. and Jonathan A. Stiber M.D.
Duke University Medical Center

Duchenne's muscular dystrophy (DMD) is a progressive and fatal disorder arising from a mutation in dystrophin resulting in altered membrane integrity and muscle wasting. The pathophysiology of muscular dystrophy is multifaceted and includes both increased susceptibility to oxidative stress and abnormal calcium signaling. We recently found that Homer expression is decreased in mouse models of muscular dystrophy, and that mice lacking Homer 1 exhibited a myopathy characterized by a gain of function of TRP channel activity

and dysregulation of calcium influx. These findings provided evidence that Homer 1 functions as an important scaffold for TRP channels and regulates their effects on calcium signaling in skeletal muscle. We hypothesized that the ability of Homer isoforms to serve as scaffolds for signaling proteins would be influenced by oxidative stress. We have found by standard SDS-PAGE of lysates from adult mouse skeletal muscle exposed to air oxidation that Homer migrates as both a dimer and monomer in the absence of reducing agents and solely as a monomer in the presence of a reducing agent: suggesting that Homer dimers exposed to oxidation could be modified by the presence of an inter-molecular disulfide bond. Analysis of the entire peptide sequence of Homer 1b revealed the presence of only two cysteine residues located at positions 246 and 365 adjacent to the C-terminal coiled-coil domain, and addition of biotin-HPDP to native recombinant Homer 1b confirmed that these two cysteine residues are available for formation of disulfide bonds. HEK 293 cells were then transfected with WT and cysteine mutant forms of Homer 1b and exposed to oxidative stress by addition of menadione which resulted in the formation of disulfide bonds except in the double mutant (C246G, C365G). Exposure of C2C12 myotubes or intact adult mouse muscle to oxidative stress resulted in decreased solubility of endogenous Homer isoforms which was dependent on the disulfide bond formation. We also provide evidence that Homer is able to form a mixed disulfide bond with the actin binding protein Drebrin. Our results suggest that oxidative stress results in disulfide cross-linking of a Homer polymeric network which may provide a link between oxidative stress and abnormal calcium signaling.

87. JON TINSLEY, SUMMIT PLC.

Daily treatment with SMTC1100, a novel small molecule utrophin upregulator, dramatically reduces the dystrophic symptoms in the mdx mouse.

Jon Tinsley[1]#, Richard Storer[1], Rebecca Fairclough[2], Fraser Wilkes[1], Allison Potter[2], Sarah Squire[2], Francis Wilson[1], Graeme Wynne[1], Annamaria De Luca[3], Kay E. Davies[2]#
1 Summit plc, Milton Park, Abingdon, Oxfordshire, OX14 4RY 2 MRC Functional Genomics Unit, Department of Physiology Anatomy and Genetics, University of Oxford OX1 3PT 3 Unit of Pharmacology, Department of Pharmaco-biology, University of Bari "A. Moro", Italy

Utrophin is expressed during early myotube formation and is then transcriptionally down regulated as the fibre matures. Using genetic tricks, utrophin, a protein closely related by function to dystrophin, can essentially cure the dystrophin deficient mdx mouse model. In order to affect this rescue one has to divorce utrophin expression away from its normal transcriptional regulation. Our approach uses small chemical entities to re-programme utrophin transcription such that utrophin RNA and therefore protein is continually made even in mature fibres. In these fibres in the absence of dystrophin, utrophin can confer the same functional attributes and prevent muscle necrosis. Over two years, around 30 different optimised compounds were tested in the mdx. This culminated in the nomination of a clinical development candidate; SMTC1100 which when dosed orally in sedentary and exercised mdx mice has significant beneficial effects on the muscular dystrophy. Treatment attenuates calcium imbalance and fibre damage which in turn significantly reduces the profound secondary events such as muscle fibrosis, inflammation, and degeneration all of which leads to increase grip strength and resistance to fatigue (a murine model of the six minute walk test). In July 2008, Summit signed an exclusive worldwide licensing agreement with BioMarin Pharmaceutics Inc. where it subsequently entered into Phase I clinical trials in January 2010. In August 2010, after assessment of data, BioMarin took the decision to discontinue its development citing pharmaceutical and pharmacokinetic challenges related to plasma concentrations of the compound. No safety issues or adverse events were reported from this trial. Summit is committed to working in DMD and believes that use of an appropriate formulation has the potential to produce a viable medicine. A successful outcome from this safety trial should enable clinical trials in DMD to start in 2012 and thus bring into being the first potentially novel disease modifying treatment for all DMD boys. The preclinical efficacy data and future plans will be discussed in more detail.

88. JACQUES P. TREMBLAY, LAVAL UNIVERSITY

Laminin-111: a potential therapeutic agent to treat Duchenne muscular dystrophy

Sébastien Goudenege1, Yann Lamarre1, Nicolas Dumont2, Joël Rousseau1, Jérôme Frenette2, Daniel Skuk1 and Jacques P. Tremblay1
1- Unité de recherche de recherche en Génétique Humaine, Centre de recherche de CHUL, CHUQ, Faculté de médecine, Université Laval, Sainte-Foy, Québec, Canada.
2- Unité CRML, Centre de recherche du CHUL, CHUQ, Faculté de médecine, Université Laval, Ste-Foy, QC, Canada

Duchenne muscular dystrophy (DMD) still need effective treatments and myoblast transplantation (MT) is considered as an approach to repair damaged skeletal muscles. DMD is due to the loss of dystrophin and the dystrophin glycoprotein complex from muscles. The lack of link between the contracting apparatus and the extracellular matrix leads to frequent damage to the sarcolemma triggering muscle fiber necrosis. Laminins are major proteins in the extracellular matrix. Laminin-111 is normally present in skeletal and cardiac muscles in mice and humans but only during embryonic development. In the present study we wanted to further evaluate the benefits of laminin-111 to improve the muscle pathology in mdx mice and to evaluate the benefit on strength and resistance. Moreover, we also used laminin-111 as a co-adjuvant in MT.

89. JACQUES P. TREMBLAY, LAVAL UNIVERSITY

Meganucleases could be used to correct the reading frame or delete stop codons in the dystrophin gene.

Jacques P. Tremblay 1, Joel Rousseau 1, Pierre Chapdelaine 1, Sébastien Boivert 1, Luciana Almeida 1, Jacques Corbeil 1, Alexandre Montpetit 2 and Frédéric Pâques 3
1 Université Laval, Québec, Canada, 2 McGill University and 3 Cellectis inc. Romainville, France.

Several hereditary diseases are due either to a nonsense codon or to a frame shift mutation. Meganucleases (MGNs) are enzymes, which can be engineered to induce double strand break at a specific DNA sequence. These breaks are repaired by Homologous Recombination (HR) or by Non Homologous End Joining (NHEJ), which results in insertions or deletions (indels) of a few base pairs. We have tested three methods to detect, characterize and quantify the frequency of the indels produced by various MGNs targeting the Rag1 (Recombination activating gene 1) or the dystrophin gene. The Surveyor enzyme method was not very sensitive and permitted at best to determine whether abundant indels had been produced. For the other two methods, the DNA region targeted by the MGN was amplified by PCR. For one of them, the subtractive colony hybridization, the amplicons were cloned in the pDrive plasmid and bacteria colonies were grown. The colonies still containing the non-mutated sequences were detected by hybridization with a radioactive oligonucleotide complementary to the wild type sequence. Only the non-radioactive colonies were sequenced to identify indels. For the third method, called deep sequencing, the PCR primers include the adapters for Illumina deep sequencing, a multiplexing marker and a sequence to amplify the target gene. Roughly 15,000 to 65,000 amplicons were sequenced on the Illumina platform for each experimental condition. Indels of a variable number of base pairs were obtained. Some of these indels changed the reading frame by 1 or 2 bp while other did not. Different MGNs produced micro-deletions with different size distributions. These experiments are thus a proof of principle that MGNs, adequately engineered to target appropriate exon sequences should be able to: 1) restore the normal reading of a gene with a frame shift mutation by inducing indels with the appropriate number of bp; 2) delete a non-sense codon while maintaining the normal reading frame by inducing micro-deletions, which are multiple of 3 bp and 3) knockout a gene by inducing indels, which are not a multiple of 3 bp.

90. DOMINICO TRICARICO, UNIVERSITY OF BARI

Bendroflumethiazide, ethoxzolamide, acetazolamide and dichlorphenamide are potent openers of the human(hslo) calcium-activated-K+channel: a novel mechanism of action explaining their effects in neuromuscular disorders.

Domenico Tricarico1, Antonietta Mele1, Gianluigi Cannone1,

Giulia Maria Camerino1, Marilena Dinardo1, Ramon Latorre2 and Diana Conte Camerino1.
Affiliations: 1Department of Pharmacobiology, Faculty of Pharmacy, University of Bari, Bari, Italy. 2Centro de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaiso, Valparaiso, Chile.
corresponding author: prof. Domenico Tricarico, Department of Pharmacobiology, Faculty of Pharmacy, University of Bari, Bari, Italy

Benzo-thiadiazine/thiazole sulphonamides as ethoxzolamide(ETX) and bendroflumethiazide(BFT) are largely prescribed as diuretics and anti-hypertensive drugs. They are known to inhibit thiazide-sensitive Na(+)-Cl(-) cotransporter(TSC) at submicromolar concentrations with diuretic effects. They also show spasmolitic actions which is however unexplained. Other sulphonamides derivatives as acetazolamide(ACTZ) and dichlorphenamide(DCP) are potent inhibitors of carbonic anhydrase enzyme at nanomolar concentrations used to treat ocular edema, epilepsy and neuromuscular disorders. These drugs are indeed the drug of choice in periodic paralysis(PP)(Platt and Griggs, Curr Opin Neurol 22:524-531, 2009). Previous work have shown that all these drugs activate the BK channels of skeletal muscle fibers in rats and their effects are structure-related(Tricarico et al., FASEB J 18:760-761, 2004). They are capable to prevent the paralytic attacks of weakness induced by insulin/glucose injections in K-depleted rats, an animal model of the hypokalemic periodic paralysis(hypoPP) (Tricarico et al., Neuromuscul Disord 16:39-45). Their effects on human BK channels are, however, not known. In the present work the effects of acetazolamide, dichlorphenamide, methazolamide(MTZ), ethoxzolamide, bendroflumethiazide and hydrochlorthiazide on the human alpha subunit(hslo) of the BK channel expressed in HEK293 cell line were investigated using the patch-clamp technique. The results were compared with the effects of NS1619, an alpha subunit-selective channel opener, and with those of the flavonoids quercetin(QUERC) and resveratrol(RESV). We showed that the hslo channel subunit was activated by all drugs in the range of concentrations tested(10-10-10-3M) with different potency and efficacy, with the exception of HCT molecule. The potency ranking of the openers expressed as DE50 at -60 mV(Vm) was BFT(7.45x10-10)>ETX(2.1x10-7)>DCP(6.3x10-7)>ACTZ(8.71x10-7)>QUERC(1.5±x10-6)> MTZ (1.47x10-5) > NS1619(5.5x10-5)>RESV(7.5x10-5). Therefore, BFT and ETX were the most powerful drugs in comparison with others BK channel openers. BFT activates the human channel at subnanomolar concentrations at all voltage membranes indicating that it can be the primary target for the drug action in diseases that can benefit from the activation of BK channels such as PP. BFT and ETX can represent alternative drugs in the treatment of PP in those patients intolerant to ACTZ or DCP. Supported by Telethon GGP10101.

91. SILVÈRE VAN DER MAAREL, LEIDEN UNIVERSITY MEDICAL CENTER

The pathogenesis of facioscapulohumeral muscular dystrophy

Silvère M van der Maarel1, Dan Miller2, Gala Filippova3, and Stephen J. Tapscott3, Rabi Tawil4
1Leiden University Medical Center, Leiden, Netherlands. 2. University of Washington, Seattle, WA 3. Fred Hutchinson Cancer Research Center, Seattle, WA 4. University of Rochester Medical Center, Rochester, NY

Most patients with autosomal dominant facioscapulohumeral muscular dystrophy (FSHD1) carry a contracted D4Z4 repeat array of 1-10 units in the subtelomere of one of their chromosomes 4. A contacted array, which normally varies between 11-100 units, has a more open chromatin structure facilitating the transcriptional activity of the double homeobox gene DUX4 encoded within each unit. However, only D4Z4 contractions on specific chromosomal backgrounds lead to FSHD. Our recent data show that these FSHD permissive backgrounds have specific genetic polymorphisms that allow for the stabilization of the DUX4 transcript through the usage of a unique exon immediately distal to the repeat. Non-permissive backgrounds fail to stabilize these DUX4 transcripts in the absence of a polyadenylation signal in this unique distal exon. Indeed, 1:1,000 nuclei of differentiated FSHD myoblasts show abundant levels of DUX4 protein. We have generated transgenic mice that carry a patient allele of 2½ repeat units. In the absence of an overt muscle phenotype these mice show all the genetic, epigenetic and transcriptional attributes of FSHD alleles. D4Z4 repeats in these mice contain the genetic polymorphisms of permissive alleles allowing for the stabilization of DUX4 transcripts, have an open chromatin structure and express abundant levels of DUX4 in 1:1,000 nuclei of

differentiated myoblasts. We propose that these mice represent a unique model that allows for the study of the molecular mechanism underlying FSHD and the testing of new therapeutic intervention strategies.

92. ADELINE VULIN, CENTER FOR GENE THERAPY

Mutation-directed studies on the function of the dystrophin ZZ domain

Vulin A1, Maiti B2, Taylor L1, Kaminoh Y1, Simmons T1 and Flanigan K1.
1Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, Columbus. OH 43205
2 Department of Neurology, Washington University in St. Louis, School of Medicine, St. Louis, MO 63110.

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder due to mutations in the DMD gene encoding the dystrophin protein. DMD is typically associated with the loss of dystrophin, which plays an important role in skeletal and cardiac muscle fiber integrity via interactions with β-dystroglycan and other members of a transmembrane glycoprotein complex. Mutations in DMD also cause the milder Becker Muscular Dystrophy, typically due to mutations that result in the presence of some dystrophin, albeit of diminished size or in diminished amounts. Although the dystrophin protein has been studied in increasing detail, little is known about the function of the ZZ domain of the protein, a cysteine-rich zinc-finger domain near the C-terminus. This domain has been implicated in forming a stable interaction between dystrophin and β-dystroglycan, but other potential binding partners have not been well investigated. Missense mutations in DMD are quite rare, accounting for only 1.4% of all dystrophinopathy mutations; most are associated with BMD, and only 0.3% of DMD cases are due to missense mutations. 11 point mutations have been identified in the ZZ domain but their consequence is not well studied. To date, studies have only addressed effects on β-dystroglycan binding, with discrepant results. Here we seek to delineate the effect of three ZZ mutations on several known and candidate binding partners: βdystroglycan, myospryn and calmodulin. Via site-directed mutagenesis, we have introduced ZZ mutations into constructs encompassing the dystrophin WW, EF, ZZ and C terminal domains. Constructs are cloned into an expression vector and transfected in 293FT cells, following which we perform co-immunoprecipitation of native or co-expressed candidate binding partners. Our preliminary data suggest ZZ mutations have no effect on binding to known partners; confirmatory experiments are in progress. In some ZZ missense patients, a sizeable amount of dystrophin localizes to the membrane, suggesting β-dystroglycan may be intact, suggesting that the absence of another unknown binding partner may be responsible for disease. We will present our work in progress, including our mass spectrometry approach to identifying novel binding partners, and tertiary structure modeling of the effect of missense mutations on C-terminal folding.

93. DA-ZHI WANG, CHILDREN'S HOSPITAL BOSTON AND HARVARD MEDICAL SCHOOL

MicroRNA-1 and miR-206 Regulate Skeletal Muscle Satellite Cells

Jian-Fu Chen and Da-Zhi Wang
Cardiovascular Research Division, Children's Hospital Boston, Harvard Medical School, 320 Longwood Avenue, Boston, MA 02115

Skeletal muscle satellite cells are adult stem cells responsible for postnatal skeletal muscle growth and regeneration. Paired-box transcription factor Pax7 plays a central role in satellite cell survival, self-renewal and proliferation. However, how Pax7 is regulated during the transition from proliferating satellite cells to differentiating myogenic progenitor cells is largely unknown. In this study, we find that miR-1 and miR-206 are sharply up-regulated during satellite cell differentiation and down-regulated after muscle injury. We show that miR-1 and miR-206 facilitate satellite cell differentiation by restricting their proliferative potential. We identify Pax7 as one of the direct regulatory targets of miR-1 and miR-206. Inhibition of miR-1 and miR-206 substantially enhances satellite cell proliferation and increases Pax7 protein level in vivo. Conversely, sustained Pax7 expression due to the loss of miR-1 and miR-206 repression elements at its 3'UTR significantly inhibits myoblast differentiation. Our studies therefore suggest that miRNAs participate in a regulatory circuit that

allows rapid gene program transitions from proliferation to differentiation.

94. GUQI WANG, CAROLINAS MEDICAL CENTER

Development of novel nitric oxide based compounds for muscular disorders.

Guqi Wang and Qi Lu
McColl-Lockwood laboratory, Carolinas medical center, Charlotte, NC, USA

Skeletal health is exquisitely dependent on proper growth and repair of muscle. NO mediates activation of satellite precursor cells to enter the cell cycle. Such process provides new precursor cells for skeletal muscle growth and muscle repair in response to injury or disease. NO also functions as a signaling molecule responsible for regulating blood flow, oxygen delivery, glucose uptake, muscle contraction, muscle fatigue resistance and force output. Thus it has major physiological and cellular impacts in muscle growth and repair. Moreover, NO inhibits cellular ubiquitin-mediated proteasome degradation to arrest muscle atrophy and dystrophy commonly caused by muscle disuse, aging, and diseases. In most myopathic biopsies, sarcolemma-localized nNOS is either reduced or not detected, contributing to the disease progression. Diseased muscle fibers also exhibit an increased susceptibility to contraction-induced membrane damage which is directly correlated with the magnitude of mechanical stress during contraction. Muscle relaxants function to prevent muscle wasting by decreasing nerve impulses and reducing calcium influx that regulate tensing or tightening of muscle fibers. Supplement of NO and reduction in contraction-related stress have therefore been considered potential therapeutic strategy. We have recently developed a new class of nitric esters that combines NO and muscle relaxation pharmacologically. These compounds promise significant benefits to muscle growth, repair, and functions in normal and dystrophic mouse models. The lifespan of muscular dystrophic mice was significantly extended. The potentials of the new class drugs for treating muscular disorders will be discussed.

95. XIN WANG, BRIGHAM AND WOMEN'S HOSPITAL

Neuroprotection of n-acetyl-l-tryptophan in models of amyotrophic lateral sclerosis

Ana C. Sirianni1, Jiying Jiang1, Jiang Zeng2, David Li1, Sergei Baranov1, Lilly L. Mao3, Shuanhu Zhou1, Peter Sugerbaker1, Bruce Kristal1, Mohammed Bouhajib3, Robert M. Friedlander5, and Xin Wang1,*
1Brigham and Women's Hospital, Boston, MA, United States, 2University of Toronto, Mississauga, ON, Canada, 3Pharma Medica Research Inc, Mississauga, ON, Canada, 4University of Pittsburgh, Pittsburgh, PA, United States
Email address for correspondence: xwang@rics.bwh.harvard.edu
Keywords: N-acetyl-L-tryptophan, neuroprotection, mSOD1 G93A mice/NSC34 motor neurons, anti-apoptotic mechanisms

Substance P antagonist N-acetyl-tryptophan, an inhibitor of release of cytochrome c, is a potential therapeutic agent in the Neurodegenerative Drug Screening Consortium's library of 1040 compounds. However, there is no report on its neuroprotection in Amyotrophic Lateral Sclerosis (ALS). In addition, the action mechanisms of N-acetyl-tryptophan are poorly understood. We aim to evaluate the mechanisms of action and possible neuroprotective efforts of N-acetyl-tryptophan in models of ALS. We demonstrate that N-acetyl-L-tryptophan, but not N-acetyl-D-tryptophan, is neuroprotective in H2O2-mediated NSC34 motor neurons. The finding is supported by chemistry structure and activity relation analysis with molecular docking and energy minimization. Furthermore, we report that N-acetyl-L-tryptophan delays disease onset and extends survival in mSOD1G93A ALS transgenic mice. Our results indicate that N-acetyl-L-tryptophan inhibits the secretion of Substance P and IL-1beta, and N-acetyl-L-tryptophan provides protection not only through the prevention of mitochondrial factor cytochrome c/smac/AIF release, mitochondrial membrane potential dissipation, and caspase-9/-3/-1 activation but also through the correction of proteasomal dysfunction. However, N-acetyl-L-tryptophan is not a mitochondrial permeability transition inhibitor in isolated mitochondria. Our investigation elucidates the molecular mechanisms of neuroprotective effects of N-acetyl-L-tryptophan. We also demonstrate that N-acetyl-L-tryptophan could be used in potential therapies for ALS and other neurodegenerative diseases that are

characterized by inappropriate activation of cell-death pathways and/or oxidative stress. Acknowledgements
This work was supported by grants from MDA (to X.W.), NIH/NINDS (to X.W., R.M.F.).

96. ELLEN M. WELCH, PTC THERAPEUTICS

Identification and characterization of small molecules for the treatment of Duchenne/Becker muscular dystrophy.

Westley Friesen, Vara Prasad, Marla Weetall, Klara Pendrak1, H.Lee Sweeney2 and Ellen M. Welch
PTC Therapeutics, 100 Corporate Court., South Plainfield, NJ and 3Univ. of Pennsylvania, Dept of Physiology, Philadelphia, PA

PTC Therapeutics, Inc. (PTC) is engaged in the discovery of new drugs to treat Duchenne/Becker muscular dystrophy (DBMD). Several targets (myostatin, alpha-7 integrin, muscle-specific IGF1 (mIGF1), utrophin, and SERCA2a) were selected to enter the drug discovery program based on functional validation from animal studies. Using a proprietary drug discovery platform technology, referred to as GEMS™ (Gene Expression Modulation by Small-molecules), we sought to identify small molecules that upregulate the production of these protein targets to identify potential treatments for DBMD. Constructs containing a reporter gene flanked by the 5′ and 3′ untranslated regions (UTR) specific for each of the targets were stably transfected in human muscle (RD) or kidney (293H) cells and used in HTS. We identified hits that demonstrate concentration dependent activities in cell-based reporter assays and in assays that measure endogenous protein levels. Further, a number of molecules exhibit attractive pharmacological properties (e. g., low cytotoxicity, microsome metabolic stability, oral exposure). Presently, we are focused on optimizing the activity, potency and pharmacological properties of our lead chemical scaffolds. The ultimate goal of this drug discovery and development effort is to identify small molecules that can specifically modulate the production of a number of proteins that can be used to treat Duchenne/Becker muscular dystrophy.

97. YEFEI WEN, PURDUE UNIVERSITY

Notch regulation of stem cell fate and development in mouse skeletal muscles

Yefei Wen and Shihuan Kuang
Department of Animal Science and Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA.

Notch signaling is a conserved regulator of cell fate determination and pattern formation during development in metazoan. It has also been implicated in postnatal tissue regeneration, adult stem cell differentiation and cancer. Using skeletal muscle stem cells (satellite cells) as a model, here we investigate how Notch signaling regulates adult stem cell fate choice between self-renewal and differentiation. We demonstrate that constitutive activation of Notch signaling in myogenic progenitors results in decreases of skeletal muscle mass and embryonic lethality in mice. Strikingly, Notch activation increased satellite cell number but decreased the number of differentiated myonuclei, suggesting that Notch signaling inhibits muscle stem cell differentiation but promotes their self-renewal. Consistently, de novo activation of Notch signaling abolished MyoD expression and inhibited myoblast differentiation in culture. In contrast, the expression of self-renewal marker Pax7 was upregulated, suggesting Notch signaling directly promotes self-renewal. Interestingly, constitutive activation of Notch in later stage of muscle development (in differentiated myocytes) promoted cell fusion as indicated by increased small branches on newly formed multinuclei muscle fibers, which was confirmed by observation of increased expression of some myogenesis requested genes. Together, these results demonstrate that Notch signaling plays multiple roles in skeletal muscle development and stem cell fate choice.

98. LUKE WITHERSPOON, CHILDREN'S HOSPITAL OF EASTERN ONTARIO RESEARCH INSTITUTE

FDA drug mining for novel therapeutics for Myotonic Muscular Dystrophy Myotonic dystrophy type

1 (DM1) is caused by a pathogenically expanded CTG repeat in the 3' untranslated region of the Myotonic dystrophy protein kinase(DMPK) gene.

Luke Witherspoon, Dr. Alex MacKenzie

Mice null for DMPK develop a mild late onset myopathy, this fact suggests that the down regulation of DMPK mRNA, particularly in cases of congenital DM with profoundly amplified CTG tracts and very limited lifespan, represents a valid therapeutic avenue. It has been recognized in recent years that small molecules can affect a substantial proportion of the human transcriptome in ways that are both currently unknown and difficult to predict. In this regard, Justin Lamb (Broad Institute) has provided us with the rough Affymetrix Array data from the Connectivity map (1) showing DMPK mRNA expression in cancer cell lines cultured in the presence of 1278 drugs (10 uM, 6 hours). Twenty three drugs were selected for DMPK mRNA suppressive potency as well as availability and screened for their effect on DMPK mRNA levels in cultured murine C2C12 myoblast cells at three concentrations (25µm, 1µm, 50nM). Some drugs had no effect, others showed actual induction but ten(tomelukast, sulindac sulfide, procainamide, prilocaine, splitomicin, mexiletine,simvastatin, nalidixic acid, methylbenzethonium chloride and metoprolol) of the 23 compounds resulted in 30% or less of normal DMPK mRNA levels at least one of the two concentrations. The sole drug class over represented among these putative DMPK suppressants is, interestingly, one which has been used for DM1 treatment for decades, sodium channel blockers. Further analyses of these drugs evaluating the ability of these compounds to suppress pathologically expanded DMPK mRNA is in both patient cell lines as well as murine disease models. References 1) Lamb, J., Crawford, E., Peck, D., Modell, J., Blat, I., Wrobel, M., Lerner, J., Brunet, J.P., Subramanian, A., Ross, K.N., Reich, M., Hieronymus, H., Wei, G., Armstrong, S.A., Haggarty, S.J., Clemons, P.A., Wei, R., Carr, S.A., Lander, E., Golub, T.R. 2006. The connectivity map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 313 (29): 1929-1935.

99. YONGPING YUE, UNIVERSITY OF MISSOURI

Genetic expression of a near-full length dystrophin at the borderline level improves muscle force and lifespan in a strain of severely affected dystrophin/utrophin double knockout mice

Dejia Li, Yongping Yue, Dongsheng Duan
Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO, USA

Utrophin/dystrophin double knockout mdx (u-dko) mice display severe clinical symptoms and die prematurely as in Duchenne muscular dystrophy (DMD) patients. Here we tested the hypothesis that minimal level dystrophin expression can improve the clinical outcome of u-dko mice. It has been shown that mdx3cv (3cv) mice express a near-full length dystrophin protein at ~ 5% of the normal level. We crossed utrophin-null mutation (all utrophin isoforms are inactivated) to the 3cv background. The resulting uko/3cv mice expressed the same level of dystrophin as 3cv mice but utrophin expression was completely eliminated. Surprisingly, uko/3cv mice showed a much milder phenotype. Compared to u-dko mice, uko/3cv mice had significantly higher body weight and stronger specific muscle force. Most importantly, uko/3cv outlived u-dko mice by several folds. Our results suggest that a threshold level dystrophin expression may provide vital clinical support in a severely affected DMD mouse model. This finding may hold clinical implications in developing novel DMD therapies.

100. AVA YUN LIN, UNIVERSITY OF MINNESOTA

Structural dynamics of the actin-binding domains in dystrophin and utrophin

Ava Yun Lin, Ewa Prochniewicz, Zach James, Davin Henderson , James Ervasti and David D. Thomas

We are using time-resolved phosphorescence anisotropy (TPA) and dipolar electron-electron-resonance (DEER) to determine and compare the structural dynamics of the actin-binding domains in dystrophin and utrophin. Our goal is to study the fundamental characteristics of the dystrophin-actin and utrophin-actin interaction for better understanding of the pathophysiology of muscular dystrophy and assist in rational drug design. Dystrophin and utrophin bind actin in vitro with similar affinities, but with different molecular contacts (Rybakova et al, 2006, J. Biol. Chem). We hypothesize that these differences alter the elasticity of dystrophin-actin and utrophin-actin linkages to the sarcolemma, affecting the cell's response to muscle stretches, with important implications for muscular dystrophy and its therapy. Our previous TPA studies, detecting the microsecond dynamics of phosphorescent-labeled actin, showed that both proteins have novel effects on actin flexibility, with utrophin more effective than dystrophin (Prochniewicz et al., 2009, PNAS). We have now compared the effects of the isolated actin-binding domains of dystrophin, ABD1 and ABD2. TPA shows that the enhanced rate of actin rotational dynamics is induced primarily by ABD1, while both ABD1 and ABD2 contribute to the restriction in rotational amplitude. Disease-causing point mutations in ABD1 decrease the effects on actin's rotational rate. We propose that this in turn causes the dystrophin-actin complex to be less resilient and thus less able to prevent damage to the muscle cytoskeleton during contraction. Finally, we have attached probes directly to ABD1 in dystrophin and utrophin, to detect changes in structure upon actin binding. High-resolution distance measurements, provided by DEER, show that the two lobes (calponin-homology domains) within ABD1 undergo a dramatic opening upon actin binding, helping to resolve a previous controversy. Analogous studies with dystrophin are in progress.

114. VIRGINIA KIMONIS, UNIVERSITY OF CALIFORNIA, IRVINE

VCP Syndrome associated with hereditary Inclusion body myopathy, Paget disease of bone, frontotemporal dementia, and amyotrophic lateral sclerosis.

VE Kimonis1, S Donkervoort1, A Wang2, A Nalbandian1, M Badadani1, GD Watts2, B Martin4, C Smith4, V Caiozzo3, T Mozaffar2
1 Division of Genetics and Metabolism, Department of Pediatrics, University of California-Irvine, CA, USA
2 Departments of Neurology, University of California-Irvine, California
3 School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, Norfolk, UK
4 Department of Orthopedic Surgery, University of California, Irvine, CA, USA
5 Department of Neurology, University of Kentucky Medical School, Lexington, KY, USA.

Hereditary inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia is an increasingly recognized disorder caused by VCP mutations. VCP mutations have been identified in 2% of familial amyotrophic lateral sclerosis (ALS). VCP is at the crossroads of many cellular functions including ubiquitin proteasome-mediated degradation, and p62 associated autophagy. Pathology includes ubiquitin and TDP43 positive inclusions also seen in ALS and other proteinopathies. Genotype-phenotype correlations reveal marked intrafamilial variations and varied phenotypes including cardiomyopathy, ALS, Parkinson's, myotonia, cataracts and gastrointestinal complications.
Our R155H VCP knock-in heterozygous mice are a good model for preclinical studies and demonstrate progressive muscle, bone and brain pathology. Mice have progressive weakness, vacuolization of myofibrils, centrally located nuclei, and cytoplasmic accumulation of TDP-43 and ubiquitin in myofibrils and in the brain. Bone micro-CT morphometrics shows decreased trabecular pattern and increased cortical wall thickness, and histology reveals increased osteoclastogenesis. Homozygosity of the R155H mutation is lethal by 21 days; these mice reveal abnormal skeletal muscle architecture and large vacuoles in the cardiac muscle.
Myoblasts from patients identified large ubiquitin containing vacuoles that accumulate LC3, a marker for autophagy. The vacuoles fuse with lysosomes as indicated by LAMP-1 and LAMP-2-staining; these proteins however are differentially N-glycosylated. Additionally, mutant myoblasts show decreased proliferation activity, and increased apoptosis.

We have recently performed comprehensive evaluations in 12 individuals and have identified ALS findings in two individuals. Case 1 was a 48 year old woman with a VCP disease mutation (R155C, 463 C>T), who was evaluated for rapidly progressive weakness. She developed weakness four years prior and became wheelchair bound over the preceding year and a half. She had severe dysarthria and dysphagia with a 23-pound weight loss in the past year. She had dyspnea with activity and orthopnea. Her past medical history was significant for metastatic thymoma in full remission. Her father, brother, sister and daughter have myopathy and several family members had frontotemporal dementia. Examination revealed nasal speech and spastic dysarthria. There was facial, tongue and neck flexor and extensor weakness, tongue fasciculations and positive jaw jerk. She had diffuse atrophy, fasciculations and weakness with retained reflexes. IBM functional rating scale was 3. Bedside pulmonary function tests revealed a spontaneous nasal inspiratory pressure of -11 cm and vital capacity of 0.46 liters (13% predicted). Electromyography revealed ongoing denervation and reinnervation in three body segments consistent with a disorder of the motor neurons and its axons. Creatine kinase as 37 U/L. She was diagnosed with fulminant ALS and is since deceased at the age of 48 y. The autopsy revealed classical IBM changes in the muscle and loss of myelin in the lateral columns of the spinal cord. The spinal cord also revealed scattered hyaline eosinophilic inclusions in the anterior horns of the spinal cord and occasional glassy cytoplasm in the motoneurons and some Bunina bodies. Ubiquitin immunostain shows rare nuclear staining, cytoplasmic staining in numerous motoneurons and scattered positive inclusions in the neuropil of the anterior horns.

Case 2 is a 42 year old male (R155H) with IBM and Paget disease in his hemipelvis and skull who has had a rapid progression of his weakness and pulmonary function studies. He has respiratory difficulties and electromyography revealed chronic denervation and reinnervation suggestive of motor neuron disease. EMG findings in the other individuals revealed mixed neurogenic-myogenic or myogenic pattern.
Our studies in this fascinating disorder in patients, myoblasts and the knock-in VCP R155H mouse model adds some insight in VCP disease. VCP is at the crossroads of pathology involving progressive muscle, bone and brain disease and understanding the pathogenesis has implications for more common related disorders such as ALS.

Contacts

Abdel-Hamid, Hoda
University of Pittsburgh
hza1@pitt.edu

Abresch, Richard
UC Davis
tabresch@ucdavis.edu

Acsadi, Gyula
Connecticut Children's Medical Center; University of Connecticut
gacsadi@ccmckids.org

Adams, Marvin
University of Washington
marva@uw.edu

Aguilan, Jennifer
Albert Einstein College of Medicine
jaguilan@aecom.yu.edu

Akaaboune, Mohammed
University of Michigan
makaabou@umich.edu

Alfano, Lindsay
Nationwide Childrens Hospital
lindsay.alfano@nationwidechildrens.org

Allen, Hugh
Nationwide Children's Hospital
hugh.allen@nationwidechildrens.org

Allen, Ronald
University of Arizona
rallen@ag.arizona.edu

Anderson, Judy
University of Manitoba
janders@ms.umanitoba.ca

Androphy, Elliot
Indiana Univ. School of Medicine
eandro@iupui.edu

Anglister, Lili
Hebrew University Medical School
lili.anglister@huji.ac.il

Antonellis, Anthony
University of Michigan
antonell@umich.edu

Apkon, Susan
Seattle Children's Hospital
susan.apkon@seattlechildrens.org

Appel, Stanley
Methodist Neurological Institute
sappel@tmhs.org

Armstrong, Dustin
4s3 Bioscience
dustin.armstrong@4s3bioscience.com

Arnett, Andrea
University of Washington, MSTP
alarnett@uw.edu

Askanas, Valerie
University of Southern California Keck School of Medicine
askanas@usc.edu

Atchison, William
Michigan State University
atchiso1@msu.edu

August, Paul
Sanofi-Aventis
paul.august@sanofi-aventis.com

Banks, Glen
University of Washington
banksg@uw.edu

Barnum, Jamie
The Ohio State University
barnum.15@buckeyemail.osu.edu

Barry, Michael
Mayo Clinic
mab@mayo.edu

Baumbach-Reardon, Lisa
University of Miami School of Medicine
Lbaumbac@med.miami.edu

Bean, S. Charles
Nemours Al Dupont
cbean216@verizon.net

Beggs, Alan
Children's Hospital / Harvard Medical School
beggs@enders.tch.harvard.edu

Beilsmith, Susan
Muscular Dystrophy Association
sbeilsmith@mdausa.org

Belayew, Alexandra
University of Mons
alexandra.belayew@umons.ac.be

Bennett, Lindsey
Children's Hospital of MI
LBennett2@dmc.org

Bennett, Frank
Isis Pharmaceuticals
fbennett@isisph.com

Berry, Suzanne
University of Illinois
seberry@life.uiuc.edu

Bertoni, Carmen
UCLA
cbertoni@ucla.edu

Beson, Brent
INTEGRIS Neuromuscular Center
besonneuro@coxinet.net

Bidichandani, Sanjay
Muscular Dystrophy Association
sbidichandani@mdausa.org

Blonsky, Karla
Marigold Foundation
kblonsky@biobasix.com

Bodensteiner, John
Barrow Neurological Institute
jbodens@chw.edu

Bogdanik, Laurent
The Jackson Laboratory
laurent.bogdanik@jax.org

Boulis, Nicholas
Emory University School of Medicine
nboulis@emory.edu

Braunstein, Joel
Tivorsan Pharmaceuticals, Inc.
jbraun@tivorsan.com

Bravver, Elena
Carolinas Medical Center
elena.bravver@carolinas.org

Bromberg, Mark
University of Utah
Mark.Bromberg@hsc.utah.edu

Brooks, Benjamin Rix
Carolinas Medical Center
benjamin.brooks@carolinashealthcare.org

Brown, Susan
Royal Veterinary College
scbrown@rvc.ac.uk

Burghes, Arthur
Ohio State University
burghes.1@osu.edu

Burkin, Dean
University of Nevada, Reno
dburkin@medicine.nevada.edu

Butterfield, Russell
University of Utah, Pediatric Neurology
russell.butterfield@hsc.utah.edu

Campbell, Kevin
HHMI/University of Iowa
kevin-campbell@uiowa.edu

Chamberlain, Joel
University of Washington
jrcham@uw.edu

Chamberlain, Jeffrey
University of Washington
jsc5@uw.edu

Chicoine, Louis
Nationwide Childrens Hospital
LOUIS.CHICOINE@NATIONWIDECHILDRENS.ORG

Childers, Martin
Wake Forest University Batpist Medical Center
mchilder@wfubmc.edu

Chimanji, Raj
The Ohio State University
chimanji.1@osu.edu

Chin, Wei-Chun
University of California-Merced
wchin2@ucmerced.edu

Chu, Mary Lynn
NYUMC
marylynn.chu@nyumc.org

Clemens, Paula
University of Pittsburgh
pclemens@pitt.edu

Cleveland, Don W.
Ludwig Institute for Cancer Research/UCSD
dcleveland@ucsd.edu

Cloud, Richard
Prothelia Inc.
rich.cloud@prothelia.com

Conwit, Robin
NIH/NINDS
rc296d@nih.gov

Cortopassi, Gino
UC Davis
gcortopassi@ucdavis.edu

Cossu, Giulio
San Raffaele Scientific Institute
cossu.giulio@hsr.it

Crosbie, Rachelle
UCLA
rcrosbie@physci.ucla.edu

Cudkowicz, Merit
Massachusetts General Hospital
mcudkowicz@partners.org

Cwik, Valerie
Muscular Dystrophy Association
vcwik@mdausa.org

Dabrowski, Edward
Childrens Hosp. of Michigan, Wayne State University
edabrows@dmc.org

Darvish, Daniel
HIBM Research Group
ddarvish@hibm.org

Davies, Kay
University of Oxford
kay.davies@dpag.ox.ac.uk

De Haro, Maria
Baylor College of Medicine
mh135822@bcm.edu

Deka, Priti
The Scripps Research Institute
deka@scripps.edu

Delfín, Dawn
The Ohio State University, College of Medicine
delfin.1@osu.edu

Desai, Urvi
Carolinas Neuromuscular ALS-MDA Clinic
urvi.desai@carolinashealthcare.org

Dialynas, Georgios
University of Iowa
georgios-dialynas@uiowa.edu

Dick, Arthur
University of Kansas Medical Center
awhited@kumc.edu

Discher, Dennis
Univ. Pennsylvania
discher@seas.upenn.edu

Doe, Jinger
University of Nevada Reno
jdoe@medicine.nevada.edu

Dowling, James
University of Michigan
jamedowl@umich.edu

Drachman, Daniel
Johns Hopkins School of Medicine
dandrac@aol.com

Duan, Dongsheng
University of Missouri
duand@missouri.edu

Durham, Heather
McGill University
heather.durham@mcgill.ca

Durrant, Andrea R.
Dept. Med. Neurobio., Inst. Med. Res. (IMRC),
aviva007_israel@yahoo.com

Engel, W. King
University of Southern California Keck School of Medicine
askanas@usc.edu

Eynott, Paul
sanofi-aventis, US
paul.eynott@sanofi-aventis.com

Fallon, Justin
Brown University
Justin_Fallon@brown.edu

Felice, Kevin
Hospital for Special Care
FELICEK@hfsc.org

Figlewicz, Denise Anne
ALS Society of Canada
daf@als.ca

Filippova, Galina
Fred Hutchinson Cancer Research Center
gfilippo@fhcrc.org

Finanger Hedderick, Erika
Oregon Health & Sciences University
hedderic@ohsu.edu

Flanigan, Kevin
Center for Gene Therapy
kevin.flanigan@nationwidechildrens.org

Forbes, Sean
University of Florida
scforbes@ufl.edu

Foster, Keith
Royal Holloway University of London
keith.foster@rhul.ac.uk

Fraidenraich, Diego
UMDNJ
fraidedi@umdnj.edu

Frewing, Kent
Kurt+Peter Foundation
kfrewing@gmail.com

Froehner, Stanley
University of Washington
froehner@uw.edu

Ganguly, Ranjit
The Ohio State University
ganguly.11@osu.edu

Gangwani, Laxman
Texas Tech. University Health Science Center
laxman.gangwani@ttuhsc.edu

Garbedian, Chris
AVI BioPharma
rmonaghan@avibio.com

Garbuzova-Davis, Svitlana
University of South Florida College of Medicine
sgarbuzo@health.usf.edu

Geng, Linda
Fred Hutchinson Cancer Research Center
Lgeng@fhcrc.org

Genge, Angela
Montreal Neurological Institute
angela.genge@mcgill.ca

Gilchrist, James
Rhode Island Hospital/Brown University
jgilchrist@lifespan.org

Girgenrath, Sweta
Boston University
swetag@bu.edu

Godfrey, Earl
Eastern Virginia Medical School
godfreew@evms.edu

Goldstein, Edward
Childrens Healthcare of Atlanta
egoldstein@childneuro.org

Gornstein, Eric
Acceleron Pharma
egornstein@acceleronpharma.com

Goudenege, Sebastien
CHUQ
goudenege@hotmail.com

Goyal, Namita
Massachusetts General Hospital/ Harvard Medical School
nagoyal@partners.org

Goyenvalle, Aurelie
University of Oxford
aurelie.goyenvalle@dpag.ox.ac.uk

Grange, Robert
Virginia Tech.
rgrange@vt.edu

Gray, Jennifer
St. Charles Hospital
Jennifer.Gray@chsli.org

Gregorevic, Paul
Baker IDI heart & Diabetes Institute
paul.gregorevic@bakeridi.edu.au

Grosmann, Carla
UCSD/Rady Children's Hospital
cgrosmann@chsd.org

Gubitz, Amelie
NINDS/NIH
gubitza@ninds.nih.gov

Guenzel, Adam
Mayo Clinic
guenzel.adam@mayo.edu

Guo, Ling
University of California, San Diego
liguo@ucsd.edu

Gupta, Rishi
OrbiMed Advisors
rgupta@orbimed.com

Gutmann, Laurie
Department of Neurology, WVU School of Medicine
lagutmann@hsc.wvu.edu

Guttridge, Denis
Ohio State University
denis.guttridge@osumc.edu

Habeeb-Louks, Elizabeth
Muscular Dystrophy Association
elouks@mdausa.org

Hadwen, Jeremiah
Children's Hospital of Eastern Ontario Research Institute
hadwen.jeremiah@gmail.com

Hakim, Chady
University of Missouri
hakimch@health.missouri.edu

Hall, David
Charcot Marie Tooth Association
dave@cmtausa.org

Han, Jay
University of California Davis Med. Center
jay.han@ucdmc.ucdavis.edu

Han, Renzhi
Loyola University Medical Center
renhan@lumc.edu

Harper, Amy
Carolinas Pediatric Neurology Care Charlotte
amy.harper@carolinashealthcare.org

Hauschka, Steve
University of Washington
haus@u.washington.edu

Haviland, Kate
PTC Therapeutics
khaviland@ptcbio.com

Hawkins, Al
Bioenterprise
phawkins@bu.edu

Hayden, Sarah
Muscular Dystrophy Association
shayden@mdausa.org

Heatwole, Chad
The University of Rochester
chad_heatwole@urmc.rochester.edu

Heller, Kristin
Nationwide Childrens Hospital/ The Ohio State University
kristin.heller@nationwidechildrens.org

Henricson, Erik
University of California, Davis
erik.henricson@gmail.com

Hensley, Kenneth
University of Toledo Medical Center
kenneth.hensley@utoledo.edu

Hesterlee, Sharon
Parent Project Muscular Dystrophy
sharon@parentprojectmd.org

Hillestad, Matthew
Mayo Clinic
hillestad.matthew@mayo.edu

Hodges, Bradley
Prothelia
brad.hodges@prothelia.com

Homma, Sachiko
Boston Biomedical Research Institute
homma@bbri.org

Horak, Holli
University of Arizona
hhorak@uph.org

Howard, James
The University of North Carolina at Chapel Hill
howardj@neurology.unc.edu

Howell, R. Rodney
National Institutes of Health
howellr@mail.nih.gov

Huard, Johnny
University of Pittsburgh/ Children's Hospital of Pittsburgh of UPMC
jhuard@pitt.edu

Ingersoll, Evan
Knopp Biosciences LLC
eingersoll@knoppbio.com

Jacob, Dena
Thomas Jefferson University
dena.jacob@jefferson.edu

Jahnke, Vanessa
Center for Genetic Medicine, Children's National Medical Center
vjahnke@cnmcresearch.org

Janas, Joanne
Child Neurology Associates
jjanas6533@aol.com

Jani-Acsadi, Agnes
University of Connecticut SOM Dept. Neurology
Jani-acsadi@uchc.edu

Janssen, Paul
The Ohio State University
janssen.10@osu.edu

Jarecki, Jill
Families of SMA
Jill@fsma.org

Jasmin, Bernard
University of Ottawa
jasmin@uottawa.ca

Jiang, Huiyuan
Children's Hospital of Michigan, Wayne State University
hjiang@med.wayne.edu

Karachunski, Peter
University of Minnesota
karac001@umn.edu

Kaspar, Brian
The Research Institute at Nationwide Children's
Brian.Kaspar@NationwideChildrens.org

Kassir, Allison
MDA
crobinson@mdausa.org

Kawahara, Genri
Children's Hospital Boston
gkawahara@enders.tch.harvard.edu

Kayali, Refik
University of California, Los Angeles
rkaylai@ucla.edu

Keller, Erica Goude
UCDMC
erica.goude@ucdmc.ucdavis.edu

Kennedy, Annie
Muscular Dystrophy Association
akennedy@mdausa.org

Khemani, Suresh
Knopp Biosciences
suresh.khemani@knoppbio.com

Kimonis, Virginia
UC Irvine
vkimonis@uci.edu

Kong, Jiming
University of Manitoba
kongj@cc.umanitoba.ca

Kornegay, Joe
University of North Carolina at Chapel Hill
joe_kornegay@med.unc.edu

Kothary, Rashmi
Ottawa Hospital Research Institute
rkothary@ohri.ca

Krainer, Adrian
Cold Spring Harbor Laboratory
krainer@cshl.edu

Krull, Catherine
University of Michigan
krullc@umich.edu

Kuang, Shihuan
Purdue University
skuang@purdue.edu

Kumar, Ajay
Boston University
ajaysar@bu.edu

Kumar, Ashok
University of Louisville School of Medicine
ashok.kumar@louisville.edu

Kunkel, Louis
Children's Hospital Boston
kunkel@enders.tch.harvard.edu

López Castel, Arturo
Valentia Biopharma S.L.
a.lopez@valentiabiopharma.com

Lachey, Jennifer
Acceleron Pharma
jlachey@acceleronpharma.com

Ladha, Shafeeq
Barrow Neurological Institute
shafeeq.ladha@chw.edu

Lange, Dale
Hospital For Special Surgery/ Weill Medical College of Cornell University
langed@hss.edu

Lawlor, Michael
Children's Hospital of Boston/ Harvard Medical School
mlawlor@enders.tch.harvard.edu

Leshner, Robert
Rady Children's Hospital---San Diego
rleshner@cnmc.org

Li, Juan
University of North Carolina at Chapel Hill
juanl@email.unc.edu

Li, Dejia
University of Missouri
lide@missouri.edu

Lin, Ava
University of Minnesota
yl@ddt.biochem.umn.edu

Lombardo, Kellie
Neurosciences - Alberta Children's Hospital
kellie.lombardo@albertahealthservices.ca

Lomen-Hoerth, Catherine
UCSF
catherine.lomen-hoerth@ucsf.edu

Lowes, Linda
Nationwide Children's Hospital
linda.lowes@nationwidechildrens.org

Lu, Qi
Carolinas Medical Center
qi.lu@carolinashealthcare.org

Lynch, Gordon
The University of Melbourne
gsl@unimelb.edu.au

Lynch, David
Children's Hospital of Philadelphia
lynchd@mail.med.upenn.edu

MacKenzie, Don
Marigold Foundation
admin@marigoldfoundation.org

MacKenzie, Alex
Children's Hospital of Eastern Ontario Research Institute
Mackenzie@cheo.on.ca

Mah, Jean
Alberta Children's Hospital
jean.mah@albertahealthservices.ca

Mallela, Krishna
University of Colorado Denver
krishna.mallela@ucdenver.edu

Mathews, Katherine
University of Iowa
katherine-mathews@uiowa.edu

Mattaliano, Robert
Genzyme Corporation
bob.mattaliano@genzyme.com

Matthews, Dennis
The Children's Hospital, University of Colorado
matthews.dennis@tchden.org

Mazzini, Letizia
Eastern Piedmont University; "Maggiore della Carità" Hospital
mazzini.l@libero.it

McCall, John
Pharmac LLC
john.m.mccall@pharmacLLC.com

McNally, Elizabeth
University of Chicago
emcnally@uchicago.edu

Meadows, Eric
Nationwide Children's Hospital
eric.meadows@nationwidechildrens.org

Mendell, Jerry
Nationwide Children's Hospital
Jerry.Mendell@nationwidechildrens.org

Menhart, Nick
Illinois Institute of Technology
menhart@iit.edu

Michele, Daniel
University of Michigan
dmichele@umich.edu

Michelson, David
Loma Linda University School of Medicine
dmichels@llu.edu

Miller, Timothy
University of Arizona / Genzyme
timothy.miller@genzyme.com

Miller, Robert
Forbes Norris MDA/ALS Research Center
millerrx@sutterhealth.org

Miller, Daniel
University of Washington and Seattle Childrens Hospital
dgmiller@u.washington.edu

Minnerly, Carolyn
Muscular Dystrophy Association
cminnerly@mdausa.org

Mishra, Shri
MDA clinic Olive View UCLA Medical center
smishra@usc.edu

Mitsumoto, Hiroshi
Columbia University
hm264@columbia.edu

Molina-Campos, Elizabeth
Michigan State University
molinac1@msu.edu

Moore, Mark
Glialogix
mwmoore880@comcast.net

Moreno, Carlos
Muscular Dystrophy Association
cmoreno@mdausa.org

Morgan, Michelle
Muscular Dystrophy Association
mmorgan@mdausa.org

Mozaffar, Tahseen
University of California, Irvine
mozaffar@uci.edu

Muhlrad, Paul
Muscular Dystrophy Association
pmuhlrad@mdausa.org

Nagaraju, Kanneboyina
Children's National Medical center
knagaraju@cnmcresearch.org

Naryshkin, Nikolai
PTC Therapeutics
nnaryshkin@ptcbio.com

Nelson, Sally
University of Colorado Denver
Sally.Nelson@UCDENVER.edu

Nguyen, Thien
Johns Hopkins Hospital
tnguyena@jhmi.edu

Nicorici, Alina
UC Davis Medical Center
alina.nicorici@ucdmc.ucdavis.edu

Niu, Li
State University of New York at Albany
lniu@albany.edu

Nogalska, Anna
USC Neuromuscular Center
nogalska@usc.edu

Odom, Guy
University of Washington
godom@uw.edu

Oleszek, Joyce
The Children's Hospital
oleszek.joyce@tchden.org

Osman, Erik
MU-Columbia/Dept. of Mol. Microbiology & Immunology
eo8q5@mail.missouri.edu

Pandey, Udai
Louisiana State University Health Sciences Center
upande@lsuhsc.edu

Parker, Maura
Fred Hutchinson Cancer Research Institute
mparker@fhcrc.org

Parsons, Julie
The Children's Hospital
parsons.julie@tchden.org

Partridge, Terence
Children's National Medical Center
tpartridge@cnmcresearch.org

Pasinetti, Giulio
The Mount Sinai School Of Medicine
giulio.pasinetti@mssm.edu

Pattee, Gary
UNMC
gpattee@pol.net

Paushkin, Sergey
SMA Foundation
spaushkin@smafoundation.org

Peltier, Wendy
Medical College of Wisconsin
wpeltier@mcw.edu

Peltz, Stuart
PTC Therapeutics
speltz@ptcbio.com

Percival, Justin
University of Washington
justinp2@uw.edu

Perrin, Steven
ALS Therapy Development Institute
sperrin@als.net

Peterson, Jennifer
The Ohio State University
jennifer.peterson@osumc.edu

Pierson, Chris
The Research Institute at Nationwide Children's Hospital
christopher.pierson@nationwidechildrenshospital.org

Pines, Mark
Volcani Center
pines@agri.huji.ac.il

Porensky, Paul
Ohio State University
paul.porensky@osumc.edu

Porter, John
NINDS/NIH
porterjo@ninds.nih.gov

Puri, Pier Lorenzo
Sanford-Burnham Medical Research Institute
lpuri@sanfordburnham.org

Rafael-Fortney, Jill
College of Medicine, The Ohio State University
rafael-fortney.1@osu.edu

Ravits, John
Benaroya Research Institute
jravits@benaroyaresearch.org

Rawls, Alan
Arizona State University
alan.rawls@asu.edu

Rayavarapu, Sree Veera
Children's National Medical Center
srayavarapu@cnmcresearch.org

Rebolledo, Daniela
University of Washington
drebol@uw.edu

Reed, Patrick
University of Maryland, Baltimore
preed001@umaryland.edu

Rhoads, Timothy
Oregon State University
rhoadst@onid.orst.edu

Ricketts, David
Muscular Dystrophy Association
dricketts@mdausa.org

Rodino-Klapac, Louise
Research Institute at Nationwide Children's Hospital
Louise.Rodino-Klapac@nationwidechildrens.org

Rolon, Desiree
Michigan State University
rolondon@msu.edu

Rooney, Jachinta
University of Nevada, Reno
jachinta@medicine.nevada.edu

Rosen, Lindsay
PTC Therapeutics
lrosen@ptcbio.com

Rosenfeld, Jeffrey
UCSF - Fresno
jrosenfeld@fresno.ucsf.edu

Rothstein, Jeffrey
Johns Hopkins university
Jrothstein@jhmi.edu

Rowland, Lewis P.
Columbia University
lpr1@columbia.edu

Ruf-Zamojski, Frederique
California Institute of Technology
fruf@caltech.edu

Rusche, James
Repligen Corporation
jrusche@repligen.com

Russman, Barry
OHSU and Shriners Hospital-Portland
russmanb@ohsu.edu

Sacco, Alessandra
Sanford-Burnham Medical Research Institute
asacco@sanfordburnham.org

Sahenk, Zarife
Nationwide Children's Hospital and Research Institute
zarife.sahenk@nationwidechildrens.org

Sampson, Jacinda
University of Utah
jacinda.sampson@hsc.utah.edu

Scaramuzzino, Chiara
Fondazione Istituto Italiano di Tecnologia
chiara.scaramuzzino@iit.it

Scherer, Katalin
University of Arizona
kscherer@uph.org

Schill, Kevin
The Ohio State University
schill.9@osu.edu

Schnier, Joachim
University of California Davis
jbschnier@ucdavis.edu

Seto, Jane
University of Washington Shool of Medicine
jtseto@uw.edu

Seehra, Jasbir
Co-Founder, Acceleron Pharma
seehrajj@verizon.net

Shieh, Perry
UCLA
pshieh@mednet.ucla.edu

Shin, Jin-Hong
University of Missouri
shinzh@missouri.edu

Shiue, Lily
UC Santa Cruz
lshiue@ucsc.edu

Shrewsbury, Stephen
AVI BioPharma
rmonaghan@avibio.com

Skerjanc, Ilona
University of Ottawa
iskerjan@uottawa.ca

Soltys, Jindrich
Saint Louis University
jsoltys@slu.edu

Sparks, Susan
Carolinas Medical Center
susan.sparks@carolinas.org

Spurney, Christopher
Children's National Medical Center
cspurney@cnmc.org

Stern, Lawrence
The University of Arizona/MDA
lawjacq@msn.com

Stiber, Jonathan
Duke University Medical Center
stibe001@mc.duke.edu

Strober, Jonathan
UCSF
stroberj@neuropeds.ucsf.edu

Sufit, Robert
Northwestern University Feinberg School of Medicine
r-sufit@northwestern.edu

Svendsen, Clive
Cedars-Sinai Regenerative Medicine Institute
clive.svendsen@cshs.org

Swisher, Anne
Muscular Dystrophy Association
aswisher@mdausa.org

Swoboda, Kathryn
University of Utah -School of Medicine
swoboda@genetics.utah.edu

Tabebordbar, Mohammadsharif
Harvard Medical School
tabebord@fas.harvard.edu

Tesi Rocha, Carolina
Childrens National Medical Center
ctesiroc@cnmc.org

Thompson, Tom
University of Cincinnati
tom.thompson@uc.edu

Thornton, Charles
University of Rochester Medical Center
charles_thornton@urmc.rochester.edu

Thrush, Philip
Nationwide Children's Hospital
philip.thrush@nationwidechildrens.org

Tinsley, Jon
Summit plc
jon.tinsley@summitplc.com

Toshav, Effie
AVI BioPharma
rmonaghan@avibio.com

Tremblay, Jacques P.
Laval University
jacques-p.tremblay@crchul.ulaval.ca

Tricarico, Domenico
Dept. of Pharmacobiology, Fac. of Pharmacy, Univ. of Bari
dtricarico@farmbiol.uniba.it

Trotti, Davide
Thomas Jefferson University
davide.trotti@jefferson.edu

Tseng, Brian
MGH
bstseng@gmail.com

Van der Maarel, Silvere
LUMC
maarel@lumc.nl

Van Remmen, Holly
University of Texas Health Science Center San Antonio
vanremmen@uthscsa.edu

Verma, Ashok
University of Miami Miller School of Medicine
averma@med.miami.edu

Vulin, Adeline
Research Institute / Center for Gene Therapy
adeline.vulin-chaffiol@nationwidechildrens.org

Wang, Da-Zhi
Children's Hospital Boston and Harvard Medical School
dwang@enders.tch.harvard.edu

Wang, Xin
Brigham and Women's Hospital/ Harvard Medical School
xwang@rics.bwh.harvard.edu

Wang, Zejing
Fred Hutchinson Cancer Research Center and University of Washington
zwang@fhcrc.org

Wang, Annabel
University of California, Irvine
akwang@uci.edu

Wang, Ching
Stanford University
wangch@stanford.edu

Wang, Eric
Massachusetts Institute of Technology
et_wang@mit.edu

Wang, Guqi
Carolinas Medical Center
guqi.wang@carolinashealthcare.org

Wiebe, Scott
Muscular Dystrophy Association
swiebe@mdausa.org

Welch, Ellen
PTC Therapeutics
ewelch@ptcbio.com

Wells, Dominic
Royal Veterinary College
dwells@rvc.ac.uk

Wen, Yefei
Purdue University
ywen@purdue.edu

Whitaker, Charles
Hospital for Special Care
WHITAKE@HFSC.ORG

Whitehead, Nicholas
University of Washington
npwhite@u.washington.edu

Williams, Jared
Oregon State University
willijar@onid.orst.edu

Wilson, James
University of Pennsylvania
wilsonjm@mail.med.upenn.edu

Wilton, Steve
University of Western Australia
swilton@meddent.uwa.edu.au

Windelborn, James
Boston Biomedical Research Institute
windelborn@bbri.org

Witherspoon, Luke
Children's Hospital of Eastern Ontario Research Institute
luke.witherspoon@gmail.com

Witt, Amanda
University of California Davis Medical Center
amandawitt80@gmail.com

Wolff, Jodi
Muscular Dystrophy Association
jwolff@mdausa.org

Wong, Joaquin
LSUHSC/Children's Hospital
jwong@lsuhsc.edu

Wright, Paul
Sanofi-Aventis
pauls.wright@sanofi-aventis.com

Wu, Bo
Carolinas Medical Center
bo.wu@carolinashealthcare.org

Wu, Jun
Purdue University
junwu@purdue.edu

Wurthner, Jens
GlaxoSmithKline
jens.u.wurthner@gsk.com

Xiao, Xiao
University of North Carolina at Chapel Hill
xxiao@email.unc.edu

Yamauchi, Jenny
Boston University
jennyy@bu.edu

Yang, Michele
The Children's Hospital Denver
yang.michele@tchden.org

Yue, YongPing
University of Missouri
yuey@missouri.edu

Sponsors

Platinum sponsor

sanofi aventis

Because health matters

ACCELERON PHARMA

Shire

To be as brave as the people we help.

AMGEN®

CYTOKINETICS

genzyme

MDA® Muscular Dystrophy Association

Fighting Muscle Disease

Jerry Lewis, National Chairman
www.mda.org • (800) 572-1717