MUSCLE STUDY GROUP MEETING, NOVEL MOLECULAR MECHANISMS OF NEUROMUSCULAR DISEASE: IMPLICATIONS FOR THERAPY, LADY MARGARET HALL OXFORD, U.K., SEPTEMBER 16–18, 2013

A – OUTCOME MEASURES

1

VALIDATION OF THE MRC MRI DIAGNOSTIC SCORE FOR INCLUSION BODY MYOSITIS

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Introduction: Whilst the MRI pattern of muscle involvement has been reported in IBM, its diagnostic utility has not been systematically assessed.

Objectives: We aimed to develop and validate an objective diagnostic MRI score based on the pattern of muscle involvement.

Methods: The MRC MRI Diagnostic Score was developed on a cohort of 20 IBM patients. The score was then applied to an independent cohort of 40 patients with IBM and 39 patients with other adult-onset myopathies by a radiologist blinded to diagnosis.

Results: The developed score consisted of 20 relative comparisons with a possible score range from −31 to +31. When applied to the independent cohort the test had good diagnostic utility with area under ROC curve of 0.80. Using a cut-off score of 10 resulted in 73% sensitivity and 80% specificity.

Conclusions: The MRC MRI Diagnostic Score is a useful adjuvant to diagnosis in IBM.

2

SYMPTOMATIC IMPACT OF CHARCOT MARIE TOOTH DISEASE

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Introduction: Charcot Marie Tooth disease (CMT) is the most common form of neuropathy. Multisystemic disease manifestations may be under-recognized.

Objective: To determine the relative impact of the symptoms most important to the CMT population's heath related quality-of-life (HRQOL).

Methods: CMT patients in the Inherited Neuropathies Consortium Rare Disease Clinical Research Network Contact Registry were surveyed. The survey was constructed from 20 themes and 214 symptoms previously identified as important in qualitative interviews with a 6-point Likert scale. Symptom impact was calculated as the prevalence multiplied by the relative importance of each symptom identified.

Results: With 405 respondents, patients identified hand-finger weakness, foot-ankle weakness, and activity limitations as the highest impact themes. Difficulty running was the highest impact symptoms.

Conclusion: There are multiple symptoms that impact CMT HRQOL. These issues must be carefully examined to develop a disease-specific patient-reported outcome measure that represents the most critical areas of CMT HRQOL.

3

COMPARISON OF THE QUANTITATIVE MYASTHENIA GRAVIS SCORE AND THE MYASTHENIA GRAVIS COMPOSITE SCALE THROUGH RASCH ANALYSIS

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Objectives: To compare two myasthenia gravis measures, the Quantitative Myasthenia Gravis Score (QMGS) and the Myasthenia Gravis Composite (MGC).

Methods: 251 patients were assessed. We studied overall fit to the Rasch model, comparing item fit, thresholds, differential item functioning (DIF), local dependency, and person separation index (PSI).

Results: Mean scores were 3.9 ± 4.3 for the MGC and 7.6 ± 4.6 for the QMGS. Neither scale fit the Rasch model ($X^2 p < 0.05$). The MGC had lower PSI (0.14) than the QMGS (0.7). There was evidence of local dependency in both scales, as well as DIF for ocular and generalized disease. Disordered thresholds were found in 6(60%) items of the MGC and in 4(31%) of the QMGS. Collapsing response options did not improve fit in either scale.

Conclusions: Neither scale fits the Rasch model, but the MGC has lower discrimination than the QMGS. New measures of body function impairment in myasthenia are needed.

4

USING QUALITATIVE METHODOLOGIES TO ENHANCE UNDERSTANDING OF NEUROMUSCULAR DISEASE: IMPLICATIONS FOR CLINICAL TRIALS

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Introduction: Qualitative research yields important patient-centered data, and has been used in OPMD, DMD, ALS and

DM1. Knowledge gained from qualitative studies informs clinical research questions, outcome measures and clinical care.

Objective: To briefly describe 3 qualitative methodologies, highlighting key findings from studies in individuals living with DM1.

Methods: Participants were enrolled in studies using: (1) Phenomenology to explore the experience of caring for someone with dysphagia (n=6), (2) Photovoice to understand the experience of living with DM1 (n=9), and (3) Grounded theory to ascertain motivations for clinic attendance and expectations for care. (n=5).

Results: Participants were highly engaged. Semi-structured interviews and focus groups were useful tools for conducting this innovative patient-centered research. Key findings will be highlighted that might inform clinical trial design.

Conclusion: Qualitative research informs needs, experiences and care expectations of individuals living with neuromuscular disease and could readily be used to create assessment tools or outcome measures for clinical trials.

5

SPINAL MUSCULAR ATROPHY: MEASURING UPPER LIMB, GROSS MOTOR, AND AMBULATORY FUNCTION

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Introduction: Functional outcome measures are included in SMA trials to evaluate clinical benefit. Combining measures of gross motor, ambulatory, and upper limb function may improve measurement sensitivity.

Methods: This was a cross-sectional study of 109 subjects age 3 to 49 years from 2 research networks.

Objective: To generate a composite score from 3 measures: Hammersmith Functional Motor Scale-Expanded, Upper Limb Module, and Six-Minute Walk Test using principal component analysis.

Results: Z-scores were calculated. The principal component analysis suggested a score that gives equal weight to the three Z-scores; this score accounted for 83% of the total variability in the three components. The composite score was especially helpful in discriminating among weaker Type 2 patients.

Conclusions: With clinical trials underway, improvement in our ability to evaluate combined groups of SMA Type 2 and 3 patients is timely. Future work is needed to evaluate the sensitivity of the composite score to change.

6

VALIDATION OF DISEASE-SPECIFIC PATIENT REPORTED OUTCOMES WITH A HOME IVIG CLINICAL MANAGEMENT RECORD (CMR) DATABASE

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Introduction: IVIG use in the US is increasing at a rate of 8–10% per year; utilization in patients with neurological conditions comprises 25% of total use. As the cost burden of IVIG increases, communication of patient outcomes between physicians and homecare can aid in management.

Objectives: Validation of homecare patient reported outcomes (PROs).

Methods: We developed a CMR platform that includes adverse event management and standard PROs for approximately 4000 IVIG patients, of which 1100 have neuromuscular disorders. In order to validate these PROs in the home infusion environment we will compare our results with duplicate PROs and objective measures obtained in physician's offices.

Results: Statistical analysis of correlation between the two sets of PROs and objective measures will determine the reliability of homecare PROs.

Conclusions: Analysis of results will define whether PROs are subject to reporting differences and clarify the value of homecare PROs for the treating clinician.

7

RASCH ANALYSIS OF THE IBMFRS

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Introduction: The Inclusion Body Myositis Functional Rating Scale (IBM-FRS) is an ordinal scale for IBM. The Rasch method is a modern psychometric approach, which transforms an ordinal score into a linear, interval-level variable.

Objectives: To apply Rasch analysis to the IBM-FRS to allow transformation of IBM-FRS scores to a linear variable to increase utility as an outcome measure in clinical trials.

Methods: IBMFRS scores were collated from sites in the UK and USA. 127 observations were analysed using RUMM2030 software.

Results: The scale demonstrated good fit and reliability of items. Participants were of higher ability than the difficulty level of the scale. Four items showed disordered thresholds that were resolved by grouping categories. The swallowing item was unrelated to the construct and was under-discriminating.

Conclusion: Transformation to an interval scale is possible. Continued inclusion of the swallowing item merits discussion as it does not fit the construct of the overall scale.

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ACTIVITY RATING SCALES IN ADULT MUSCLE DISEASE: WHAT DO THEY ACTUALLY MEASURE?

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Introduction: There is a diversity of rating scales assessing function in muscle disease. Definitive knowledge of the content covered by these scales would help in making choices.

Methods: We searched for activity rating scales used for muscle disease and assessed their content by linking scale items to the International Classification of Functioning, Disability and Health (ICF) and the muscle regions they cover.

Results: Of the 119 scales found, 19 muscle-disease specific and nine generic scales were prioritised for analysis. These 28 scales contained 457 items from which 1145 concepts were identified with160 of these being unique. 97.8% concepts could be linked to the ICF; most to the activities and participation domain (68.7%) followed by environmental factors (22.5%) and body functions (6.6%). Global muscle function was most frequently assessed, followed by lower and upper extremity function.

Conclusions: This content comparison should allow better-informed choice of activity rating scales for muscle disease.

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ACTIVITY RATING SCALES IN ADULT MUSCLE DISEASE: HOW WELL DO THEY ACTUALLY MEASURE?

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Introduction: In a previous study we reported on the content of several activity rating scales that have been used for muscle disease. In a further aid to achieving consensus we have conducted a systematic review to assess the quality of the 19 activity rating scales specifically designed for muscle disease.

Methods: We analysed the measurement properties and the feasibility of the 19 instruments. Several databases were searched for studies relating to the quality of the instruments under review. Two independent reviewers selected studies and then assessed instrument quality using pre-agreed criteria based on published frameworks.

Results: We found none of the 19 instruments have sufficiently comprehensive reporting of measurement or feasibility performance as would be required by the regulatory authorities.

Discussion: Further work is urgently required to address these deficiencies of reporting or acquiring additional data. Until then there is a major barrier to translational research to be overcome.

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SUB-REGIONAL DUAL-ENERGY X-RAY ABSORPTIOMETRY (DEXA) ANALYSIS: A POTENTIAL ENDPOINT MEASURE FOR THERAPEUTIC TRIALS IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Introduction: Progressive distal myopathy is common in DM1, but correlation of distal leg lean tissue mass (LTM) with a six-minute walk test (6MWT) has not yet been evaluated.

Objective: To determine whether distal leg LTM correlates with a 6MWT in DM1.

Methods: We performed standardized sub-regional DEXA analysis of LTM in both legs dividing each leg into thigh, distal leg, and foot segments in 70 DM1 patients at baseline and 12-month visits. LTM was correlated with a 6MWT and with quantitative strength (QMT) of dorsiflexion.

Results: Distal leg LTM significantly correlated with the 6MWT ($r = 0.61$) and with dorsiflexors QMT ($r = 0.52$) at baseline visit. There was no significant change of LTM between baseline and follow-up visits. The LTM of distal leg segments provided the strongest correlation with dorsiflexors QMT and 6MWT compared to other leg segments.

Conclusion: Sub-regional DEXA analysis is a simple, cost-effective, and time-efficient method that may be used as an appropriate endpoint for studies of disease progression and therapeutic efficacy in DM1.

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B – CLINICAL MANAGEMENT

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EVALUATION OF DIAGNOSTIC CRITERIA FOR INCLUSION BODY MYOSITIS

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Introduction: Inclusion body myositis (IBM) is an adult-onset, slowly progressive muscle disease of unknown cause without effective treatment. Many different diagnostic criteria have been proposed for IBM, however, their sensitivities and specificities have not been reported.

Objectives: Determine optimal IBM diagnostic criteria for clinical trials.

Methods: We retrospectively reviewed records of ~200 patients diagnosed as having IBM and 150 patients diagnosed with other muscle diseases, including those that may have overlapping features with IBM such as hereditary inclusion body myopathies, vacuolar myopathies, muscular dystrophies, and other forms of myositis.

Results: We applied 9 different sets of IBM diagnostic criteria comprising 24 different categories to our patients. We find that while the proposed criteria all have very high specificity, they differ markedly in their sensitivity.

Conclusions: Several proposed IBM diagnostic criteria exclude a majority of IBM patients and are not optimal for clinical trials.

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BONE-MUSCLE INTERACTION IN BOYS WITH DUCHENNE MUSCULAR DYSTROPHY

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Introduction: DMD (Duchenne dystrophy) boys are prone to bone fractures.

Objective: To assess areal and volumetric bone mineral density in DMD boys.

Methods: 93 DMD boys and their controls (ages 5–30) received DXA (dual energy x-ray absorptiometry) of the whole body and areas of interest and peripheral quantitative computerized tomography (pQCT) of the distal radius and tibia.

Results: Steroid treated DMD boys do not go through puberty and have delayed skeletal maturation. Total body bone mineral content (TBBMC) and lean body mass (LBM) in the controls ranged between 655–3943 and 14892–73314 g respectively; for DMD boys it was between 459–2067 and 13060–39347 g. However, the ratio of TBBMC/LBM was the same: 0.047 ± 0.009 in DMD and 0.049 ± 0.005 in controls ($p = 0.09$).

Conclusion: DMD boys have comparable mass per volume ratio in the shafts of long bones as compared to controls. Bones remain smaller and narrower and predispose the boys to fragility fractures.

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RESTRICTIVE LUNG INVOLVEMENT IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

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Introduction: Few prospective studies have collected pulmonary functional measures in patients with genetically confirmed fascioscapulohumerol muscular dystrophy (FSHD). Estimates for respiratory involvement have varied from 0–50%.

Objective: To evaluate the frequency and predisposing factors for respiratory involvement in FSHD patients.

Methods: We performed a prospective cross-sectional observational study of 70 participants with genetically confirmed FSHD1 (61 participants) or FSHD2 (9 participants). Participants underwent bedside pulmonary function tests, a standard clinical history and physical assessment, and formal strength testing.

Results: 61 participants completed pulmonary testing. Although, mean forced vital capacity for the population was normal, restrictive respiratory involvement was seen in 9.8% (95% CI 2.4, 17.3). These participants were more severely affected ($P = 0.005$), had weaker hip flexion ($P = 0.0007$), and were more likely to use a wheelchair ($P = 0.02$). Of the total cohort, one participant required non-invasive nocturnal mechanical ventilation (1.4% CI 0, 4.2).

Conclusion: Restrictive respiratory involvement should be considered in FSHD patients who are more severely affected, or with pelvic girdle weakness.

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EXCESSIVE SLEEPINESS IN PATIENTS WITH MYASTHENIA GRAVIS

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Introduction: Previous studies reported sleep abnormalities in patients with myasthenia gravis (MG) but few examined sleepiness in MG.

Objectives: To determine the quality of sleep disturbances in patients with MG.

Methods: Eight patients with mild to moderate MG and no known sleep disorders participated. Patients underwent maintenance of wakefulness, overnight polysomnogram and multiple sleep latency tests, and completed fatigue questionnaires.

Results: Seven patients were female, mean age of 55 ($\pm$20), average BMI of 27 ($\pm$5), and one was on prednisone. All patients were diagnosed with mild to moderate obstructive sleep apnea (OSA) but not periodic limb movement or REM sleep behaviour disorder. All patients demonstrated increased sleep tendency. Epworth scores were abnormal in three of the patients. Five patients had fatigue visual analogue score $\geq$50 (out of 100) and modified fatigue impact scale $\geq$42 (out of 84).

Conclusions: Patients with MG demonstrate evidence of excessive sleepiness, likely due to OSA.

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NERVE EXCITABILITY TESTING CAN BE DIAGNOSTIC IN PATIENTS WITH EPISODIC ATAXIA TYPE I WITHOUT KCNA1 GENE MUTATION

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Introduction: Standard nerve conduction studies, needle electromyography and muscle histology are frequently unrevealing in patients with channelopathies.

Objectives: Case Report

Methods: This case report describes a young man whose standard neurophysiologic studies were non-diagnostic at 11 months of age. Neonatally, patient demonstrated hypotonia and fine muscle twitching in his chin. Mild global delays, tight heel cords and walking at 4 yo followed. At 11 yo, examination demonstrated a rigid posture, dysfluency of speech and myokymic movements in face and hand muscles. Carbamazepine reduced stiffness of posture and stuttering. Episodes of imbalance and dysarthria lasting 30 seconds to several minutes began at 20 yo.

Results: Nerve conduction studies were normal without repetitive responses to single stimuli. Needle electromyography demonstrated myokymia and neuromyotonia with unremarkable motor unit potentials. Paraneoplastic antibody panel was normal. Perlecan and KCNA1 gene sequencing were normal.

Conclusions: Nerve excitability testing demonstrates increased superexcitability as described in KCNA1 mutation positive patients with EA I.[1]

[1]Tomlinson SE, Tan SV, Kullmann DM, Griggs RC, Burke D, Hanna MG, Bostock H. 2010 Nerve excitability studies characterize Kv1.1 fast potassium channel dysfunction in patients with episodic ataxia type 1. Brain 133(Pt 12): 3530-40.

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PERIPHERAL NEUROPATHY IN PATIENTS WITH PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA DUE TO MITOCHONDRIAL DISEASE

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Introduction: Progressive external ophthalmoplegia (PEO) is a genetically heterogeneous mitochondrial myopathy characterized by slowly progressive ptosis and reduced extraocular movements. Patients with PEO may also develop additional neurological or systemic features.

Objective: To evaluate the frequency and characteristics of peripheral neuropathy in patients with PEO.

Methods: Retrospective study of all patients with PEO and a confirmed genetic defect of either mitochondrial DNA (mtDNA) or nuclear DNA assessed at the National Hospital for Neurology and Neurosurgery.

Results/Conclusion: 116 individuals with PEO were included in the study. 90 patients had a primary mtDNA defect: 78 with a single deletion and 12 with a point mutation. 18 patients had pathogenic variants in *POLG*, *C10orf2* or *RRM2B*, and 8 had multiple deletions of mtDNA in muscle but unknown nuclear DNA defect. 77 patients had neurophysiology studies performed in the lower $\pm$ upper limbs. Of them, 16 (21%) had a sensory or sensorimotor peripheral neuropathy.

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MYOTONIC DYSTROPHY TYPE 2 AND SODIUM CHANNEL GENE MUTATION: A CASE WITH EARLY AND SEVERE MYOTONIA

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Introduction: In myotonic dystrophy type 2 (DM2), an association has been reported between early and severe myotonia and recessive chloride channel (CLCN1) mutation. No DM2 cases have been described with sodium channel gene (SCN4A) mutation.

Case Report: We describe a patient with DM2 and a suspected pathogenic variant in SCN4A gene.

Methods: This 26 year old patient complained of hand cramps and difficulty relaxing her hands after activity since she was 20 years old. Electromyography and genetic analysis for DM1, DM2, CLCN1 and SCN4A gene have been performed.

Results: Neurological examination showed thenar percussion myotonia and mild distal weakness. EMG showed myotonic discharges in all muscles examined. Genetic testing was positive for DM2 and for a variant c.215C>T (p.Pro72Leu) in SCN4A gene. Both SIFT and PolyPhen-2.2.2. predicted that this variant is pathogenetic.

Conclusions: This case suggests that modifying factors should be investigated in DM2 patients with early and severe myotonia.

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A CLINICAL STUDY OF THE PERCEPTION AND PRIORITIZATION OF COGNITIVE SYMPTOMS IN MYOTONIC DYSTROPHY TYPE-1 (DM1)

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Introduction: Cognitive symptoms are frequently reported by DM1 patients.

Objectives: To systematically identify the cognitive symptoms most prevalent and important in DM1.

Methods: We performed a large-scale survey initiative to identify the prevalence and relative importance of cognitive symptoms in DM1. We analyzed the relationships between specific cognitive symptoms and age, gender, education, employment status, and CTG repeat length.

Results: 278 DM1 patients participated. Decreased motivation was the cognitive symptom that had the greatest impact on the DM1 population. Male participants were more frequently and severely affected by cognitive issues than female participants. Participants with fewer cognitive symptoms obtained higher levels of education and were more frequently employed.

Conclusions: Cognitive symptoms are highly prevalent and generate significant disease burden in DM1.

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USE OF RITUXAN IN NEUROLOGICAL DISEASE AND OUTCOMES IN KEY DISEASE GROUPS

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Introduction: This presentation outlines patients with myasthenia gravis, dermatomyositis, neuromyelitis optica, NMDA, encephalitis, vasculitis, and CIDPN who were treated with Rituxan. These diseases are considered treatable and even have the potential to go into remission. However, many patients only improve with multiple therapies – such as steroids, azathioprine, IVIg and plasmapheresis – on a chronic basis. Rituxan is being increasingly used in order to improve the clinical status in patients who either do not respond or respond but remain dependent on these therapies long term.

Objective: This study is a description of the cases that received Rituxan and the response and lack of response as measured by clinical symptoms and dependence upon other therapies. The outcome measures include improvement to a minimally affected state and withdrawal of other therapies as an indicator of response to therapy and potential remission.

Methods: Forty patients received Rituxan after being either intolerant or poorly responsive to traditional therapies.

Results: Patients with inflammatory myopathies responded significantly to Rituxan, allowing a decrease in other therapies. Patients with myasthenia gravis demonstrated dramatic improvement in clinical symptoms and were able to reach minimal clinical symptomatology and complete remission combined with withdrawal of other therapies, particularly IVIg and plasmapheresis. Little to no response was seen in patients with CIDPN; all patients with this disorder continued previous therapy.

Conclusion: Rituxan can be safely recommended for treatment of inflammatories and myasthenia gravis, however, it is not recommended for the treatment of CIDPN based on our local experience.

Keywords: Rituxan; Inflammatory myopathies; Neuromuscular diseases; CIDPN; traditional therapies

C – MUSCLE BIOPSY

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THE IDENTITY AND DISTRIBUTION OF INFLAMMATORY CELLS IN MUSCLE BIOPSIES FROM PATIENTS WITH ANTI-HMG-COA REDUCTASE (HMGCR)-ASSOCIATED MYOPATHY

T. Chung and A. Mammen (Baltimore, MD)

Introduction: Anti-HMGCR-associated myopathy (HAM) biopsies reveal prominent myofiber necrosis; inflammatory cells are scarce on H&E staining.

Objective: To characterize the composition and distribution of immune cells in HAM.

Methods: 18 HAM and 7 dermatomyositis muscle biopsies were stained for CD3, CD4, CD8, CD20, and CD68.

Results: In HAM, endomysial CD68 positive macrophages were abundant. Scattered endomysial CD4 and CD8 positive cells were noted in half the cases; these never surrounded normal muscle fibers as in polymyositis. CD20 positive cells were rare in HAM. In contrast to dermatomyositis biopsies, which had significant accumulations of inflammatory cells in perivascular and perimysial regions, these areas were devoid of inflammatory cells in HAM.

Conclusions: In HAM, scattered CD4 and CD8 positive T cells are frequently found within the endomysium. HAM can be distinguished from polymyositis (based on the absence of primary inflammation) and from dermatomyositis (based on the paucity of perivascular and perimysial infiltrates).

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THE CONFOUNDING EFFECT OF AGE IN THE USE OF SUBSARCOLEMMAL MITOCHONDRIAL AGGREGATES (SSMA) AS A DIAGNOSTIC MUSCLE BIOPSY MARKER IN PAEDIATRIC MITOCHONDRIAL DISEASE

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Introduction: Subsarcolemmal mitochondrial aggregates (SSMA) have been proposed as a diagnostic marker for mitochondrial respiratory chain defects in children less than 16 years.

Objective: To examine the %SSMA in muscle biopsies of patients with mitochondrial disease (MD) and histologically normal/minimal change controls (CTRL), in relation to the biopsy age and tube feeding.

Methods: Retrospective analysis of diagnostic muscle biopsies (21 MD and 33 CTRL) performed at the Dubowitz Neuromuscular Centre. Biopsies were assessed semi-quantitatively and with novel image analysis technique (Definiens).

Results: Percentage of SSMA did not differ in terms of age, gender and tube feeding across groups. Significant differences were observed in MD versus CTRL for SSMA (10.9%, versus 21.3%) and lipid content (29% vs. 6%). %SSMA increased by 1.6%/year and older age at biopsy was associated with higher %SSMA.

Conclusion: Age-dependent prevalence of SSMA may limit using absolute SSMA cut-off value as a diagnostic marker of MD in paediatric muscle biopsies.

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A PRELIMINARY SCORE TOOL FOR THE EVALUATION OF MUSCLE BIOPSIES IN PATIENTS WITH INCLUSION BODY MYOSITIS - APPLICATION IN A RANDOMISED PLACEBO-CONTROLLED CLINICAL TRIAL

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Introduction: There is currently no quantitative score tool for assessing the pathological changes observed in inclusion body myositis (IBM).

Objectives: Our aim was to develop a score tool to quantify the pathological changes in muscle biopsies from patients with IBM and to apply it in a placebo-controlled trial.

Methods: An evidence-based and consensus approach was used to develop the score tool.

Results: The score tool assessed a number of pathological features considered relevant in the assessment of IBM including rimmed vacuoles, mitochondrial changes and protein accumulation. Inflammatory features such as MHC class I up-regulation and CD3, CD8, CD4, CD20 and CD68 positive cells were assessed using a modified version of the juvenile dermatomyositis score tool.(1)

Conclusions: A preliminary scoring system for muscle biopsy evaluation in patients with inclusion body myositis was developed. This new tool was applied in a placebo-controlled trial of patients with IBM.

1. Wedderburn LR et al. Arthritis Rheum 2007:57;1192–201.

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THEORETICAL SUPPORT FOR THE INFLUENCE OF MUSCLE FIBRE DIAMETER ON THE SEVERITY OF MYOTONIA CONGENITA

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Introduction: Symptom onset in the chloride channel disease, Myotonia Congenita, occurs during myofibre growth (infancy or childhood), severity is variable, and males tend to be worse affected than females.

Objectives: To calculate whether it is possible for differences in myofibre diameter *within the physiological range* to affect the propensity to myotonia.

Methods: Calculations were in Matlab with Adrian & Marshall's model of skeletal muscle.

Results: Asynchrony between surface and tubular action potentials in the model was greater at a diameter of 100 μm than 70 μm (1.7 ms and 1.1 ms respectively). The more pronounced tubule-to-surface asynchrony in the 100 μm fibre drove sustained myotonic discharges for reductions of chloride conductance that were insufficient to produce myotonia in the 70 μm diameter fibre.

Conclusions: Muscle fibre diameter may contribute to phenotypic variability in Myotonia Congenita, and muscle hypertrophy may be maladaptive, contributing to electrical instability.

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SLOW PROGRESSION OF MUSCLE IMPAIRMENT AND SPLICEOPATHY IN MYOTONIC DYSTROPHY TYPE 2 (DM2)

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Introduction: In DM2 mutant transcripts are retained in cell nuclei as ribonuclear inclusions (RIs), which alter the activity of RNA-binding proteins such as MBNL1 and CUGBP1 leading to misregulated splicing events.

Objectives: We performed a study of the progression of muscle histopathology and biomolecular findings in 5 DM2 patients over time.

Methods: We evaluate by FISH in combination with MBNL1-immunofluorescence Ris, MBNL1 foci, CUGBP1 protein expression by WB in the two biopsies and the alternative splicing of ClC1, SERCA1 and MBNL1 gene by RT-PCR splicing assay during the disease progression.

Results: Muscle histopathology shows a worsening of both type 1 and 2 fiber atrophy. Alternative splicing of all isoforms is altered and the percentage of pathological isoforms is similar or slightly increased.

Conclusion: The increase of CUGBP1 expression over time correlate significantly to worsening of muscle histopathological pattern but slightly to spliceopathy of all isoforms.

D – MODEL OF CARE

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A NOVEL METHOD FOR IDENTIFYING MYASTHENIA GRAVIS IN ADMINISTRATIVE HEALTH DATA BY LINKING TO PRIMARY CARE ELECTRONIC MEDICAL RECORDS

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Introduction: Accurate epidemiologic data is lacking in myasthenia gravis (MG). Administrative health databases may be a useful source of epidemiologic data for health care surveillance and research, and planning of health services.

Objective: To validate an algorithm for identification of patients with MG within administrative health records in the province of Ontario, Canada (population 13 million).

Methods: Potential MG patients were identified through text string searching of de-identified primary care electronic patient records. Manual chart abstraction revealed definite cases as per physician reporting. Linking to provincial administrative databases revealed the optimal algorithm for defining MG cases.

Results: Text-string searching identified 669 possible cases of MG within 128,353 electronic medical records. Of these, 55 definite cases were used to calculate the optimal algorithm.

Conclusion: We present an algorithm for accurately identifying patients with myasthenia gravis within administrative health care databases.

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CARE AND CURE FOR PATIENTS WITH NEUROMUSCULAR DISORDERS: AN OMNICOMPREHENSIVE MODEL

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Introduction: Generally patients with neuromuscular disorders are seen in the out-patient setting by Neurologists. If symptoms and signs of multiple organ involvement are present, patients are asked to see a consultant in the required field.

Objectives: To present our ideal model of care and cure for patients with neuromuscular disorders based on the experience at Omnicomprehensive NEuroMuscular (NEMO) Center in Milan, Italy.

Methods: We retrospectively analyzed clinical charts of patients with neuromuscular disorders attending the NEMO Center from December 2012 to May 2013. Limitations and advantages of this omnicomprehensive approach are discussed.

Results: 150 patients (8% < 18 years of age) with neuromuscular disorders were admitted as in-patients (ALS: 54%; muscular dystrophies, congenital myopathies, myasthenia: 13%; SMA: 7%; neuropathies: 26%); 100 new patients were admitted in the DH service and 300 as out-patients.

Conclusions: An omnicomprehensive model of care and cure for neuromuscular patients is advantageous.

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THE ITALIAN ASSOCIATION OF MYOLOGY (AIM): PROPOSALS FROM A CONSOLIDATED NATIONAL NETWORK FOR THE RESEARCH ON NEUROMUSCULAR DISORDERS

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Introduction: The Italian Association of Myology (AIM) is a multidisciplinary Scientific Society, founded in 2000. At present, it counts about 150 active members from all over the Country (adult and child neurologists, cardiologists, geneticians, basic researchers, and others), belonging to 27 Centers, twelve of them performing advanced clinical and laboratory investigations.

Objectives: Implementation of collaborative clinical research studies, with multidisciplinary teams and close exchange with basic researchers; educational programs and Registry implementations.

Results: A number of Study Groups are active on clinical research projects on various disorders (the AIM-AIG Italian Group for glycogenoses type 2, the LGMD Network, the Mitocondrial Database, the FSHD Italian Study Group, the Network for laminopathies and for congenital myopathies) with the support of Italian Telethon Foundation or other Governative Institutions and Patients Associations. This allowed the recruitment of thousands of patients, significant scientific publications and improvement of our standards of care. Single Centers are currently involved in international research activities, such as the DMD therapeutical trials. New focused Groups are on preparation (congenital myasthenias, lipid storage diseases).

Conclusion: International cooperation extended to the entire network is a major objective of AIM community

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FONDAZIONE TELETHON'S STRATEGY TO BUILD UP AND SUPPORT THE ITALIAN NEUROMUSCULAR CLINICAL PLATFORM

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Introduction and objectives: To promote quality of life studies for today's NMD patients and to ensure trial readiness, in 2002 Fondazione Telethon launched a new initiative dedicated to clinical studies on neuromuscular diseases (NMD).

Methods: To improve Investigators' performance, several actions were put in place: i) methodological support before submitting the application; ii) coordinator support to manage ongoing multicenter projects; iii) periodic analysis and corrective actions to prevent or counteract failure.

Results: After 10 years, 7.5 M€ investment and 44 projects approved (33 multicenter), major results are: 1) over 5,000 patients involved in clinical studies; 2) active clinical networks on myology, peripheral neuropathy and cardiomyology; 3) research registries and genetic/clinical characterization of large patient cohorts; 4) 155 peer-reviewed publications; 5) participation of centers in international clinical trials on DMD and SMA.

Conclusions: This investment proved to be very effective. New strategic plans are underway to consolidate this clinical platform and promote its participation in international studies as the Italian NMD consortium.

E – PATHOGENESIS

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POTENTIAL MECHANISMS IN MuSK-MYASTHENIA GRAVIS

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Introduction: Antibodies to MuSK cause MuSK-MG hallmarked by neuromuscular transmission defects and muscle weakness. MuSK, a receptor tyrosine kinase, orchestrates postsynaptic development and maintenance together with direct binding partner LRP4. Agrin, released by the motoneuron, binds to LRP4 at the postsynaptic membrane, which activates MuSK, leading to AChR clustering by downstream signaling. Antibodies to MuSK interfere with this signaling, but the pathogenic mechanisms are unknown.

Objectives: We hypothesized that the antibody blocks binding between MuSK and LRP4.

Methods: We studied plasmas from 14 patients, investigated their IgG subclass profile by flow cytometry, purified IgG, IgG4 and IgG1–3, and produced Fab fragments and assessed their impact on AChR clustering in C2C12 myotubes and MuSK-LRP4 interaction in a co-immunoprecipitation experiment.

Results: We found that patient derived IgG and IgG4 both blocked the interaction between MuSK and LRP4 and reduced agrin-induced AChR clusters. Moreover, monovalent Fab fragments were sufficient to block binding between MuSK and LRP4 and reducing agrin-induced AChR clustering.

Conclusion: The experiments suggests that IgG4 blocks binding between MuSK and LRP4 and impairs agrin-mediated AChR clustering, and divalent binding is not required for these actions.

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COLLABORATIVE DATA MINING FOR NOVEL CMT GENES IN GENOMES MANAGEMENT APPLICATION (GEM.APP)

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Introduction: The axonal forms of CMT show impressive genetic locus heterogeneity; yet, less than 30% of such cases receive a diagnosis from known genes.

Objective: Important to further gene identification are techniques such as whole exome sequencing. There is a need for novel tools that enable physicians and molecular geneticists to analyze such data and allow for secure data sharing.

Methods: We have designed GEM.app, a web-based tool that allows for seamless analysis and integration of multiple exome/genome datasets across the world.

Results: GEM.app contains >2,500 exomes from over 150 users in 20 different countries. It represents a fully functional analysis suite for exome data. More than a dozen novel genes have been identified in GEM.app by us and collaborators in the past 12 months. Examples include CMT genes *MARS* and *BICD2*.

Conclusions: This platform represents a new research resource for neuromuscular diseases, significantly simplifies the interpretation of exomes and the identification of novel CMT genes.

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DYSTOPHINOPATHIES

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Introduction: Clinical trials are rapidly moving forward in Duchenne Muscular Dystrophy (DMD). Their outcome is influenced by the DMD clinical variability. This has implications for tailored therapeutic approaches, targeting a subset of DMD patients, as randomised placebo controlled studies not always feasible.

Objectives: Patient selection and what to measure is proving fundamental. The antisense oligonucleotide approaches which restore a "Becker like" molecule in DMD patients have prompted us to reassess BMD patients with relevant deletions to assess properties of internally deleted proteins.

Methods: Dystrophin protein and RNA quantitation using western blot, quantitative immunohistrochemistry and real time PCR.

Results: BMD patients with mutations in different exons of the 3' dystrophin deletion hot spot vary in terms of the amount of protein produced, and this is associated with different clinical outcomes. RNA levels in is indistinguishable from normal.

Conclusions: The implications of these findings for DMD patients undergoing clinical trials with antisense oligonucleotides will be presented.

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GFPT1 MUTATIONS THAT UNDERLIE CONGENITAL MYASTHENIC SYNDROME REDUCE ACHR EXPRESSION

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Introduction: Mutations in glutamine:fructose-6-phosphate transaminase 1 (GFPT1) underlie a congenital myasthenic syndrome (CMS) characterised by limb-girdle pattern of muscle weakness.

Objectives: To investigate why the mutations cause a syndrome with symptoms largely restricted to neuromuscular transmission.

Methods: Acetylcholine receptor (AChR) expression levels were determined by α-bungarotoxin binding and western blots in *GFPT1*-mutated human skeletal myotubes and in a muscle cell line (TE671 DB40) with *GFPT1* expression silenced by siRNA.

Results: Cultured myotubes from *GFPT1* patients (Pt1 and Pt2) and TE671 DB40 cells with *GFPT1* expression 'knocked down' showed a significant reduction in cell surface AChR expression (Pt1 $p<0.0001$; Pt2 $p=0.0097$; TE671 DB40 $p<0.0001$). This decrease appeared to result from reduced steady-state levels of AChR α, δ, and ε subunits, but not the β subunit. AChR mRNA levels were unaffected.

Conclusion: Decreased levels of AChR at the motor endplate are likely to be a principle disease mechanism in *GFPT1* CMS.

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NDUFA4 MUTATIONS CAUSE MITOCHONDRIAL CYTOCHROME *C* OXIDASE DEFICIENCY LINKED TO HUMAN NEUROLOGICAL DISEASE

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Introduction: Cytochrome *c* oxidase (COX) deficiency is one of the most common respiratory chain defects seen in human mitochondrial disease, but the molecular basis remains undetermined in many cases.

Objective: To identify the molecular basis of isolated COX deficiency in a large consanguineous pedigree in which four affected family members had a Leigh syndrome neurological phenotype.

Methods: A combined homozygosity mapping and whole exome sequencing approach was applied.

Results: Affected individuals harboured homozygous splice donor site mutations (c.42+1G>C) in *NDUFA4,* a gene previously assigned to encode a complex I subunit. Western blot analysis of denaturing gels and immunocytochemistry revealed undetectable steady-state NDUFA4 protein levels in patient muscle, whilst one and two dimensional blue-native polyacrylamide gels confirmed an NDUFA4/COX interaction in control muscle.

Conclusions: These observations indicate *NDUFA4* encodes a COX subunit essential for the enzyme's activity. We suggest *NDUFA4* genetic analysis is performed in all patients with unexplained COX deficiency.

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PATHOGENIC MECHANISMS OF *RAPSN* MUTATIONS IN CONGENITAL MYASTHENIC SYNDROMES

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Introduction: *RAPSN* mutations contribute towards a major subtype of congenital myasthenic syndromes. Rapsyn interacts directly with acetylcholine receptors (AChR) at the postsynaptic membrane and is essential for the formation of AChR clusters. Mutations in *RAPSN* may disrupt rapsyn function through different mechanisms and cause AChR deficiencies at patient endplates.

Objectives: Our aim is to investigate the pathogenic properties of newly identified *RAPSN* mutations in patients, together with the common mutation p.N88K.

Methods: Human full-length wild-type rapsyn cDNA was cloned into expression vectors (pEGFP-N1, pBabe-PURO). Mutations identified in patients were introduced by mutagenesis. AChR clustering assay was performed by infecting these *RAPSN* variants into *RAPSN*$^{-/-}$ myoblasts; and the expression levels and the stability of the protein were determined in TE671.

Results and conclusions: When compared to wild-type rapsyn, each mutation investigated ultimately impairs cluster formation and is thus likely to be pathogenic.

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CYTOSOLIC 5'-NUCLEOTIDASE 1A AUTOIMMUNITY IN SPORADIC INCLUSION BODY MYOSITIS

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Introduction: We previously identified a circulating autoantibody against a 43 kDa muscle autoantigen (anti-IBM43) in

sporadic inclusion body myositis (IBM) and demonstrated its utility in an IBM diagnostic blood test.

Objective: Here we sought to identify the molecular target of anti-IBM43, link IBM autoimmunity and myodegeneration, and describe a high-accuracy IBM blood test.

Methods: Blood and muscle samples were studied using mass spectrometry, a synthetic human peptidome array, immunohistochemistry, and immunoblotting.

Results: Cytosolic 5'-nucleotidase 1A (cN1A; NT5C1A) is the previously identified 43 kDa IBM autoantigen. Detection of blood anti-cN1A autoantibodies was 70% sensitive and 92% specific for the diagnosis of IBM. cN1A accumulated in rimmed vacuoles in IBM muscle, localizing to areas of myonuclear degeneration.

Interpretation: Circulating autoantibodies against cN1A are common in and highly specific to IBM among muscle diseases. They provide a link between IBM's dual processes of autoimmunity and myodegeneration, and allow for blood diagnostic testing.

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CHARACTERIZATION OF CD4 AND CD8 T-CELL RESPONSES IN MuSK MYASTHENIA GRAVIS

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Introduction: T-cell functionality has not been reported in muscle specific tyrosine kinase myasthenia gravis (MMG).

Objectives: To characterize T-cell responses in MMG.

Methods: Following stimulation with CD3/CD28 and PMA/ION, intracellular cytokine production by CD4+ and CD8+ T-cells were measured in blood samples from 11 MMG patients and 4 healthy controls. All MMG patients were receiving immunosuppressive therapy. Functional regulatory T-cells were identified by the expression of CD39 and the markers CD4+CD25+FOXP3+.

Results: CD8+ T-cells from MMG patients produced higher levels of IL-2, whereas CD4+ T-cells had higher IL-2, TNF-alpha, and IL-17. MMG patients had a higher percentage of CD4+ T-cells producing combinations of IFN-gamma/IL-2/TNF-alpha, TNF-alpha/IL-2, and IFN-gamma/TNF-alpha. Regulatory T-cell numbers and function were not different from control values.

Conclusions: MMG patients had increased levels of Th1 and Th17 cytokines and were primed for polyfunctional proinflammatory responses. Further studies are necessary to determine the effect of immunosuppressive medications on these responses.

F – CLINICAL TRIALS

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RESULTS OF REVERSE-DBMD: REVATIO FOR HEART DISEASE IN DUCHENNE AND BECKER MUSCULAR DYSTROPHY

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Introduction: Duchenne muscular dystrophy (DMD) and the milder allelic disorder, Becker muscular dystrophy (BMD) are due to mutations in the gene for dystrophin. In DMD, BMD and other muscular dystrophies, there is an urgent need for efficacious and safe treatments that slow disease progression in both skeletal and cardiac muscle. In several of these disorders, including the dystrophinopathies, there is a loss of neuronal nitric oxide synthase (nNOS) from the membrane. nNOS synthesizes nitric oxide (NO) which in turn stimulates cGMP production by soluble guanylyl cyclase (sGC). The NO-cGMP signaling pathway regulates smooth muscle relaxation, cardiac and skeletal muscle contractile function and the immune response in muscle. Increasing data suggests that decreased NO-cGMP signaling in dystrophic muscle is pathogenic.

Objective: We studied the phosphodiesterase inhibitor, Revatio (Sildenafil) in adults with DMD or BMD and cardiac dysfunction.

Methods: In the placebo controlled, blinded phase of the study, subjects received 20 mg of Revatio three times a day versus placebo for 6 months. This was followed by open-label Revatio for an additional 6 months. The primary endpoint of the trial was left ventricular end systolic volume (LVESV) by cardiac MRI. Multiple secondary endpoints included additional cardiac parameters, pincher and grip strength, forced vital capacity, brachial arterial flow mediated dilation, and quality of life (SF36 and InQol). Sample size calculations suggested 30 subjects should be enrolled to detect a 10% difference in primary endpoint.

Results: A midpoint analysis of the trial included unblinding the DSMB to the LVESV data. The DSMB recommended closures of the REVERSE-DMBD trial after the midpoint analysis. The decision was based on clear lack of benefit and a nonstatistically significant increase in LVESV in the Revatio treated arm. Analysis of secondary endpoints is currently ongoing and will be presented. This trial was funded by Charley's Fund, Inc.

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TREATMENT OF SPORADIC INCLUSION BODY MYOSITIS (SIBM) WITH AN ANTI-ACTIVIN RECEPTOR II ANTIBODY

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Introduction: We found muscle SMAD2/3 phosphorylation elevated in sIBM, suggesting a role for activin type II receptor (ActRII) signaling.

Objectives: We conducted a randomized, placebo-controlled clinical trial of BYM338, a monoclonal antibody that blocks ActRII, in sIBM.

Methods: A single dose of BYM338 or placebo was administered to 14 randomized patients with sIBM. The primary outcome was the change in thigh muscle volume (TMV) by MRI at 8 weeks. Lean body mass (LBM), strength, and function were secondary outcomes. Twelve patients participated in a subsequent 16-week observation phase.

Results: BYM338-treated patients increased TMV (right leg + 6.5%, P=0.024; left leg +7.6%, P = 0.009) and LBM (+5.7%, P=0.014) at 8 weeks. BYM338-treated patients had improved 6 minute walking distance, which peaked at 16 weeks (+14.6%, P=0.008).

Conclusions: Inhibition of ActRII increased muscle mass and function in this pilot trial, offering a potential novel treatment of sIBM.