

# 13th International Conference on Myasthenia Gravis and Related Disorders

## SPEAKER ABSTRACTS

MAY 15 – 17, 2017

### Session 1: Neuromuscular Junction (NMJ) Structure and Function

**Session Chair:** Clarke R. Slater, PhD, Newcastle University, United Kingdom

8:55 AM      **Functional Organization of the Neuromuscular Junction: Introduction**

**Clarke R. Slater**, PhD

Newcastle University, Newcastle upon Tyne, United Kingdom

9:05 AM      **Presynaptic Plasticity in Myasthenia Gravis**

**Mark M. Rich**, MD, PhD and Xueyong Wang, MBBS, PhD

Wright State University, Fairborn, Ohio, United States

Using the mouse neuromuscular junction as a model system we found that block of acetylcholine receptors triggers a reversible upregulation of synaptic vesicle release that occurs and reverses within seconds of blocking or unblocking. The upregulation of release is due to a Ca<sup>2+</sup>-dependent increase in the size of the readily releasable pool (RRP). Blocking vesicle refilling prevented upregulation of quantal content (QC), while leaving baseline release relatively unaffected. This suggested that the upregulation of QC was due to mobilization of a distinct pool of vesicles that were rapidly recycled and thus were dependent on continued vesicle refilling. We term this pool the “homeostatic reserve pool.” A detailed analysis of the time course of vesicle release triggered by a presynaptic action potential suggests that the homeostatic reserve pool of vesicles is normally released more slowly than other vesicles, but the rate of their release becomes similar to that of the major pool during homeostatic upregulation of QC. Remarkably, instead of finding a generalized increase in the recruitment of vesicles into RRP, we identified a distinct homeostatic reserve pool of vesicles that appear to only participate in synchronized release following homeostatic upregulation of QC. Once this small pool of vesicles is depleted by the block of vesicle refilling, homeostatic upregulation of QC is no longer observed. This is the first identification of the population of vesicles responsible for the blockade-induced upregulation of release previously described.

9:25 AM

### **More Than a Pretzel: Spatial Distribution of the Neuromuscular Junction Components**

Marta Gawor, PhD, Paweł Niewiadomski, PhD, Patrycja Daszczuk, MSc, Krzysztof Bernadzki, MSc, and **Tomasz J. Prószyński**, PhD

Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

Mammalian neuromuscular junctions (NMJs) undergo a postnatal topological transformation from simple oval plaques to complex branch-shaped structures often referred to as “pretzels”. Although abnormalities in NMJ maturation and/or maintenance are frequently observed in neuromuscular disorders, the mechanisms that govern synaptic developmental remodeling remain poorly understood. It was reported that myotubes, when cultured aneurally on laminin-coated surfaces, form complex clusters of postsynaptic machinery, which resemble that at the NMJ. Interestingly, these assemblies undergo similar stages of developmental remodeling from “plaques” to “pretzels” to NMJ’s machinery *in vivo*. We have recently demonstrated that podosomes, actin-rich adhesive organelles, promote the remodeling process in cultured myotubes and identified a key role of one podosome component, Amotl2, a scaffold protein that regulates organization of the actin cytoskeleton and cellular signaling. Subsequent studies have demonstrated that Amotl2 and several other known podosome-associated proteins are present at the NMJ *in vivo* and are located to the sites of synaptic remodeling. Our experiments suggest that Amotl2 and its newly identified binding partner Homer1 may play important roles in the organization of the postsynaptic specialization at the neuromuscular junction.

This research was supported by grants 2012/05/E/NZ3/00487, 2013/09/B/NZ3/03524 and 2014/13/B/NZ3/00909 from the Polish National Science Centre (NCN).

9:45 AM

### **Molecular Mechanisms of the Formation and Maintenance of Neuromuscular Junction**

**Markus A. Ruegg**, PhD(1), Shuo Lin, PhD(1), Hans-Rudolf Brenner, PhD(2), Perrine Castets, PhD(1)

(1) Biozentrum, University of Basel, Basel, Switzerland

(2) Department of Biomedicine, University of Basel, Basel, Switzerland

The molecular mechanisms of neuromuscular junction (NMJ) formation are quite well understood. Of key importance is the extracellular matrix molecule agrin and its downstream signaling *via* the Lrp4-MuSK-Dok7 complex, which in turn triggers the organization of rapsyn-AChR complexes. Mutations in genes coding for these molecules cause congenital myasthenic syndrome, demonstrating the crucial role of these molecules in NMJ maintenance. Impairment of NMJ structure also correlates with the loss of muscle mass at high age, called sarcopenia. One of the important pathways that affects muscle mass and longevity includes the protein mammalian target of rapamycin (mTOR). While dampening of mTOR signaling slows-down ageing, sustained activation of mTOR results in a late-onset myopathy. In this presentation, we will report on our investigations of the role of mTOR in the maintenance of the NMJ. We find that sustained activation of mTOR results in the early de-stabilization of mouse NMJs and impairs neuromuscular transmission. In addition, muscle denervation in young mice causes the rapid precipitation of the myopathy. In summary, these data indicate that changes of mTOR signaling affect muscle mass, NMJ structure and function, suggesting that mTOR is involved in the development of sarcopenia.

10:05 AM **Is Age-related Neuromuscular Junction Fragmentation a Sign of Altered Neuromuscular Junction Function?**

Silvia Willadt, PhD(1), Mark Nash, DPhil(1), and **Clarke Slater**, PhD(2)

- (1) Novartis Pharma, Basel, Switzerland;  
(2) Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, United Kingdom

As animals and humans age, the motor nerve terminals at their neuromuscular junctions (NMJs) become increasingly branched. In parallel, the postsynaptic regions of high acetylcholine receptor (AChR) density associated with the nerve terminals become increasingly fragmented. Are these structural changes associated with impairment of neuromuscular transmission and, if so, might this contribute to age-related muscle wasting? Previous studies of age-related changes in NMJ function, including single fiber electromyography studies in humans, do not provide consistent evidence of a general decline in the efficacy of neuromuscular transmission. We have reinvestigated this issue by asking whether, at individual NMJs in mice of various ages, reduced efficacy of neuromuscular transmission is associated with increased NMJ fragmentation. We used labelling of AChRs with trace amounts of fluorescent  $\alpha$ -bungarotoxin to define NMJ structure, and two-electrode voltage clamping to define the function of the same NMJs in the diaphragms of mice from 3-28 months of age. In a sample of over 180 NMJs, although NMJ fragmentation increased continuously with age, there was no correlation between the number of fragments and the amplitude or quantal content of the evoked endplate currents. This suggests that NMJ fragmentation *per se* does not result in impaired neuromuscular transmission. It also suggests that NMJ fragmentation may often be a sign of a healthy adaptive response to aging, rather than of deterioration, of the NMJ.

## Session 2: Congenital Myasthenic Syndromes

**Session Chair:** Andrew G. Engel, MD, Mayo Clinic

10:55 AM **Presynaptic Congenital Myasthenic Syndromes Caused by Mutations in SNAP25B and MUNC13-1**

**Andrew G. Engel**, MD, Xin-Ming Shen, PhD, and Duygu Selcen, MD

Department of Neurology and Muscle Research Laboratory, Mayo Clinic, Rochester, Minnesota, United States

SNAP25B myasthenia. Ca<sup>2+</sup>-triggered exocytosis is initiated when synaptobrevin anchored to synaptic vesicles assembles with SNAP25B and syntaxin-1B anchored in the presynaptic membrane. An 11-year-old girl harboring a p.Ile67Asn variant in *SNAP25B* had severe limb-girdle weakness, joint contractures, ataxia, intellectual disability, and cortical hyperexcitability. Endplate (EP) ultrastructure and mutant SNAP25B expression were normal. EP studies showed reduced miniature endplate potential frequency and decreased quantal content (*m*) of the EP potential owing to decreased number readily releasable quanta (*n*). Transfection of bovine chromaffin cells with mutant SNAP25B markedly reduced depolarization-evoked exocytosis. The replacement of a hydrophobic isoleucine by a hydrophilic asparagine disrupts the coiled-coil configuration of the SNARE complex.

**MUNC13-1 myasthenia.** Munc18-1 binds to syntaxin 1B preventing it from entering the SNARE complex. Munc13-1 displaces Munc18 allowing syntaxin 1B to associate with SNAP25B and synaptobrevin which enables priming and docking of the synaptic vesicles. A 4-month-old microcephalic, hypotonic, blind, deaf, and paralyzed girl with abnormal cortical excitability harbored a homozygous nonsense mutation in *MUNC13-1*. EP studies showed markedly reduced *n* and normal *p*. The truncated Munc13-1 cannot displace Munc18 which consigns syntaxin 1B to a nonfunctional state. This prevents cholinergic transmission at EPs, glutamatergic transmission in brain, causes seizures, and inhibits brain development.

11:15 AM

### **The Pathophysiological Mechanisms of Congenital Myasthenia Syndromes with Acetylcholinesterase Deficiency**

S  verine Sigoillot, PhD, Jennifer Karmouch, PhD, and **Claire Legay**, PhD

University Paris Descartes, CNRS UMR 8119, Paris, France

Congenital myasthenic syndromes (CMS) are characterized by a dysfunction of the neuromuscular junction (NMJ) leading to muscle weakness and excess fatigability. One of the synaptic CMS is caused by the absence or the poor expression of acetylcholinesterase (AChE) in the synaptic basal lamina. The syndrome is due to mutations in *COLQ*, a gene encoding a specific collagen and anchors AChE in NMJ basal lamina. ColQ has 3 direct partners, AChE, MuSK (a muscle-specific tyrosine kinase receptor) and Perlecan that itself binds Dystroglycan.

A mouse model of this CMS has been generated by invalidation of the *COLQ* gene (Feng et al., 1999). As expected, the NMJ of these mice are devoided of AChE and mice exhibit myasthenic syndromes. ColQ deficient mice present a general atrophy and an hypoplasia that affects fast muscles. Besides anchoring AChE, ColQ participates in postsynaptic differentiation by regulating the expression of a subset of synaptic proteins, the density of acetylcholine receptors (AChR) and the size of the synaptic clusters (Sigoillot et al., 2010, J Neurosci., FASEB J., 2016). In the absence of ColQ, the levels of AChR mRNA are increased *in vivo* and *in vitro* in myogenic cells. The mechanisms leading to this process will be discussed and reveal that they are used more generally in neuromuscular diseases.

11:35 AM

### **Congenital Myasthenic Syndromes with Predominant Limb Girdle Weakness**

Hanns Lochmuller, MD, FAAN

Newcastle University, Newcastle upon Tyne, United Kingdom

Neuromuscular junction disorders, also called Myasthenic syndromes (MS), are a rare heterogeneous group of acquired (Myasthenia Gravis, MG) and inherited (Congenital Myasthenic Syndromes, CMS) neuromuscular disorders associated with distinctive clinical, electrophysiological, laboratory and ultrastructural abnormalities. The genetic defects in CMS either impair neuromuscular transmission directly or result in secondary impairments, which eventually compromise the safety margin of neuromuscular transmission. More recently, we have identified two genes (*DOK7*, *GFPT1*) that cause fatigable weakness of muscles in a limb-girdle distribution, but rarely affecting facial or eye muscles. Next-generation sequencing and deep phenotyping, in combination with international data sharing, reveals new genetic causes of CMS, but also unusual, overlapping clinical phenotypes which blur the boundaries with primary myopathies and motor neuropathies (*SYT2*, *GMPPB*). We will cover the significant progress made in understanding the

molecular pathogenesis of CMS, which is important for both patients and clinicians in terms of reaching a definite diagnosis and selecting the most appropriate treatment.

11:55 AM **Myasthenic Syndromes Due to Defects in COL13A1 and the N-linked Glycosylation Pathway**

**David Beeson**, PhD(1), Judith Cossins, PhD(1), Pedro Rodriguez-Cruz, MD(1), Katsiaryna Belaya, PhD(1), Susan Maxwell, MSc(1), Wei-Wei Liu, PhD(1), Yin Dong, PhD(2), Liz Carpenter, PhD(2), and Jacqueline Palace, MD(3).

- (1) Neurosciences Group, Nuffield Department of Clinical Neuroscience, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom;
- (2) Structural Genomics Consortium, Nuffield Department of Medicine, Oxford, United Kingdom;
- (3) Nuffield Department of Clinical Neuroscience, Oxford, United Kingdom

The congenital myasthenic syndromes (CMS) are hereditary disorders of neuromuscular transmission. The number of cases recognised, at around 1:100,000 in the UK, is increasing with improved diagnosis. The advent of next-generation sequencing has facilitated the discovery of many genes that harbour CMS-associated mutations. An emerging group of CMS, characterised by a limb-girdle pattern of muscle weakness, are caused by mutations in genes that encode proteins involved in the initial steps of the N-linked glycosylation pathway. Surprisingly although this pathway occurs in all mammalian cells, symptoms in many of our patients are largely restricted to neuromuscular transmission which suggests that the neuromuscular junction is particularly sensitive to defects in biochemical pathways affecting glycosylation. However, mutations in these genes may also give rise to multisystem disorders (CDGs) or muscle disorders where the myasthenic symptoms constitute only one component within a wider phenotypic spectrum. We also report a congenital myasthenic syndrome due to mutations in *COL13A1* which encodes an extracellular matrix protein that is concentrated at the neuromuscular junction and highlights the role of these extracellular matrix proteins in maintaining synaptic stability that is independent of the AGRN/MUSK clustering pathway. Knowledge of the neuromuscular synapse and the different proteins involved in maintaining its structure as well as function enables us to tailor treatments to the underlying pathogenic mechanisms.

12:15 PM **Therapeutic Strategies for Congenital Myasthenic Syndromes**

**Jacqueline Palace**, MD

John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom

The congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders which affect neuromuscular junction function. There are now more than 25 genes reported to cause CMS and the treatment paradigms vary, dependent upon CMS subtype. Indeed drugs that help some genetic CMS subtypes make others worse. Because of the rarity of CMS there are no currently licensed drugs for this condition.

The mainstay of treatment remains anticholinesterases, in particular pyridostigmine, which improves strength in acetylcholine receptor deficiency syndromes due to epsilon subunit mutations, the fast channel syndromes and CMS due to mutations in *RAPSN*, *CHAT*, and glycosylation pathways genes. 3,4-diaminopyridine can also be beneficial in the same conditions except in *CHAT* CMS.

Oral salbutamol or ephedrine are beneficial in *DOK7* and *COLQ* CMS and can improve strength in other forms of CMS where pyridostigmine is being taken at higher doses. Fluoxetine or quinidine are the main treatment options for slow channel syndrome CMS. It is important to note that pyridostigmine (and 3,4-DAP) usually worsen slow channel syndromes, *COLQ* and *DOK7* CMS and thus where CMS is suspected as a diagnosis, genetic screening of these genes should be undertaken prior to initiating anticholinesterase medication.

### Session 3: Thymus in Myasthenia Gravis

**Session Chair:** Rozen le Panse, PhD, INSERM, France

#### 2:25 PM **Thymus Involvement in Myasthenia Gravis**

**Rozen Le Panse**, PhD

UMRS 974 UPMC/INSERM/CNRS/AIM, Myology Centre for Research, Paris, France

Myasthenia Gravis (MG) due to autoantibodies against the acetylcholine receptor (AChR) is associated with thymic abnormalities. In early-onset MG, abnormal B-cell infiltrations are observed while in late onset, thymoma is quite frequent. In either cases thymectomy is strongly advised.

In early-onset MG, the thymus is often characterized by the presence of ectopic germinal centers containing anti-AChR producing B cells. The development of thymic germinal centers is the result of numerous thymic changes, such as active neoangiogenesis processes with the development of high endothelial venules and lymphatic vessels. These processes allow an active traffic of peripheral cells via the interplay of specific chemokines, such as CXCL13, CCL21, and CXCL12. All in all, the MG thymus displays the characteristics of tertiary lymphoid organs, as observed in various inflammatory diseases.

What initiates these thymic changes and the development of MG is not exactly known. However, studies have pointed out the activation of innate immunity signaling involving toll-like receptors that leads to the increased expression of type I interferons. These molecules could be the main orchestrators of thymic changes and seem central in the process of sensitization against AChR in early-onset MG but also in thymoma-associated MG.

#### 2:35 PM **MGTX Histopathology Findings**

**Alexander Marx**, MD(1), Christoph Scharff(1), Philipp Ströbel, MD(2), and Cleo-Aron Weis, MD, MSc(1)

(1) University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germany;  
(2) University of Göttingen, Göttingen, Germany

The Thymectomy Trial in Non-Thymomatous Myasthenia Gravis Patients Receiving Prednisone Therapy (MGTX) aimed to learn whether extended transsternal thymectomy combined with prednisone was more efficient than prednisone alone in terms of improving muscle weakness and quality of life, and reducing total prednisone dose. 126 non-thymomatous Myasthenia Gravis (MG) patients underwent randomization to the prednisone-only or the thymectomy group. Forty seven patients agreed to have their thymectomy specimens evaluated by reference pathologists. One patient was excluded

because of detection of a B2 thymoma. The 46 other thymectomy specimens (of 34 females, 12 males; age range 18-63 years) were extensively sampled according to a predefined protocol. Formalin-fixed paraffin embedded tissue blocks were conventionally stained and subjected to CD23 immunohistochemistry for sensitive detection and counting of lymphoid follicles in digitalized sections, and grading of lymphofollicular hyperplasia (LFH, grades 0-4). Overall atrophy (due to "fatty involution") was quantitatively assessed by image analysis, while grades of cortical atrophy (0-4) were estimated as none (adequate for age), slight, moderate, massive, and total. Results: Non-physiological LFH (grades 2-4), i.e. the hallmark of thymuses of prednisone-naïve early onset MG patients was observed in only 27% of cases, reflecting the impact of prednisone on LFH. This hypothesis is supported by the 70% of cases showing moderate or massive overall and cortical atrophy. Whether thymus histopathology at the time of surgery is correlated with patient outcome is under investigation.

2:55 PM

### **Toll-like Receptors 7 and 9 in Myasthenia Gravis Thymus**

**Paola Cavalcante**, PhD, Claudia Barzago, PhD, Fulvio Baggi, PhD, Carlo Antozzi, MD, Lorenzo Maggi, MD, Renato Mantegazza, MD, and Pia Bernasconi, PhD

Foundation Neurological Institute "Carlo Besta", Milan, Italy

Pathogen infections and innate immune signaling by Toll-like receptors (TLRs) are suspected to play a significant role into the pathogenesis of many autoimmune diseases, including myasthenia gravis (MG). Recently, we provided evidence of an active Epstein-Barr virus (EBV) infection in the thymus of MG patients, suggesting that EBV might contribute to intra-thymic autoimmunity development or maintenance in MG patients via B-cell tolerance disruption. Considerable data show that EBV can elicit and modulate TLR7 and TLR9 signaling, known to favor B-cell dysfunction and autoimmunity. We therefore investigated whether EBV infection was associated with dysregulated TLR7 or TLR9 pathways in MG thymus. Indeed, transcriptional levels of these receptors were found to be significantly increased in EBV-positive MG compared to EBV-negative control thymuses. TLR7 and TLR9 were highly expressed in B cells of MG thymic germinal centers and lymphoid infiltrates, where they co-localized with EBV proteins. Increased frequency of proliferating B cells expressing TLR7, TLR9 and EBV antigens was observed in MG thymuses, indicating that TLR7/9 signaling, along with EBV, may promote abnormal B-cell activation and proliferation. In pathological tissues, the two receptors were also over-expressed in epithelium, plasmacytoid dendritic cells and macrophages, suggesting that their activation may sustain harmful inflammation that characterizes MG thymus. Interestingly, our recent data indicate a relationship between TLR7 and TLR9 polymorphisms and the expression levels of these receptors in MG patients. Our overall findings suggest that, in the context of a genetic susceptible background, EBV-driven TLR7/9-mediated innate immune responses might contribute to the intra-thymic pathogenesis of MG.

## Session 4: Serological Phenotypes and Unique Treatment Responses

**Session Chair:** Angela Vincent, MSc, University of Oxford, Oxford, United Kingdom

3:45 PM      **Overview of Serological Classification of Myasthenia Gravis**

**Angela Vincent, MSc**

University of Oxford, Oxford, United Kingdom

4:05 PM      **Cortactin Antibodies in Myasthenia Gravis**

Elena Cortés(1,2), **Isabel Illa**, MD, PhD(1,2)

(1) Unitat Neuromuscular. Serei Neurologia. Hospital Sant Pau. Universitat Autònoma de Barcelona, Barcelona, Spain;

(2) CiBERER (Centro de Investigación Biomédica en Red)

Double-seronegative myasthenia gravis (dSNMG) includes patients with Myasthenia Gravis (MG) without detectable antibodies to AChR or MuSK. It accounts for 15% of generalized MG and 50% of ocular MG patients. Low-affinity IgG1 AChR antibodies and antibodies to LRP4 have been described in dSNMG.

Cortactin, a neuromuscular junction (NMJ) protein, was identified as a possible novel target antigen in dSNMG using a protein array approach that allowed us to study 9000 human proteins. Cortactin is an intracellular protein with phosphorylation-dependent signaling downstream from agrin/LRP4/ MuSK that promotes AChR clustering at the NMJ.

Our studies demonstrated that anti-cortactin antibodies were present in 19%-24% of patients with dSNMG whereas only 4.8%-9% of patients with AChR+MG were cortactin+ ( $p=0.002$ ). Cortactin antibodies were also found in 0%-5.2% of healthy controls and in 9%-12.5% of patients with other autoimmune disorders including inflammatory myopathies. Patients with dSNMG that were cortactin+ presented with an ocular or mild generalized phenotype of MG without bulbar symptoms. They were also negative for anti-LRP4 and anti-striated muscle antibodies.

Autoantibodies against intracellular antigens do not seem to play a pathogenic role in autoimmune diseases. However, the fact that cortactin is part of the agrin/LRP4/ MuSK complex suggests that there may be specific changes in the NMJ that promote the synthesis of anti-cortactin antibodies in dSNMG patients

In the context of MG, Cortactin autoantibodies are biomarkers that when present, suggest a mild disease course and their measurement in the routine diagnosis of dSNMG may be helpful.

4:25 PM

### **MuSK Myasthenia Gravis**

**Amelia Evoli**, MD(1), Paolo E. Alboini, MD(1), Alessia Mastroiosa, MD(1), Valentina Damato, MD(1), Emanuela Bartocconi, PhD(2), Raffaele Iorio, MD, PhD(1).

(1) Institute of Neurology, Catholic University, Roma, Italy

(2) Institute of General Pathology, Catholic University, Roma, Italy

The management of myasthenia gravis with antibodies to MuSK (MuSK-MG) has gradually changed through the last decade in concert with an improved understanding of the disease pathogenesis. In our MuSK-MG population, we evaluated how the disease severity and outcome had changed over the years and observed a steady reduction of respiratory crises and a significant improvement of outcome measures. These findings appear to be related to earlier diagnosis and better treatment.

We performed a multiple logistic regression analysis to assess the impact of demographics, disease characteristics and treatment timing on the response to conventional immunosuppression. We found a positive, though not significant, association of early-onset and less severe disease with remission, while an indolent course with slow progression to maximum disease severity was significantly associated with refractoriness ( $p=0.005$ ). Nine patients, all but one with refractory disease, received one or two courses of rituximab. Seven patients (77.7%) showed a good response, defined as achievement and maintenance of minimal manifestation status or better, together with a  $\geq 50\%$  reduction of steroid dose, withdrawal of immunosuppressants, no need for plasma-exchange or intravenous immunoglobulin treatments. The time to relapse after a single course of rituximab ranged 1.5-5 years, irrespective of circulating B lymphocyte (CD19) count normalization and MuSK antibody persistence.

4:45 PM

### **A Unique Sub-phenotype of Myasthenia Gravis**

**Jeannine M. Heckmann**, PhD, and Melissa Nel, MBChB

University of Cape Town, Cape Town, South Africa

While extraocular muscles (EOMs) are affected early in generalized Myasthenia Gravis (MG) and respond to treatment much like non-ocular muscles, we have identified a unique endophenotype of MG which is characterised by treatment-resistant ophthalmoplegia (OP-MG). This OP-MG endophenotype most commonly affects subjects with African genetic ancestry who present with juvenile-onset acetylcholine receptor-antibody positive MG. However, a few OP-MG cases have been identified with MuSK-antibodies and one case with triple-seronegative MG. In some OP-MG cases, the EOM treatment-resistance is apparent at diagnosis, while in others the dysfunction only manifests at a later critical period in the disease course which often coincides with treatment interruption.

The management of OP-MG is an unmet need. Although cases with complete ophthalmoplegia do not have diplopia, they have severe ptosis which results in functional blindness. Surgical elevation of ptotic lids has been beneficial, although it is frequently contraindicated due to concomitant myasthenic facial weakness. In contrast, surgical realignment for partial ophthalmoplegia has rarely showed favourable long-term outcomes.

The pathogenesis of OP-MG remains unresolved. We have used an 'extreme phenotype' approach to dissect the molecular genetics using whole exome sequencing. Unbiased prioritization of potentially pathogenic variants ranked by the significance of their association with OP-MG and expression in human EOMs, has implicated genes related to complement protection and muscle remodeling. Preliminary functional studies in patient-

derived transdifferentiated muscle models have captured dynamic biological signals *in-vitro* which suggest that potentiated muscle damage and altered myogenesis pathways contribute to the postulated synaptopathy at the EOM endplates in individuals with OP-MG.

5:05 PM

### Autoantibodies to Agrin in Myasthenia gravis

Arthur Melms, MD

Universitätsklinikum Erlangen, Erlangen, Germany

C. Gasperi(1), **Arthur Melms, MD**(2), B. Schoser(3), Y. Zhang(1), J. Meltoranta(1), V. Risson(5), L. Schaeffer(5), B. Schalke(4) , and S. Kröger(1)

- (1) Institute for Physiology; Dept. of Physiological Genomics; Ludwig-Maximilians-University, Munich, Germany;
- (2) Dept of Neurology, University Medical Center Erlangen and Dept of Neurology, University Medical Center Tübingen, Tübingen, Germany;
- (3) Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians University, Munich, Germany;
- (4) Department of Neurology, University of Regensburg, Regensburg, Germany;
- (5) Laboratory of Molecular Biology of the Cell, Neuromuscular Differentiation Group, University of Lyon, France

**Objective:** We tested the hypothesis that the extracellular matrix protein agrin which is essential for the formation and maintenance of the neuromuscular junction is a potential target for autoantibodies in Myasthenia gravis (MG).

**Methods:** We used a solid-phase ELISA with a purified human recombinant agrin domain and examined serum samples from patients with generalized MG for the presence of anti-agrin antibodies. Nine of 54 sera were from patients with autoantibodies to the acetylcholine receptor (AChR), 15 had autoantibodies to the muscle-specific tyrosine kinase (MuSK) and 30 were negative both for antibodies to AChR and MuSK.

**Results:** We identified five samples with antibodies to agrin. Four of the five agrin-positive samples had also autoantibodies to MuSK, one had autoantibodies to AChR. Some of the sera stained adult mouse neuromuscular junctions and native mini-agrin expressed in 293HEK cells.

**Conclusions:** These results provide evidence for agrin as a novel target protein for autoantibodies in myasthenia gravis. In this selected, non-representative series anti-agrin antibodies were always detected in the presence of autoantibodies against MuSK or AChR suggesting antigen spreading at the neuromuscular junction in a minority of MG cases. It remains to be shown whether a double hit of two autoantibodies have an influence on the clinical phenotype.

## Session 5: Mechanisms of Autoimmunity (basic, including diseases other than Myasthenia Gravis)

**Session Chair:** Sonia Berrih-Aknin, DSc, INSERM, France

### 8:30 AM Introduction to Autoimmunity

**Sonia Berrih-Aknin, DSc,**

Center of Research in Myology, Sorbonne Universités, UPMC — Inserm UMRS 974, CNRS FRE3617, Institute of Myology, G.H. Pitié-Salpêtrière, Paris, France

Myasthenia gravis is a model of organ-specific autoimmune disease (AID). Even if the target organ differs from one AID to another, many mechanisms are shared in common, that involves a combination of predisposing factors, triggering components and their interactions with the immune system.

Many of the genes and components that may predispose to AIDs are similar. These include genetic, epigenetic, hormones, vitamin D or microbiota. The mechanisms of tolerance are also a part of predisposing factors in so far as AIRE that controls the thymic expression of the tissue-specific antigens is not equally produced in all individuals. Recently it was shown that females express less AIRE than males in the thymus, providing a new explanation for the high predisposition of women to autoimmunity.

Triggering factors could be infections or drugs and could affect the immune system, inducing in most cases a bias toward an inflammatory immune response. The dysregulation of the immune cells concerns both the innate and acquired immune systems. Well-known mechanisms are the imbalance between Treg cells and pathogenic cells, namely Th17 cells, the generation of germinal centers, as well as the network of cytokines and chemokines. Treg cells play a significant role in most AIDs, but there is evidence that their functional defects are not intrinsic but rather the result of the inflammatory environment.

All these aspects will be discussed during this session.

### 8:40 AM **Keynote Address: Pathogenic and Regulatory T Cells in Central Nervous System Autoimmunity**

**Vijay Kuchroo, DVM, PhD,** Harvard Institutes of Medicine

Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, United States

Recently a subset of interleukin (IL)-17-producing T cells (T<sub>H</sub>17) distinct from T<sub>H</sub>1 or T<sub>H</sub>2 cells was described and shown to have a crucial role in the induction of autoimmune tissue injury. Accumulating data suggests that there are three distinct steps in Th17 differentiation: Induction, Amplification and Stabilization mediated by distinct cytokines. Whereas TGF- $\beta$  + IL-6 or IL-1 + IL-6 induces them, IL-21 amplifies Th17 cells, IL-23 stabilizes the phenotype of Th17 cells. Loss of any of the cytokines (TGF- $\beta$ , IL-1, IL-6, IL-21 or IL-23) in the pathway results in a defect in generation of Th17. However not all

Th17 cells are pathogenic and induce autoimmunity, IL-23 is a key cytokine that induces pathogenicity in Th17 cells. Using expression profiling at very high temporal resolution, novel computational algorithms and innovative nano-wire based “knock-down” approaches, we have developed a regulatory network that governs the development of Th17 cells. In addition to high-density temporal microarray analysis, we have performed single-cell RNA-seq of Th17 cells in order to characterize cellular heterogeneity, identify subpopulations, functional states and learn how gene expression variation affects Th17 effector functions. We have identified novel regulators of Th17 cells both *in vivo* and *in vitro* that do not affect Th17 differentiation but affect pathogenic vs. non-pathogenic functional states of Th17 cells. Some of the regulators that make Th17 cells non-pathogenic are also utilized by CD8+ T cells to induce T cell “exhaustion” or “dysfunction”. These novel inhibitory molecules cooperate with other known “check-point” co-inhibitory receptors to suppress anti-tumor immunity.

9:10 AM **Regulatory B Cells in Health and Disease**

**Claudia Mauri**, David Isenberg, and Madhvi Menon

Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom

CD24<sup>hi</sup>CD38<sup>hi</sup> regulatory B-cells (Bregs) have been shown to be critical in restraining excessive inflammation and to ameliorate autoimmune related disorders. Breg numerical and functional deficiency is associated with several human autoimmune diseases. The majority of data demonstrating the importance of Breg in disease, derived principally from mouse studies, however the functional mechanism of these cells, and the role that Bregs play in human in autoimmune disease is beginning to emerge. We will discuss our latest findings showing how Bregs maintain homeostasis and tolerance in healthy individuals and how multiple immunological processes are altered in patients with lupus. In particular, we will provide evidence showing the existence of a novel feedback loop between plasmacytoid dendritic cells (pDCs) and Bregs that contributes to the regulation of pro-inflammatory signals.

9:30 AM **Estrogen-mediated Downregulation of AIRE Influences the Gender-bias in Autoimmune Diseases**

**Nadine Dragin**(1,2), Geraldine Cizeron-Clairac(2), Jacky Bismuth(2), Rozen Le Panse(2), and Sonia Bérrih-Aknin(2)

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Autoimmune diseases affect 5–8% of the population, and women are more susceptible to these diseases than men. Here, we analyzed human thymic transcriptome and revealed gender-associated differences in the expression of tissue-specific antigens that are controlled by the autoimmune regulator (AIRE), a key factor in central tolerance. We hypothesized that the level of AIRE is linked to the gender-bias susceptibility to autoimmune disease. In human and mouse thymus, females expressed less AIRE (mRNA and protein) than males after puberty. These results were confirmed in purified murine thymic epithelial cells (TECs). We also demonstrated that AIRE expression is

related to sexual hormones, as male castration decreased AIRE thymic expression, and estrogen receptor  $\alpha$ -deficient mice did not show a gender disparity for AIRE expression. Moreover, estrogen treatment resulted in downregulation of AIRE expression in cultured human TECs, human thymic tissue grafted to immunodeficient mice, and in murine fetal thymus organ cultures. AIRE levels in human thymus grafted in immunodeficient mice depended upon the gender of the recipient. Estrogen also upregulated the number of methylated CpG sites in the *AIRE* promoter. Together, our results indicate that, in females, estrogen induces epigenetic changes in the *AIRE* gene, leading to reduced AIRE expression under a threshold that increases the female susceptibility to autoimmune diseases.

9:50 AM

### Expanding Field of IgG4 Autoimmune Diseases

**Maartje G. Huijbers**, PhD, Yvonne E. Fillié-Grijpma, Inge E. Stienstra-van Es, Jaap J. Plomp, PhD, Silvère M. van der Maarel PhD, and Jan J. Verschuuren, MD, PhD

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Myasthenia gravis (MG) is hallmarked by fluctuating fatigable muscle weakness and is caused by auto-antibodies binding essential neuromuscular junction proteins. Acetylcholine receptor (AChR) MG is caused by IgG1 and IgG3 antibodies inducing complement-mediated muscle membrane destruction, antigenic modulation and internalization. The majority of antibody-mediated autoimmune diseases is caused by IgG1 antibodies and induces disease through a similar pathophysiological mechanism. MG can also be caused by auto-antibodies against muscle-specific kinase (MuSK). These auto-antibodies are mainly of the IgG4 subclass and induce MG through a distinct pathomechanism. IgG4 is unable to activate complement, has low affinity for Fc $\gamma$  and is functionally monovalent. These characteristics render it unable to induce pathology through the same mechanisms as IgG1 and IgG3. Instead in MuSK MG, IgG4 physically obstructs essential protein-protein interactions causing inhibition of MuSK signaling and AChR clustering. The antibody isotype is thus essential in the pathomechanism of disease.

In recent years several new antigenic targets have been described for a variety of antibody-mediated autoimmune diseases. With the identification of these antigens a new niche of IgG4-mediated autoimmune diseases was identified. Currently, at least 13 autoimmune diseases are hallmarked by predominant involvement of IgG4 auto-antibodies. These diseases affect a range of organs and include the skin blistering disease pemphigus vulgaris, chronic inflammatory demyelinating polyneuropathy with antibodies against contactin1 and limbic encephalitis with LG11 antibodies. In this presentation we will discuss these diseases, their similarities and differences.

## Session 6: Mechanisms of Myasthenia Gravis Autoimmunity — Updates

**Session Chair:** Kevin C. O'Connor, PhD, Yale School of Medicine

10:40 AM **Introduction to Myasthenia Gravis Autoimmunity**

**Kevin C. O'Connor**, PhD, Yale School of Medicine

10:50 AM **Mechanisms of B Cell and T Cell Immunopathology in Myasthenia Gravis**

Panos Stathopoulos, MD, PhD(1), Aditya Kumar, MD(1), Jason A. Vander Heiden, BSc(2), Steven H. Kleinstein, PhD(2,3), Richard J. Nowak, MD(1), and **Kevin C. O'Connor**, PhD(1)

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- (3) Departments of Pathology and Immunobiology, Yale School of Medicine, New Haven, Connecticut, United States

We propose a model of autoantibody production in myasthenia gravis (MG), which is founded on experience with the use of CD20-mediated B-cell depletion therapy (BDT) in MG. BDT reduces the autoantibody titer in many MG patients, thus it likely targets autoantibody-producing cells. Accordingly, our proposed model posits that disease pathology in MG begins with a self-reactive pool of naïve B-cells. With help from antigen-specific T-cells, these naïve B-cells supply a subset of MG-specific expanded memory B-cell clones. Two populations of B-cells then contribute to the production of autoantibodies: circulating short-lived plasmablasts and long-lived plasma cells, the latter of which reside in the bone marrow, and are present in the thymus of only some MG patients. Given that plasmablasts and plasma cells are CD20-negative, clinical improvement and reduced autoantibody titer following BDT may be associated with elimination of the autoimmune CD20-positive memory reservoir, causing production of short-lived plasmablasts to cease. We have tested the components of this model in both untreated and MG subjects receiving BDT, and have thus far demonstrated: (i) Abnormally high frequencies of self-reactive naïve B-cells accumulate in MG, indicating a breach in immune tolerance. (ii) Consistent with these tolerance defects is a distorted naïve B-cell repertoire revealed by high-throughput B cell sequencing. (iii) Antigen-specific Th17 cells provide pro-inflammatory support for B-cell activation. (iv) Circulating, antibody-producing memory B cells and plasmablasts are present in some MG subjects. (iv) The influence of BDT on these components of our model is underway using highly novel technologies.

11:10 AM **Immune Checkpoint Markers in Myasthenia Gravis Patients**

**John S. Yi, PhD**, Melissa A. Russo, Nathan Van Landingham, Jeffrey T. Guptill, MD

Duke University Medical Center, Durham, North Carolina, United States

The immune system is equipped with checkpoints that enforces immune homeostasis. Our immune system utilizes these checkpoints as a balance to prevent an immune response

against self-antigens and thus the onset of autoimmune diseases like myasthenia gravis (MG). Based on recent evidence of cancer patients developing MG as an adverse effect of checkpoint immunotherapy, the expression of checkpoint inhibitors may play a significant role in restricting the generation of MG-specific responses. To investigate the role of checkpoint markers in MG, we performed an immune profiling study to characterize the expression of checkpoint markers and inhibitory receptors in a cohort of MG patients. High-dimensional flow cytometry analysis identified the expression patterns of BTLA, CTLA-4, PD-1, TIGIT, and TIM-3 on B cells, CD4, and CD8 T cells. In parallel, 14 soluble checkpoint markers (BTLA, GITR, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD28, CD80, CD137, CD27, and CD152) were quantified from plasma samples in a Luminex multiplex assay. Immune profiling was also performed, in a longitudinal analysis, on a patient presenting with MG after Nivolumab treatment (anti-PD-1 monoclonal antibody). With the rising use of immunotherapy in the treatment of cancer, the incidence of MG will only increase. Therefore, a better understanding of the mechanisms controlled by immunotherapy could be critical in identifying immunological signatures of MG.

11:30 PM

### **Cytokine Production in Myasthenia Gravis Patients**

**Vuslat Yilmaz**(1,4), Piraye Oflazer(2), Fikret Aysal(3), Hacer Durmus(2), Yesim Parman(2), Erdem Tuzun(4), Feza Deymeer(2), and Guher Saruhan-Direskeneli(1)

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- (4) Department of Neuroscience, Institute for Experimental Medical Research, University of Istanbul, Istanbul, Turkey

Myasthenia gravis (MG) is an autoimmune disease, which is mainly mediated by pathogenic antibodies that are produced by T-cell-dependent and B-cell-mediated mechanisms. Cytokines play a crucial role in antigen presentation, T- and B-cell activation and antibody production and thus, modulation of immune responses by cytokines contributes to MG pathogenesis.

In a study performed to evaluate T cell derived cytokine production in MG, plasma levels of Th1, Th2 and Th17 related cytokines were overall not significantly different between MG patients with acetylcholine receptor (AChR) and muscle specific kinase (MuSK)-antibodies and healthy controls. By contrast, anti-CD3-stimulated peripheral blood mononuclear cells (PBMC) of MuSK-MG but not AChR-MG patients produced higher levels of IL-17A, IFN- $\gamma$  and IL-21. Moreover, immunosuppression was found to inhibit Th1 cytokine and enhance Th2 cytokine production in AChR-MG but not MuSK-MG patients.

*In vitro* activation model of directly isolated B cells from peripheral blood can be used to determine B-cell derived cytokine production and its potential role in MG. B-cell derived IL-10, IL-6 and TNF- $\alpha$  are down-regulated in MG, irrespective of antibody subgroups. Ineffective cytokine production by B-cells may be a susceptibility factor in dysregulation of autoantibody production.

Considering the substantial progress in B-cell targeting therapy approaches in recent years, the impact of naive or memory B-cells and B-cell cytokine dysregulation on MG development warrants further investigation. Better characterization of cytokine patterns in

AChR-MG and MuSK-MG is also recommended for development and innovation of potential future cytokine-specific therapies designed for MG.

11:50 AM

### **Circulating MicroRNAs in Myasthenia Gravis**

**Anna Rostedt Punga**, MD, PhD, Uppsala University, Sweden

Carl Johan Molin, MD, Liis Sabre, MD, PhD, Tanel Punga, PhD, and **Anna Rostedt Punga, MD, PhD**

Uppsala University, Uppsala, Sweden

Since reliable biomarkers for monitoring of MG patients are lacking, circulating microRNAs (miRNAs) could serve as potential novel disease-specific biomarkers due to their altered levels in the diseased state. AChR antibody seropositive (AChR+) MG differs from MuSK antibody seropositive (MuSK+) MG with regard to antibody subclass, clinical phenotype and thymus histopathology. We previously analyzed circulating miRNAs in sera of AChR+, AChR- and MuSK+ patients with and without immunosuppressive therapy. Quantitative analysis of 179 circulating miRNAs revealed elevated levels of the immuno-miRNAs miR-150-5p and miR-21-5p specifically in AChR+ and AChR- MG patients compared to patients with other autoimmune disorders. Further, these miRNA levels were lower in MG patients undergoing immunosuppression. On the other hand, in MuSK+ MG patients another profile of circulating microRNAs was detected, including elevated levels of the let-7 miRNA family. We also specifically analyzed longitudinal levels of miR-150-5p and miR-21-5p in patients in the prospective thymectomy trial (MGTX) study, comparing AChR+ patients that underwent extended transsternal thymectomy with patients that received only prednisone with a follow-up period of 3 years. Here, significantly lower levels of miR-150-5p were observed at 2 years after thymectomy, in parallel with clinical improvement, whereas miR-150-5p levels were unchanged in patients receiving only prednisone.

This presentation will summarize the latest results of our studies on the disease specific circulating miRNA profiles in different subgroups and novel longitudinal data regarding influences by different treatment in MG. These data especially support the role of circulating miR-150-5p as a disease-specific biomarker in AChR+ MG.

Acknowledgements: Part of the work was done with the support of NIH grant U01 NS 42685 and the MGTX study group.

## Session 7: Day 2 Hot Topic Short Talks (Selected from Submitted Abstracts)

Session Chair: Maria Elena Farrugia, MD, MRCP, Southern General Hospital, United Kingdom

### 1:55 PM **Interleukin-23 Deregulation Contributes to Thymic Inflammation in Myasthenia Gravis Patients**

**Jose Adolfo Villegas MSc**(1), Rozen Le Panse PhD(1), Sonia Berrih-Aknin PhD(1), and Nadine Dragin PhD(1,2)

- (1) Center of Research in Myology, Sorbonne Universités, UPMC INSERM UMRS 974, Institute of Myology, G.h. Pitié-Salpêtrière, Paris, France
- (2) Inovarion, Paris, France

Overexpression of IL-17 related genes and IL-23R have been reported in T-cells of AChR<sup>+</sup> MG patients. CD4<sup>+</sup> T cell subtypes related to these genes, (i.e. Treg and Th17 cells) are dysfunctional and contribute to the general inflammation in MG AChR<sup>+</sup> thymuses (Gradolatto et al., 2014). In addition, a group of cytokines (Interleukin 6, 21, 23 (IL-6, IL-21, IL-23), and TGF- $\beta$ 3) has been described as inducers of pathogenic CD4<sup>+</sup> Th17 cells (Lee et al., 2012).

Here, we investigate the involvement of IL-6, IL-21, IL-23 and TGF- $\beta$ 3 in MG T cells subset disequilibrium and dysfunction. We found that all these cytokines are significantly overexpressed in AChR<sup>+</sup> MG thymuses. However, only IL-23 remained increased in the periphery. In addition, we observed that thymic epithelial cells (TEC) from MG patients overproduced IL-23. In order to identify factors sustaining IL-23 overexpression, we analyzed the effect of inflammatory molecules in normal TEC cultures. We found that interferon type 1 (molecules overexpressed in MG thymuses) and IL-17 stimulated IL-23 expression by TEC, while interferon type 2 or LPS had no effect.

Our data show that IL-23 deregulated expression may be involved in the inflammatory process in MG thymuses and promote pathogenic Th17 cells differentiation. We also show that IFN type 1 contribute to IL-23 overexpression by TEC. Therefore, in AChR<sup>+</sup> MG thymuses, the global inflammation sustains IL-17 expression by CD4<sup>+</sup> T cells. Hence, a development of a continuous inflammatory loop between TEC and IL-17 producing cells can appear and explain the chronic inflammation observed in MG thymuses.

### 2:05 PM **Rituximab is an Effective Treatment for Anti-MuSK Myasthenia Gravis**

**Michael K. Hehir, MD**(1), Lisa D. Hobson-Webb, MD(2), Michael Benatar, MD, PhD(3), Carolina Barnett, MD, PhD(4), Nicholas J. Silvestri, MD(5), James F. Howard Jr., MD(6), Diantha Howard MA, MS(1), Amy Visser, MD(7), Brian A. Crum, MD(7), Richard Nowak, MD, MS(8), Rachel Beekman, MD(8), Aditya Kumar, MD(8), Katherine Ruzhansky, MD, MS(9), I-Hweii Amy Chen, MD, PhD(9), Michael T. Pulley, MD, PhD(10), Shannon M. LaBoy MD, MS(10), Melissa A. Fellman, MD(3), Shane M. Greene MD(1), Mamatha Pasnoor, MD(11), and Ted M. Burns, MD

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- (2) Duke University School of Medicine Durham, North Carolina, United States
- (3) University of Miami Health System, Miami, Florida, United States
- (4) University of Toronto School of Medicine, Toronto, Ontario, Canada
- (5) SUNY Buffalo Jacobs School of Medicine, Buffalo, New York, United States
- (6) UNC School of Medicine, Chapel Hill, North Carolina, United States
- (7) Mayo Clinic, Rochester, Minnesota, United States
- (8) Yale School of Medicine, New Haven, Connecticut, United States

- (9) Medical University of South Carolina, Charleston, South Carolina, United States  
University of Florida, Jacksonville, Florida, United States
- (10) University of Kansas Medical Center, Kansas City, Kansas, United States
- (11) University of Virginia School of Medicine, Charlottesville, Virginia, United States

**Objective:** Evaluate the efficacy of rituximab in treatment of anti-muscle specific kinase (MuSK) myasthenia gravis (MG).

**Methods:** Multi-center, blinded prospective review, comparing anti-MuSK positive MG patients treated with rituximab to those not treated with rituximab. The primary clinical endpoint was the Myasthenia Gravis Status and Treatment Intensity (MGSTI), a novel outcome that combines the Myasthenia Gravis Foundation of America (MGFA) post intervention status (PIS) and the number and dosages of other immunosuppressant therapies used. A priori, an MGSTI of Level  $\leq 2$  was used to define a favorable outcome. Secondary outcomes included: modified MGFA PIS of Minimal Manifestations or better, mean/median prednisone dose, and mean/median doses of other immunosuppressant drugs.

**Results:** Seventy-seven of 119 anti-MuSK MG patients evaluated between 1/1/2005 and 1/1/2015 at 10 neuromuscular centers were selected for analysis after review of limited clinical data by a blinded expert panel. An additional 22 subjects were excluded due to insufficient follow-up. Baseline characteristics were similar between the rituximab-treated ( $n=24$ ) and the control participants ( $n=31$ ). Median follow-up duration was  $>3.5$  years. At last visit, 58% (14/24) of rituximab-treated patients reached the primary outcome compared to 16% (5/31) of controls ( $p = .002$ ). Number needed to treat (NNT) for the primary outcome is 2.4. At last visit 29% of rituximab treated subjects were taking prednisone (mean dose 4.5mg/day) compared to 74% of controls (mean dose 13mg/day) ( $p = .001$  and  $p = .005$ ).

**Conclusion:** This multi-center, blinded prospective review provides class II evidence that rituximab is an effective treatment for anti-MuSK MG.

2:15 PM

### Engineered Agrin Attenuates the Severity of Experimental Autoimmune Myasthenia Gravis

Shafeeq Ladha, MD, Barrow Neurological Institute

Zhiguo Li, MS(1,2), Minshu Li, MS(1,2), Kristofer Wood, BS(1), Stefan Hettwer, PhD(3), Suraj A. Muley, MD(1), Fu-Dong Shi, MD, PhD(1,2), Qiang Liu, MD, PhD(1,2), **Shafeeq S. Ladha, MD(1)**

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- (2) Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin, China
- (3) Neurotune AG, Schlieren-Zurich, Switzerland

**Background and Purpose:** To determine the effects of a soluble C-terminus fragment of neural agrin (NT-1654) on the severity of experimental autoimmune myasthenia gravis (EAMG). Compared to prior agrin agonists which had poor tissue penetration, NT-1654 is resistant to degradation and highly soluble owing to induced mutations at the neurotrypsin and heparin binding sites, respectively.

**Method:** EAMG was induced in female Lewis rats by immunization with the Torpedo acetylcholine receptor (tAChR) and complete Freund's adjuvant. A total of forty-eight rats used in this study were assigned to three EAMG groups receiving vehicle phosphate-buffered saline (disease control), 2 mg/kg NT-1654, or 6 mg/kg NT-1654, and a naïve control group (n =12 per group). NT-1654 was dissolved in PBS and injected daily s.c. into tAChR immunized rats during the first 10 days after immunization, and then every other day for the following 20 days. EAMG clinical scores, body weight, repetitive nerve stimulation, and muscle histopathology were assessed as readouts.

**Results:** NT-1654 reduced clinical severity of EAMG in the rats as evidenced by higher EAMG clinical scores and improved weight gain. Administration of NT-1654 also effectively promoted the clustering of AChRs at NMJs, alleviated the neurophysiologic impairment of NMJ transmission and up-regulated the expression of muscle-specific kinase (MuSK).

**Conclusion:** These findings suggest that NT-1654 may be a promising agent to induce repair and augment synaptic strength at NMJs in EAMG. Future investigations are needed to test the potential of NT-1654 to treat human myasthenia gravis or other neuromuscular disorders.

2:25 PM

### **Oral Administration of Lactobacilli and Bifidobacteria Prevents Experimental Autoimmune Myasthenia Gravis in the Lewis Rat**

**Elena Rinaldi**, MSc, Neurological Institute 'Carlo Besta', Italy

Alessandra Consonni, PhD(1), Chiara Cordiglieri, PhD(1), **Elena Rinaldi**, MSc(1), Roberta Marolda, PhD(1), Elena Guidesi, PhD(2), Marina Elli, PhD(2), Renato Mantegazza, MD(2), and Fulvio Baggi, PhD(1)

- (1) Preclinical Neuroimmunology Laboratory, Neurological Institute "Carlo Besta", Milan, Italy
- (2) AAT-Advanced Analytical Technologies, Fiorenzuola d'Arda, Piacenza, Italy

Oral probiotic administration is able to modify host's gut microbiome and induce different immunoregulatory activities by triggering dendritic cell (DC) maturation, modifying T effector cell balance and inducing regulatory T cell (Treg). In this work, clinical efficacy of two lactobacilli (LB) strains (*L. crispatus* and *L. rhamnosus*), and two bifidobacteria (BF) strains (*B. breve* and *B. animalis* subsp. *Lactis*) were evaluated in the TACHR-EAMG model in the Lewis rat, via preventive and therapeutic protocols. EAMG clinical manifestations were significantly ameliorated by LB and BF, compared to vehicle-fed animals. Significant reduction of serum anti-AChR Ab titers was observed in the BF-treated group ( $18.9 \pm 4.5$  pmol/ml,  $p < 0.05$ ) and in LB-treated group ( $19.8 \pm 6.2$  pmol/ml,  $p < 0.05$ ) compared with vehicle-fed EAMG group ( $30.9 \pm 6.5$  pmol/ml). Muscle AChR content in BF-treated EAMG rats ( $91.4 \pm 11.1$  fmol/g) and in LB-treated rats ( $61.6 \pm 5.7$  fmol/g) was found increased compared to vehicle-fed EAMG animals ( $49.2 \pm 6.6$  fmol/g).  $CD4^+CD25^+$  and  $FoxP3^+CD4^+CD25^+$  T cells were found increased in mesenteric LNs, PPs and PBL from probiotic-fed naïve animals. LB and BF promoted the maturation of BMDCs and the acquisition of an immunomodulatory phenotype, characterized by increased mRNA expression of  $TGF\beta$  and  $CCR7$ , and by reduced  $TLR4$  levels, compared to control DC cultures. Up-regulation of  $TGF\beta$  mRNA was confirmed by ELISA assay on culture medium. We are investigating the microbiome composition of LB- and BF-fed rats by NGS technique, to study the modulation of the gut microbiome. Probiotic supplementation could contribute to systemic immunomodulation and immunological tolerance and could represent a promising therapeutic approach for MG.

## Session 8: Clinical Trials Update

Session Chair: Henry Kaminski, MD, George Washington University

2:35 PM **Clinical Trials for Myasthenia Gravis**

**Henry Kaminski, MD**

George Washington University School of Medicine & Health Sciences, Washington District of Columbia, United States

The start of our new century has brought what can be considered the golden age of clinical trials for myasthenia gravis. With the MG Foundation of America Task Force guidelines dedicated to improving the rigor of clinical research, a consensus for clinical classification, outcome measures, and expectations for statistical analysis was established and has gained global acceptance. Although not necessarily positive in all cases, several randomized, controlled trials have come to successful conclusions. Pharmaceutical and biotechnology companies have taken an interest in myasthenia gravis because of its well-defined pathophysiology, to not only apply their novel technologies, but to serve as proof of concept to ultimately validate agents for use in other autoimmune disorders. As an orphan disease, drug development for myasthenia gravis is provided advantages in patent life by the United States Federal Drug Administration and similar organizations in other countries, which further contributes to the increase in clinical trials as well as pre-clinical investigations. This session will provide overviews of three clinical trials that have therapies in early phase to completed Phase 3 assessments, one of which will change standard of care.

2:45 PM **Eculizumab Results in Improvement in Activities of Daily Living and Muscle Strength in Refractory Generalized Myasthenia Gravis Patients Compared with Placebo**

**James F. Howard, Jr, MD(1), Fanny O'Brien, PhD(2), Jing Jing Wang, MD(2), Renato Mantegazza, MD(3), and the REGAIN Study Group**

- (1) Department of Neurology, University of North Carolina, Chapel Hill, North Carolina, United States;
- (2) Alexion Pharmaceuticals, New Haven, Connecticut, United States;
- (3) Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy

Refractory generalized myasthenia gravis (rgMG) is an ultra-rare patient group who have significant unmet need despite multiple MG therapies. Previously presented Phase III REGAIN study results comparing the effect of eculizumab, a terminal complement inhibitor, vs. placebo in rgMG patients showed that 18 of 22 pre-specified primary and secondary analyses, including activities of daily living (MG-ADL) and muscle strength (QMG) achieved  $p$ -values  $<0.05$ , favoring patients treated with eculizumab. The objective of this analysis is to evaluate the improved response, as evaluated by MG-ADL and QMG responder analyses, in rgMG patients.

Acetylcholine receptor antibody-positive patients  $\geq 18$  years of age with rgMG, a MG-ADL screening/baseline score of  $\geq 6$  were evaluated and randomized 1:1 to receive intravenous infusion of eculizumab ( $n=62$ ) for 26 weeks or placebo ( $n=63$ ). Rescue therapy was permitted with physician discretion. Responder analyses of the MG-ADL and QMG were evaluated.

The proportion of patients without use of rescue therapy and who achieved a clinically relevant response in both (MG-ADL:  $\geq 3$  point change; QMG:  $\geq 5$ ) were consistently greater in the eculizumab group compared with the placebo group at week 26 (all  $p < 0.05$ ). There was an observed difference in the proportion of patients achieving a response between the eculizumab and placebo groups at Week 2 that was sustained through Week 26.

Refractory gMG patients treated with eculizumab achieved early and sustained clinically relevant improvements compared with placebo on both MG-ADL and QMG through 26 weeks. These data, combined with previously reported data, further highlight the clinically meaningful effect of eculizumab in rgMG.

This study (NCT01997229) was sponsored by Alexion Pharmaceuticals (New Haven, CT, USA).

3:05 PM

### MGTX Primary and Secondary Outcomes

**Gil I. Wolfe**, MD, Henry J Kaminski, MD, Inmaculada B. Aban, PhD, Greg Minisman, MA, Hui-Chien Kio, MS, Alexander Marx, MD, Philipp Ströbel, MD, Claudio Mazia, MD, Joel Oger, MD, J. Gabriel Cea, MD, Jeannine M. Heckmann, MBChB, PhD, Amelia Evoli, MD, Wilfred Nix, MD, Emma Ciafaloni, MD, Giovanni Antonini, MD, Rawiphan Witoonpanich, MD, John O. King, MD, Said R. Beydoun, MD, Colin H. Chalk, MD, Alexandru C. Barboi, MD, Anthony A. Amato, MD, Aziz I. Shaibani, MD, Bashar Katirji, MD, Bryan R. F. Lecky, MD, Camilla Buckley, MD, Elza Dias-Tosta, MD, PhD, Hiroaki Yoshikawa, MD, PhD, Marcia W. Cruz, MD, Michael T. Pulley, MD, PhD, Michael H. Rivner, MD, Anna Kostera-Pruszyk, MD, Robert M. Pascuzzi, MD, Carlyne E. Jackson, MD, Guillermo S. Garcia Ramos, MD, Jan J.G.M. Verschuuren, MD, Janice M. Massey, MD, John T. Kissel, MD, Lineu C. Werneck, MD, PhD, Michael Benatar, MD, PhD, Richard J. Barohn, MD, Rup Tandan, MD, FRCP, Tahseen Mozaffar, MD, Robin Conwit, MD, Joanne Odenkirchen, MPH, Joshua R. Sonnett, MD, Angela Vincent, MBBS, Alfred Jaretzki III, MD, John Newsom-Davis, MD, Gary R. Cutter, PhD, and the MGTX Investigators

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Since first utilized in 1941, a benefit from thymectomy in non-thymomatous myasthenia gravis (MG) has been claimed by numerous studies. In concert with calls dating back a half century, a randomized study employing standardized medical therapy was organized. MGTX was a randomized, rater-blinded trial whose aim was to answer the following: Does extended transsternal thymectomy (ETTX) combined with a strictly defined prednisone protocol, when compared with prednisone alone a three years result in (i) greater improvement in MG weakness based on time-weighted average of the Quantitative MG (QMG) score, (ii) a lower prednisone exposure, and (iii) a lower adverse event burden? Patients with acetylcholine receptor antibody-positive generalized non-thymomatous myasthenia gravis, age 18 to 65 years with MGFA disease class 2 to 4 and duration less than five years were enrolled.

A total of 126 patients were randomized at 36 sites. Over three years, thymectomized patients showed improved clinical status based on QMG score (6.15 vs. 8.99;  $p < 0.001$ ) and a lower average alternate-day prednisone requirement, 44 mg vs. 60 mg ( $p < 0.001$ ). Fewer surgical patients required immunosuppression with azathioprine (17% vs. 48% of subjects,  $p < 0.001$ ) and hospitalizations for exacerbations (9% vs. 37% of subjects,  $p < 0.001$ ). Treatment-associated complications were not different ( $p = 0.73$ ), but the thymectomy group reported fewer treatment-associated symptoms ( $p < 0.001$ ) and lower distress levels ( $p = 0.003$ ).

Thymectomy demonstrated several benefits over three years in generalized non-thymomatous MG, and should be considered early in management decisions.

The study was registered on [clinicaltrials.gov](https://clinicaltrials.gov), identifier NCT00294658 and was supported by NIH/NINDS U01 NS042685, the MDA, and the MGFA.

3:25 PM

### **A Phase 1b Clinical Trial of CV-MG01, Acetylcholine Receptor Mimetic Peptides**

**Rudy Mercelis**, MD(1), Jonathan Baets, MD(1), Stephane Huberty, MD(2), Nicolas Havelange(2), Ellen Strijbos, MD(3), Maartje Huijbers, PhD(3), and Jan Verschuuren, MD(3)

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- (2) CuraVac Europe, Rixensart, Belgium;
- (3) Leiden University Medical Center, Leiden, The Netherlands

In contrast to most other autoimmune disorders, the target of the autoimmune response in myasthenia gravis (MG) is well known. In theory, this offers the possibility of a targeted immune therapy specific for autoimmune MG. So far, the mainstay of the therapy for this disorder consists of symptomatic or general immunosuppressive treatment.

Patients with acetylcholine receptor (AChR) MG have antibodies directed against the main immunogenic region (MIR) and in particular to the amino acid sequence 61 to 76 of the alpha subunit. Based on the molecular recognition theory a complementary peptide to this amino acid sequence of the MIR was synthesized, the B-peptide. Antibodies against this B-peptide are expected to behave as anti-idiotypic antibodies against the pathogenic AChR antibodies. In a similar way a complementary peptide to a T-cell epitope (amino acid 100–116 of the alpha subunit) on the AChR was synthesized, the T-peptide. Antibodies against this T-peptide are expected to interfere with the activation of anti-AChR specific T-cells.

*In vitro* and *in vivo* studies in rats with induced MG and in dogs with spontaneous MG showed a beneficial effect of the injection of these peptides on the course of the disease.

An international consortium ([www.myasterix.eu](http://www.myasterix.eu)) supported by the European Commission under the Health priority of 7th Framework program has developed the use of a combination of both peptides for human administration. A phase 1b clinical trial is ongoing and is intended to be completed in the spring of 2017. The preliminary safety, immunological and clinical data will be presented.

## **Session 9: Upcoming, Ongoing or Future Trials in Myasthenia Gravis**

**Session Chair:** Jeffrey Guptill, MD, Duke University

4:15 PM

### **Overview Introduction**

**Jeffrey Guptill**, MD, Duke University Medical Center

4:25 PM

### **B-cell Targeted Treatment in Myasthenia Gravis (BeatMG): A Phase 2 Trial of Rituximab in Myasthenia Gravis**

**Richard J. Nowak**, MD(1), Christopher Coffey, PhD(2), Jonathan M. Goldstein, MD(3), Mazen M. Dimachkie, MD(4), Michael Benatar, MD(5), Safawa N. Huq, BA(6), Brenda Pearson, BS(2), Laura Herbelin, BS(4), Kevin C. O'Connor, PhD(1), Robin Conwit, MD(7), John T. Kissel, MD(8), David A. Hafler, MD(1), Richard J. Barohn, MD(4), Merit E. Cudkowicz, MD(6), and the NeuroNEXT NN103 BeatMG Study Team\*

- (1) Yale School of Medicine, New Haven, Connecticut, United States;
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\* Additional contributors will be acknowledged during presentation.

The specific primary objective of the BeatMG study is to determine whether rituximab is a safe and beneficial therapeutic for MG that warrants further study in a phase 3 efficacy trial. The study is coordinated by NeuroNEXT (Network for Excellence in Neuroscience Clinical Trials) with support and funding from the National Institute of Neurological Disorders and Stroke. The primary outcome measure is steroid sparing effect, defined as the proportion of participants achieving a  $\geq 75\%$  reduction in mean daily prednisone dose in the four weeks prior to week 52. Secondary outcomes focus on whether there is a trend toward clinical benefit as measured by the MG Composite and the Quantitative MG scores. This study also affords a unique opportunity to study both drug and disease mechanisms. Biomarker work has received support from the Myasthenia Gravis Foundation of America and the National Institute of Allergy and Infectious Diseases. The BeatMG study is a multicenter randomized, double-blind, placebo controlled phase 2 clinical trial utilizing a futility design and includes participants with acetylcholine receptor (AChR) antibody positive generalized MG on at least 15 mg/day of prednisone. We planned to enroll a total of 50 participants (1:1 randomization) with a follow-up of 52 weeks. The study was opened to enrollment in May 2014 and completed screening visits for 68 participants resulting in 52 total randomizations. Randomization target was reached within the projected two-year enrollment window. The study is now closed to enrollment while study visits remain in progress. Baseline study data will be presented.

4:45 PM

### **AChR-specific Immunosuppressive Therapy of Myasthenia Gravis**

**Jon M. Lindstrom**, PhD and Jie Luo, PhD

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Myasthenia gravis (MG) and its animal model experimental autoimmune myasthenia gravis (EAMG) are caused by antibody-mediated autoimmune responses to the extracellular domain of muscle acetylcholine receptors (AChRs). The ideal therapy for MG would quickly and permanently suppress only the pathological autoimmune response to AChRs. We have developed a therapy for EAMG that specifically suppresses the pathological autoimmune response to the extracellular domain of AChRs by immunizing rats with the cytoplasmic domains of the subunits of human muscle AChR. The therapy prevents induction of EAMG, rapidly suppresses ongoing EAMG, is potent, robust, long lasting, safe

because it uses antigens that do not induce EAMG, and likely to be free of the side effects of nonspecific immunosuppressive therapy. Mechanisms of suppression may involve both antibody-mediated feedback suppression and induction of regulatory T-cells. Therapy prevents re-induction of EAMG by immunization with Torpedo AChR six months later through causing switching of antibodies to the main immunogenic region in the extracellular domain to isotypes that fix complement poorly.

5:05 PM

### **Learning from the Past: Reflections on Recently Completed MG Trials**

**Michael Benatar, MD, PhD**(1), James F. Howard, Jr, MD(2), Richard Barohn, MD(3), and Gil Wolfe, MD(4)

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- (2) University of North Carolina, Chapel Hill, North Carolina, United States
- (3) University of Kansas Medical Center, Kansas City, Kansas, United States
- (4) University at Buffalo SUNY, Buffalo, New York, United States

Recently completed clinical trials have varied widely by design, but also perhaps in less explicit ways. This presentation will explore the ways in which these design characteristics may have impacted results and the implications for forthcoming studies. Eligibility criteria may yield study populations with characteristics that are, at least partially, unintended. For example, limiting the permissible number of prior immunosuppressive drugs is likely to yield a population with relatively shorter disease duration. While eligibility criteria aim to define the population in which the clinical trial will be performed, they also impact generalizability of results and define the ultimate therapeutic indication. Eligibility criteria that are too restrictive may impede recruitment, but other factors (e.g. placebo, access to open-label drug, etc.) may also adversely affect recruitment. Eligibility criteria also determine the characteristics of the study population, and these may in turn influence the ability of a trial to demonstrate a therapeutic effect. For example, patients with relatively mild disease may have less potential for further lowering of the Quantified Myasthenia Gravis (QMG) scores. Other aspects of study design – e.g. treatment duration, selection of primary outcome measure, strategy for prednisone tapering, statistical methodology – may profoundly influence results. Steroid-sparing effects may not be apparent if treatment duration is too short, for example,. Similarly, steroid tapering strategies require only clinical improvement rather than minimal manifestation status, might differentially impact ability to detect a steroid-sparing effect. Finally, variability in study design characteristics are also likely responsible for varying magnitude of placebo effects.

5:25 PM

### **Myasthenia Gravis Foundation of America Philanthropist of the Year Award**

#### **Presentation: Honoring Mona Roth**

Nancy Law, Myasthenia Gravis Foundation of America, and Robert Ruff, MD, PhD, Case Western Reserve University School of Medicine

## Session 10: Acetylcholine Receptor Animal Models

**Session Chair:** Linda L. Kusner, PhD, George Washington University

### 8:30 AM **Acetylcholine Receptor Animal Models and Complement**

**Linda L Kusner**, PhD, Manjitha Sengupta, PhD, and Henry J. Kaminski, MD

George Washington University, Pharmacology and Physiology Department, Neurology, Washington, DC, United States

The animal models of myasthenia gravis (MG) have been useful in understanding the pathophysiology of myasthenia gravis and in therapeutic assessment. The prime disease mechanism in acetylcholine receptor-specific MG is the destruction of the neuromuscular junction through complement-mediated injury. Experimental autoimmune MG (EAMG) and passive transfer MG (PTMG) have clear defined use and limitations. Several studies confirm the activity of complement at the neuromuscular junction in EAMG and MG. As well, complement regulatory deficient mice show alterations in cytokine profile and immunoglobulin subclass switch due to EAMG induction. Also, complement regulatory proteins and complement components alter T cells responses. Therapeutics directed at complement inhibition ablates the weakness in EAMG and PTMG through the reduction in the membrane attack complex. Complement involvement in animal models of MG demonstrates the complexity of the disease.

### 8:50 AM **Pathogenic Mechanisms and Treatment Strategies for AChR-MG in Preclinical Models**

**Pilar Martinez Martinez** and Mario Losen

Department of Psychiatry and Neuropsychology, School of Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands

Myasthenia gravis (MG) with antibodies against the acetylcholine receptor (AChR-MG) is an autoimmune disease characterized by the reduction of functional muscle AChRs at the postsynaptic membrane. Rapsyn is crucial for the stabilization of the AChR and has an important role in the maintenance of the adult neuromuscular junction (NMJ). In rats and mice, expression of rapsyn increases with age and thereby stabilizes the AChR. In a passive transfer rat model for MG, increased expression of rapsyn at the NMJ induces resistance against AChR antibodies by reducing antibody-induced AChR internalization. Conversely, rapsyn upregulation has a detrimental effect in the chronic experimental autoimmune myasthenia gravis (EAMG) model where endplates are already substantially damaged. In chronic EAMG, increased rapsyn expression increases AChR turnover by antibodies. Interestingly reduced expression of rapsyn without the autoimmune attack leads to a compensatory increase of postsynaptic folding, thus highlighting the plasticity of the adult NMJ. Similarly, reduced expression of Dok-7 (downstream of kinase 7) increases the susceptibility to passive transfer MG, by rendering AChR clusters less resistant to the autoantibody attack. Besides their importance for determining susceptibility and resilience of the NMJ, both the EAMG as well as the passive transfer MG model are also useful to study experimental treatment strategies. These strategies include for example killing autoreactive plasma cells with proteasome inhibitors and protecting the AChR with monovalent antibody formats that block binding of pathogenic autoantibodies.

9:10 AM

### **Toll-like Receptor (TLR) Agonists to Induce Experimental Autoimmune Myasthenia Gravis (EAMG)**

Marieke Robinet, MSc, Bérengère Villeret, MSc, Solène Maillard, MSc, Mélanie Cron, MSc, Sonia Berrih-Aknin, PhD, and **Rozen Le Panse**, PhD

<sup>1</sup>UMRS 974 UPMC/INSERM/CNRS/AIM, Myology Centre for Research, Paris, France

Myasthenia Gravis (MG) positive for anti-acetylcholine receptor (AChR) antibodies is very often associated with ectopic germinal center development (thymic hyperplasia). Anti-AChR antibodies are also observed in the classical experimental autoimmune MG (EAMG) mouse model obtained by immunizations with purified torpedo AChR (T-AChR) but unfortunately mice do not display thymic abnormalities.

We previously demonstrated that injections of Poly(I:C), a synthetic double-stranded RNA mimicking viral infection, induce thymic changes (increased expressions of  $\alpha$ -AChR, interferon- $\beta$  and chemokines such as CXCL13 and CCL21 leading to B-cell recruitment) and induce MG symptoms in C57Bl6 mice. In order to develop an experimental MG model associated with thymic hyperplasia, we used Poly(I:C) in the EAMG model induced by T-AChR injections. We observed that Poly(I:C) strongly favored the development of MG as nearly all mice displayed MG symptoms just after the second T-AChR immunization. Nevertheless, we did not observe any thymic abnormalities.

We next challenged mice with Poly(I:C) together with other toll-like receptor (TLR) agonists known to be involved in germinal center development and whose receptors were overexpressed in MG thymuses. Imiquimod that activates TLR7 signaling did not induce thymic changes. In contrast, LPS that activates TLR4, potentiated Poly(I:C) effects and induced a huge expression of CXCL13 in the thymus associated with a higher recruitment of B cells. Further experiments are ongoing to determine if prolonged injections of LPS together with Poly(I:C) could lead to thymic germinal center development as this model appears to better mimic the human pathology than the classical EAMG model.

9:30 AM

### **Electrophysiology of Myasthenia Gravis Animal Models**

**Jaap J. Plomp**, PhD

Leiden University Medical Center, The Netherlands

Electrophysiological investigations have been crucial for the understanding of the basic and plasticity mechanisms of neurotransmission at synapses. Historically, the neuromuscular junction (NMJ) has served as a model synapse, owing to its relatively easy experimental accessibility. Consequently, the electrophysiological dysfunction of the NMJ in myasthenia gravis (MG), due to the autoimmune attack on acetylcholine receptors (AChRs) or other crucial NMJ components such as muscle-specific kinase (MuSK), has been well-investigated and described in great detail. Many aspects of the fatigable muscle weakness in MG can thus be explained by the observed aberrant NMJ electrophysiology. In this presentation I will review the electrophysiological methods that can be used to assess NMJ function and discuss their application in the study of NMJs in animal models for MG. Special focus will be on homeostatic changes observed at myasthenic NMJs. For instance, a compensatory increase in presynaptic acetylcholine release occurs at NMJs in AChR MG, apparently in an attempt to counteract the primary postsynaptic defect. However, such a homeostatic response seems not or less clearly present at NMJs in MuSK MG. This suggests that MuSK or one of its interacting partner molecules may be involved in the homeostatic mechanism that senses loss of postsynaptic neurotransmitter sensitivity and instructs the nerve terminal to increase acetylcholine release.

## Session 11: Other Animal Models in Myasthenia Gravis

**Session Chair:** William Donald Phillips, PhD, University of Sydney, Australia

### 10:20 AM **Does Anti-MuSK Cause Myasthenia Simply by Inhibiting Agrin/MuSK Signaling?**

**William (Bill) D. Phillips**, PhD(1), Stephen W. Reddel, FRACP, PhD(2), Marco Morsch, PhD(1,3), Nazanin Ghazanfari, PhD(1), and Sofie Trajanovska, PhD(1)

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- (2) Concord Hospital, Sydney, New South Wales Australia;
- (3) Macquarie University, Sydney, New South Wales, Australia

MuSK autoantibodies are predominantly IgG4. They block the assembly/activation of the agrin-LRP4-Muscle Specific Kinase (MuSK) transmembrane protein complex. Repeated injections IgG from anti-MuSK-positive myasthenia gravis (MG) patients into mice resulted in declines in the number of postsynaptic acetylcholine receptors (AChR) and in the endplate potential amplitude. This culminated in failure of neuromuscular transmission and whole body weakness. Treating such mice with the cholinesterase inhibitor, pyridostigmine, exacerbated the loss of AChR. These findings are consistent with our developing understanding of the physiological mechanisms that control the extent of postsynaptic differentiation at the neuromuscular junction. In anti-AChR MG the reduced postsynaptic responsiveness to acetylcholine triggers an increase in the amount of acetylcholine released by the nerve terminal. This presynaptic adaptation failed in mouse models of anti-MuSK MG, suggesting that postsynaptic MuSK helps provide adaptive feedback to the nerve terminal. Adeno-associated viral vectors were used to supplement expression of MuSK in mouse muscles. In our mouse model, this recombinant MuSK protected motor endplates from anti-MuSK-induced loss of AChRs. Experimentally elevating MuSK expression in muscles of healthy mice caused unexpected modifications in nerve-evoked muscle contraction, suggesting an expanded role for MuSK in the physiological regulation of neuromuscular transmission.

### 10:40 AM **Anti-MuSK IgG4 in the Passive Transfer Model**

**Jan Verschuuren**, MD, PhD(1), Jaap J. Plomp, PhD(1), Steve Burden, PhD(2), Wei Zhang, PhD(2), Yvonne Fillié-Grijpma(1), Inge Stienstra-van Es(1), Erik Niks, PhD(1), Mario Losen, PhD(3), Silvère M. van der Maarel, PhD(1), and Maartje Huijbers, PhD(1)

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- (3) Maastricht University Medical Center, Maastricht, The Netherlands

Myasthenia gravis (MG) with antibodies to muscle-specific kinase (MuSK) is characterized by fluctuating fatigable weakness that is clinically different from the acetylcholine receptor (AChR) MG. In MuSK MG involvement of bulbar muscles, neck, shoulder and respiratory weakness is more prominent than in AChR MG. MuSK antibodies are of the IgG4 subclass, which is unable to activate complement, in contrast to the mainly IgG1 and IgG3 complement activating antibodies in AChR MG. IgG4 has a low affinity for Fcγ and is functionally monovalent. Passive transfer studies using plasmapheresis fluid from MuSK MG patients showed that the purified IgG4 fraction was able to induce myasthenic disease in NOD/SCID mice, with clear clinical, electrophysiological as well as histological signs of

disease. The human MuSK IgG4 antibodies were shown to interfere with agrin-dependent association between MuSK and Lrp4. The IgG4 MuSK antibodies did not inhibit MuSK dimerization, nor did we find indications of depletion of MuSK cell surface expression. The human IgG1-3 MuSK antibodies did not show any of these pathogenic activities. A role for IgG1 MuSK antibodies cannot be excluded, but seems rather small or only relevant in rare patients. Thus, blocking of the essential MuSK-Lrp4 interaction seems to be the most important mechanism in human MuSK MG. Human MuSK antibodies preferentially bind the first Iglike-1 domain of MuSK, and are significantly correlated with disease severity, in contrast to antibodies binding to epitopes outside this domain. Altogether this provides a rationale for epitope specific treatment strategies.

11:00 AM

### Experimental Studies on Seronegative Myasthenia

**Angela Vincent**<sup>1</sup>, Saif Huda, Michelangelo Cao, Hakan Cetin, Richard Webster, Judith Cossins, and David Beeson

Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

Seronegative myasthenia gravis is still not fully understood. Recent developments in cell-based assays for antibodies to clustered AChR, MuSK and LRP4, have led to an increase in the number of patients in whom an antibody is detected, but the serum levels are often relatively low, and it is not always easy to assess their importance. Passive transfer studies are one way of demonstrating pathogenicity (e.g. Viegas et al. *Exp Neurol*, 2012; Jacob et al. *JAMA Neurol*, 2012) but they require large amounts of antibody which are seldom available except when plasmapheresis has been performed. By contrast, the C2C12 mouse myotubes have proved a major source of understanding of the agrin/LRP4/MuSK/Dok7 clustering pathway. Incubation in agrin for a few hours leads to clusters of AChRs on the surface that can be counted. The effects of MuSK and LRP4 antibodies on the clustering process can be measured, and the interactions between components of the pathway, and their phosphorylation, can be studied by immunoprecipitation and western-blotting. Using these approaches, we have shown that both IgG1-3 and IgG4 subclasses can inhibit AChR clustering but only IgG4 MuSK antibodies inhibit the LRP4/MuSK interaction and MuSK phosphorylation, suggesting that IgG1-3 MuSK antibodies act by a different mechanism. Nevertheless, a phosphatase inhibitor that enhances MuSK phosphorylation increases AChR clustering in the absence or presence of MuSK serum antibodies, and could provide a useful adjunctive therapy for MuSK-MG. These and further studies looking for evidence and effects of other antibody targets are in progress.

11:20 AM

### **Using the Mouse Passive Transfer Model of Lambert–Eaton Myasthenic Syndrome (LEMS) to Explore Disease Pathology and Test New Therapeutic Leads**

**Stephen D. Meriney**, PhD(1), Tyler B. Tarr, PhD(1), Man Wu, MS(1), Kristine S. Ojala, BS(1), Christopher J. Meriney, BS(1), David Lacomis, MD(2), and Stephen W. Reddel, MD(3)

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- (3) Department of Clinical Neurology, Concord Hospital, Sydney, NSW, Australia

LEMS is an autoimmune disorder that targets presynaptic proteins at the neuromuscular junction (NMJ) (notably presynaptic calcium channels). Passive transfer of LEMS to mice has helped to confirm that IgG is responsible for the neuromuscular weakness, and has documented many pathological details of LEMS, including reduced action potential-triggered transmitter release, reduced frequency of spontaneous release, disruption in the ordered array of active zone proteins, reduction in the number of active zone proteins, reduced calcium entry into the nerve terminal, and changes in the types of calcium channels expressed at motor nerve terminals. This mouse model also presents the opportunity to evaluate novel symptomatic treatment approaches for LEMS in an animal model that recapitulates the IgG-mediated attack at the NMJ. We previously reported the development of novel Cav2 gating modifiers (GV-58) that prolong channel deactivation. These compounds stabilize the open configuration of the channel, increasing calcium flux during an action potential. Currently, patients are often treated using diaminopyridine (DAP), which provides modest symptomatic relief for LEMS patients. Using this mouse model, we have shown that a combination of DAP plus GV-58 works synergistically to provide a more complete relief of neuromuscular weakness. We are currently using the LEMS passive transfer model in mice to examine the *in vivo* efficacy of these symptomatic treatment approaches on behavior. Limitations of this approach include the need for significant quantities of LEMS patient serum or plasma, and generally mild behavioral alterations in mice that are highly dependent on the specific patient serum used for passive transfer.

11:40 AM

### **Lrp4 and Agrin Antibodies in Myasthenia Gravis**

**Lin Mei**, MD, PhD, Min Yan, MD, Jinxiu Pan, MS, Brandy Quarles, BS, Michael Rivner, MD, and Wen-Cheng Xiong, MD, PhD

Department of Neuroscience and Regenerative Medicine, Department of Neurology, Medical College of Georgia, Augusta University, Augusta, Georgia, United States

Myasthenia gravis (MG) is the most common disorder of the neuromuscular junction (NMJ). MG can be diagnosed by detecting anti-AChR or MuSK antibodies (Abs). However, some MG patients lack these Abs (thereafter called double negative MG, DNMG). We have detected Agrin and LRP4 Abs in DNMG patients in line with independent reports, identifying novel potential causes to DNMG. Indeed, we showed that inducing LRP4 Abs in mice or transferring rabbit anti-LRP4 Abs to mice leads to muscle weakness and NMJ degradation. However, the clinical significance of these findings is unclear because the Ab prevalence is not available for Agrin Abs and is variable for LRP4 Abs, probably due to geographic or ethnic differences, limited numbers of DNMG patients, or variation in diagnosis criteria. We have established a collaboration with 16 MG centers in the United States with >700 DNMG patients. Characterization of this large cohort will better characterize these Abs and contribute to the development of better diagnostic and therapeutic strategies against DNMG. To determine whether Agrin Abs are pathogenic to

MG, we injected mice with recombinant Agrin. We found that Agrin-injected mice produced anti-Agrin Abs and reduced in muscle strength. Concomitantly, NMJs became fragmented and neuromuscular transmission was reduced. Intriguingly, these deficits were observed only in mice injected with neural Agrin, but not those injected with muscle Agrin that lacks 8-residue insert. *In vitro*, anti-neural agrin Abs inhibited agrin-induced AChR clustering in muscle cells. Together, these observations indicate that anti-agrin autoantibodies may be causal to MG.

12:00 PM      **Remembering Claudio Mazia, MD, University of Buenos Aires, Argentina**

## **Session 12: Day 3 Hot Topic Short Talks (Selected from Submitted Abstracts)**

**Session Chair:** Vera Bril, FRCPC, MD, University of Toronto

1:30 PM      **Rituximab Downregulates Antigen-specific T Cell Repertoire in Myasthenia Gravis Patients**

Mariapaola Marino, MD, PhD(1), Alessia Piermattei, PhD(1), Mariagrazia Valentini, PhD(1), Gabriele Di Sante, MD, PhD(1), Paolo Emilio Alboini, MD(2), Konstantinos Lazaridis, PhD(3), Socrates Tzartos, PhD(3), Francesco Ria, MD, PhD(1), Amelia Evoli, MD(2), and **Emanuela Bartocconi**, PhD(1)

- (1) Institute of General Pathology, School of Medicine, Università Cattolica S. Cuore, Rome, Italy;
- (2) Institute of Neurology, School of Medicine, Università Cattolica S. Cuore, Rome, Italy;
- (3) Department of Neurobiology, Hellenic Pasteur Institute, Athens, Greece

Rituximab (RTX), a monoclonal anti-CD20 immunoglobulin, proved effective in a high proportion of myasthenia gravis (MG) patients refractory to conventional immunosuppression, with greater benefit being reported in those with antibodies to muscle specific kinase (MuSK-MG). Interestingly, the therapeutic benefit of B cell depletion appears disproportionately larger than the effect on circulating autoantibody titers. Here we tested the hypothesis that a change of T cell receptor (TCR) repertoire occurs in MuSK-MG patients after RTX therapy. We used CDR3 TRBV-TRBJ spectratyping (immunoscope) to profile TCR response to recombinant human MuSK in PBMCs from five HLA-DQ5+ MuSK-MG patients before and after treatment with Rituximab. A specific set of four TCR VJ rearrangements (TRBV29-TRBJ2.5, TRBV28-TRBJ2.1, TRBV3-TRBJ1.2, TRBV28-TRBJ1.2) was analyzed, based on our previous observation of a restricted TCR repertoire. We confirmed that these semiprivate rearrangements were differently shared by all five patients in response to MuSK stimulation before RTX administration. When the same rearrangements were interrogated in samples collected after therapy, no expanded peak in response to stimulation with the antigen was observed in most cases; this effect was still evident after six and twelve months from drug infusion. Far from being mere antibody-producing cells, B cells are also able of antigen-presentation. Our results suggest that a global disruption of B cell activity, and in particular of T and B cell crosstalk and cooperation, contributes to the success of B cell depleting therapeutic strategies. Alternatively, a direct action of RTX on autoreactive T cell cannot be ruled out.

1:40 PM

**Presynaptic Congenital Myasthenic Syndrome with a Homozygous Sequence Variant in *LAMA5* Combines Myopia, Facial Tics and Failure of Neuromuscular Transmission**

**Ricardo A. Maselli**, MD(1), Juan Arredondo, PhD(1), Jessica Vázquez, BS(1), Jessica X. Chong, PhD(2), Michael J. Bamshad, MD(2,3,4), Deborah A. Nickerson, PhD(3), Marian Lara, BS(1), Fiona Ng, BS(1), Victoria Lee Lo, BS(1), Peter Pytel, MD(5), and Craig M. McDonald, MD(6)

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- (6) Department of Medicine and Rehabilitation, University of California Davis, Sacramento, California, United States

Defects in genes encoding the isoforms of the laminin alpha subunit have been linked to various phenotypic manifestations, including brain malformations, muscular dystrophy, ocular defects, cardiomyopathy and skin abnormalities. We report here a severe defect of neuromuscular transmission in a consanguineous patient with a homozygous variant in the laminin alpha-5 subunit gene (*LAMA5*). The variant c.8046C>T (p.Arg2659Trp) is rare and has a predicted deleterious effect. The affected individual, who also carries a rare homozygous sequence variant in *LAMA1*, had muscle weakness, myopia and facial tics. Cognitive function was intact, but magnetic resonance imaging of brain showed mild volume loss and periventricular T2 prolongation. Failure of neuromuscular transmission was demonstrated by repetitive nerve stimulation at 2 Hz, which showed 50% decrement of compound muscle action potential amplitudes and 250% facilitation immediately after exercise, similar to that seen in the Lambert Eaton Myasthenic Syndrome (LEMS). Endplate studies performed in an anconeus muscle biopsy revealed profound reduction of the endplate potential quantal content, but normal amplitudes of miniature endplate potentials. Electron microscopy showed endplates with increased postsynaptic folding that were denuded or only partially occupied by small nerve terminals. Expression studies revealed that p.Arg2659Trp caused decreased binding of laminin alpha-5 to SV2A and impaired laminin-521 cell-adhesion and cell projection support in primary neuronal cultures. In summary, this report describing severe neuromuscular transmission failure in a patient with a *LAMA5* mutation expands the list of phenotypes associated with defects in genes encoding alpha-laminins.

1:50 PM

### **Salbutamol Sustains the Beneficial Effects of Pyridostigmine on Muscle Fatigue and Neuromuscular Junction Structure in Myasthenic Mice**

**An E. Vanhaesebrouck**, DVM(1), Richard Webster, PhD(1), Jonathan Cheung, PhD(1), Hakan Cetin, MD(1), Susan Maxwell(1), James Wickens, PhD(2), and David Beeson, PhD(1)

- (1) Neurosciences group, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom;
- (2) Department of Chemistry, University of Oxford, Oxford, United Kingdom

Recent reports describe patients with congenital myasthenic syndrome benefiting from the add-on of salbutamol ( $\beta$ 2-adrenergic agonist) to their routine treatment of pyridostigmine. The clinical efficacy of acetylcholinesterase inhibitors is known to decline over time. The mechanism for how salbutamol improves muscle fatigue is unclear.

The aim of this study was to investigate in a mouse model of acetylcholine receptor (AChR) deficiency whether salbutamol added to pyridostigmine resulted in improvement of muscle fatigue, neuromuscular transmission and/or neuromuscular junction (NMJ) structure.

The 3-month trial consisted of 4 groups of 15 myasthenic mice, receiving either no treatment, pyridostigmine alone, salbutamol alone, or pyridostigmine to which salbutamol was added mid-trial.

Following an initial improvement in muscle fatigue on the inverted screen test, a gradual decline in the effect of pyridostigmine was observed in mice treated with pyridostigmine alone. Salbutamol, as an add-on drug, significantly counteracted this decline in performance. An improved performance was also observed in the salbutamol-only group compared to the untreated group. Recordings from diaphragm-phrenic nerve preparations suggested that salbutamol enhances neuromuscular transmission maintenance at high-frequency nerve stimulation. Staining of AChRs showed significantly smaller NMJ areas in limb muscles of pyridostigmine-treated mice. Salbutamol, as an add-on drug, counteracted this decrease in NMJ size.

This study demonstrates that salbutamol sustains the beneficial effects of pyridostigmine on muscle fatigue over the long-term, by reversing the structural changes at the NMJ. We hypothesize that  $\beta$ 2-adrenoreceptor activation can play a crucial role in maintaining NMJ stability where the underlying genetic disorder or treatment disturbs synaptic structure.

2:00 PM

### **Inhibition of SHP2 Reverses the Pathogenic Effects of MuSK Antibodies *In Vitro***

**Saif Huda**, MD(1,2), Michelangelo Cao, MD(1,3), Anna De Rosa, MD(4), Mark Woodhall, PhD(1), Judith Cossins, PhD(1), Michelangelo Maestri, MD(4), Roberta Ricciardi, MD(4), David Beeson, PhD(1), and Angela Vincent, FRCPath(1)

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- (3) Department of Neuroscience, University of Padua, Padua, Italy;
- (4) Department of Clinical and Experimental Medicine, Neurology Unit, Pisa, Italy

**Background:** Muscle-specific kinase (MuSK) antibodies (Abs) inhibit the agrin/LRP4/MuSK/DOK7 AChR clustering pathway through MuSK/LPR4 disruption. MuSK

phosphorylation (MuSK-P) is a critical step in this pathway and is negatively regulated by SH2 domain-containing phosphatase (SHP2). We assessed whether SHP2 inhibition alleviated negative effects of MuSK-Abs on MuSK-P and AChR cluster formation, dispersal, and recovery in cultured myotubes.

**Methods:** C2C12 and DOK7 overexpressing myotubes were incubated with agrin, the SHP2 inhibitor NSC-87877 (100 $\mu$ M; Tocris), purified IgG, IgG1-3, IgG4 fractions from MuSK-MG plasmas (n=2), or MuSK-MG sera (n=31; 0.25-24.96nM). To assess MuSK-P, MuSK expression and tyrosine phosphorylation were detected by western blotting, and phosphorylation expressed as the ratio of MuSK phosphotyrosine to total MuSK expression. For AChR cluster quantification, myotubes were labelled with  $\alpha$ -bungatatorxin Alexa Flour-594 followed by image acquisition and analysis with ImageJ software.

**Results:** MuSK-MG IgG4 fractions reduced agrin dependent MuSK-P compared to agrin treated myotubes (p=0.03), which was increased with addition of NSC-87877 (p<0.0001). NSC-87877 increased the number of agrin induced AChR clusters in myotubes incubated with MuSK-MG sera (1:30; p<0.0001). SHP2 inhibition also prevented dispersal of preformed agrin dependent AChR clusters in C2C12 (p=0.0003) and DOK7 overexpressing myotubes (p<0.0001). Lastly, following dispersal by MuSK-MG sera (1:30), AChR cluster reformation was enhanced by NSC-87877 (p=0.008).

**Conclusions:** MuSK-IgG4 inhibits agrin dependent MuSK phosphorylation but this can be rescued by NSC-87877. SHP2 inhibition restores formation, blocks dispersal and increases recovery of AChR clusters following dispersal by MuSK antibodies. Targeted modulation of the agrin/MuSK/LRP4/DOK7 pathway may represent a novel therapeutic strategy in MuSK-MG.

2:10 PM

### **Hinge-deleted IgG4 Blocker Therapy for Acetylcholine Receptor Myasthenia Gravis in Rhesus Monkeys**

Mario Losen, PhD, Maastricht University, the Netherlands

**Mario Losen**, PhD(1)\*, Aran F. Labrijn, PhD(2)\*, Vivianne H. van Kranen-Mastenbroek, MD, PhD(3)\*, Maarten L. Janmaat, PhD(2), Krista G. Haanstra, PhD(4), Frank J. Beurskens, PhD(2), Tom Vink, PhD(2), Margreet Jonker, PhD(4,5), Bert A. 't Hart, PhD(4,6), Marina Mané-Damas, MSc(1), Peter C. Molenaar, PhD(1), Pilar Martinez-Martinez, PhD(1), Eline van der Esch(1), Janine Schuurman, PhD(2), Marc H. de Baets, MD, PhD(1,7), and Paul W.H.I. Parren, PhD(2,5)

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Autoantibodies against ion channels are the cause of numerous neurologic autoimmune disorders. Frequently, such pathogenic autoantibodies have a restricted epitope-

specificity. In such cases, competing antibody formats devoid of pathogenic effector functions (blocker antibodies) have the potential to treat disease by displacing autoantibodies from their target. Here, we have used a model of the neuromuscular autoimmune disease myasthenia gravis in rhesus monkeys (*Macaca mulatta*) to test the therapeutic potential of a new blocker antibody: MG was induced by passive transfer of pathogenic acetylcholine receptor-specific monoclonal antibody IgG1-637. The effect of the blocker antibody (IgG4Δhinge-637, the hinge-deleted IgG4 version of IgG1-637) was assessed using decrement measurements and single-fiber electromyography. Three daily doses of 1.7 mg/kg IgG1-637 (cumulative dose 5 mg/kg) induced impairment of neuromuscular transmission, as demonstrated by significantly increased jitter, synaptic transmission failures (blockings) and a decrease in the amplitude of the compound muscle action potentials during repeated stimulations (decrement), without showing overt symptoms of muscle weakness. Treatment with three daily doses of 10 mg/kg IgG4Δhinge-637 significantly reduced the IgG1-637-induced increase in jitter, blockings and decrement. Together, these results represent proof-of principle data for therapy of acetylcholine receptor-myasthenia gravis with a monovalent antibody format that blocks binding of pathogenic autoantibodies.

## Session 13: Treatment Guidelines from Around the World

**Session Chair:** Donald B. Sanders, MD, Duke University Medical Center

2:50 PM **International Consensus Guidance Statements for Myasthenia Gravis Treatment**

**Donald B. Sanders**, MD(1), Gil I. Wolfe, MD(2), and Pushpa Narayanaswami, MBBS,DM(3), for the Myasthenia Gravis Foundation of America Task Force on MG Treatment Guidance

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In 2013, the Myasthenia Gravis Foundation of America appointed a Task Force to develop guidance statements for myasthenia gravis (MG) based on expert consensus. A panel of 14 international experts was selected based on clinical experience and publications on the treatment of MG. The RAND/UCLA appropriateness method (RAM) was used to develop consensus, and a non-voting moderator facilitated this process. Standard definitions were first developed for the following terms to be used in the consensus guidance statements during subsequent deliberations: goals of MG treatment, minimal manifestations, remission, ocular MG, impending crisis, crisis and refractory MG. At an in-person meeting, the panel then identified the following 7 treatment topics for which consensus guidance statements were developed: symptomatic and immunosuppressive treatments, intravenous immunoglobulin and plasma exchange, management of impending and manifest myasthenic crisis, thymectomy, juvenile MG, MG associated with antibodies to muscle specific tyrosine kinase, and MG in pregnancy. Draft guidance statements for each of these topics were developed based on published literature, existing national and regional MG treatment guidelines, and personal experience of the panel. Consensus guidance statements for each topic were finalized with up to three rounds of anonymous e-mail votes, in each of which modifications were made based on panel input, using the RAM method for calculating agreement. An Executive Summary of the consensus process and final guidance statements to guide clinicians caring for MG patients worldwide was published

with open access in August 2016 (*Neurology* 2016;87:419-425). The full document with extensive background information is available on-line.

3:00 PM

### **Treatment Guidelines for Myasthenia Gravis in Japan**

**Hiroyuki Murai, MD, PhD(1) and Kimiaki Utsugisawa, MD, PhD(2)**

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Treatment of myasthenia gravis (MG) has depended on high-dose oral corticosteroids. Although this procedure remarkably reduced the mortality rate and increased the improvement rate of MG, it brought up issues such as adverse-effects of steroids and impairment of quality of life (QOL). Along with the recent discovery of novel autoantibodies, numbers of alternative therapeutic options have become available. The recent Japanese clinical guidelines for MG were published in 2014, and they proposed a novel strategy utilizing these aggressive therapeutic options in the early stage of the disease, later nomenclated as the “early fast-acting treatment (EFT)” strategy, which may replace high-dose oral steroids. According to the recent multi-center study in Japan, the EFT strategy achieved the treatment goal (minimal manifestation status or better, with prednisolone dose of 5mg or lower) earlier and more frequently compared to non-EFT group with a hazard ratio of 1.98. As the fundamental philosophy, the guidelines recommend are as follows: Since full remission is difficult to achieve in adult-onset MG, treatment strategies should take into account the fact that treatment might be prolonged, and aim for maintaining health-related QOL and mental health. To fulfill this recommendation, the EFT strategy might be one of the good choices. Regarding thymectomy, the recommendations are: thymectomies might be effective and could be considered for non-thymomatous early onset MG patients in cases of anti-AChR antibody positive and thymic hyperplasia in the early stages of the disease. Thymectomy is not considered first-line treatment for non-thymomatous late-onset MG, and the procedure should be considered carefully in these cases.

3:20 PM

### **The Myasthenia Gravis Guidelines of the Association of British Neurologists**

**Jon D. Sussman, MB, ChB, PhD, FRCP(1), Maria E. Farrugia, DPhil, MD(2), Paul Maddison, MBBS, PhD, FRCP(3), M. Isabel Leite, MD, DPhil(5), and David Hilton-Jones, MA, MB, ChB, MD, FRCPE, FRCP(5)**

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Myasthenia gravis is an autoimmune disease for which many therapies were developed before the era of evidence-based medicine. The basic principles of treatment are well known, however, patients continue to receive suboptimal treatment as a result of which a myasthenia gravis guidelines group was established under the aegis of The Association of British Neurologists.

The guidelines attempt to steer a path between evidence-based practice where available, and established best practice where evidence is unavailable. Where there is insufficient evidence or a choice of options, the guidelines invite the clinician to seek the opinion of a myasthenia expert. The guidelines support clinicians not just in using the right treatments in the right order, but in optimising the dosing of well-known therapeutic agents. The guidelines have been designed to help clinicians avoid the most common errors of myasthenia management, including inappropriate expectations of Pyridostigmine, inadequate dosing, too-rapid tapering of corticosteroids, and inappropriate dosing and expectations of immunosuppressive agents.

3:40 PM

### Unique Treatment Challenges in South America

**Valeria L. Salutto**, MD(1), Valeria C. Alvarez, MD(2), Gabriel Cea, MD(3), Mariela Bettini, MD(4), Florencia Aguirre, MD(5), Marcelo Rugiero, MD(4), Márcia Waddington-Cruz, MD, PhD(6), Oscar del Bruto, MD(7), Abayuba Perna, MD(8), Ricardo Reisin, MD(9), Roberto Rey, MD(10), Fabio Barroso, MD(11), Martín Nogués, MD(11), and Claudio G. Mazia, MD(1)

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- (11) FLENI, Buenos Aires, Argentina

**Introduction:** Myasthenia gravis (MG) is the most common autoimmune disease of the neuromuscular junction. The epidemiology of MG in South America (SA) is incompletely known, therefore we analyzed MG prevalence and incidence, and the challenges physicians face in treating MG patients in several South American countries.

**Materials and Methods:** We searched PubMed and Lilacs for studies of prevalence of MG published in English, Spanish and Portuguese. We also conducted a survey among neuromuscular specialists to ascertain the prevalence of MG and the challenges they face in treating MG.

**Results:** We found data from Colombia (6°N/75°W) that show a prevalence of 2.77/100,000 and from Chile (33°N/ 70°W) where the prevalence is 8.36/100,000. In the northwest of Argentina (28°S/ 62°W) the prevalence rate was 37.5/100,000 and the incidence was 5.5/100,000. In the city of Buenos Aires (34°S/ 58°W) the prevalence was 36.71/100,000 and the incidence 3.7/100,000. Surveys results identified problems in diagnosis and treatment of MG. Some countries lack a national register. Anti-MuSK tests are only available in Argentina and Brazil, limiting patient categorization. Most specialists in MG are based in the capital cities in the huge South American territory (17.84 million-km<sup>2</sup>). Generic drugs that are commonly used may not have the same therapeutic effect as brand drugs: this could explain why we sometimes use high steroids doses.

**Conclusions:** The epidemiological data suggest a probable South-North gradient between parallels 34°S and 6°N. Many patients do not have access to adequate diagnosis and

treatment and there may be high financial costs for patients. Enhancing the network of neuromuscular specialists could help to improve the management of MG.

4:00 PM

**Panel Discussion:**

**Comparing and Contrasting Treatment Strategies around the World**

**Moderator:** Henry Kaminski, MD, George Washington University

**Panelists:**

Hiroyuki Murai, MD, International University of Health and Welfare, Japan

Donald B. Sanders, MD, Duke University Medical Center

Jon Sussman, PhD, FRCP, Greater Manchester Neuroscience Centre, United Kingdom

Gil I. Wolfe, MD, University of Buffalo