myopathy called relatively relaxed or ryr. Among other hits, we found up-regulation of several genes associated with oxidative stress. We thus hypothesized that abnormalities in oxidative stress may be a key aspect of this disease. We then tested our hypothesis using several measures of oxidative stress in ryr, and found significant increases in all of them. Based on these data, we next studied the effect of antioxidant therapy on the ryr motor phenotype. We determined that N-acetylcysteine reduced oxidative stress, improved endurance, and ameliorated certain pathologic changes in the fish. Finally, we related these findings back to patients with RYR1 mutations. Using myocytes derived from patient biopsies from a range of RYR1 myopathies, we detected significantly increased levels of basal oxidative stress and increased sensitivity to oxidant exposure. Both abnormalities were reversed with N-acetylcysteine exposure. In all, we demonstrated that increased oxidative stress is an important aspect of the pathogenesis of RYR1-related myopathies, and showed that antioxidant treatment is a viable potential treatment strategy for patients affected with these conditions.

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P4.55
Developing AMPA receptor aptamers as new drug candidates for ALS
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Developing aptamer drugs for a new therapy for amyotrophic lateral sclerosis (ALS) is our ultimate goal. The aptamers are nanomolar affinity, water-soluble RNA inhibitors of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, a subtype of glutamate ion channel receptors. Excessive activation of AMPA receptors induces calcium-mediated excitotoxicity, one of the leading causes underlying the selective death of motor neurons in ALS. To date, we have successfully identified aptamers that inhibit every AMPA receptor subunit – these are competitive and noncompetitive type. We have also identified a non-competitive aptamer that exclusively inhibits GluR2, a key AMPA receptor subunit that mediates excitotoxicity. Because these aptamers possess unparalleled properties, i.e. nanomolar potency, subunit or subtype selectivity and water solubility, as compared to all other existing AMPA receptor inhibitors, they are promising drug candidates for a new and potentially more effective ALS therapy. To turn these aptamers into potentially useful drugs, we have also successfully generated a class of chemically modified aptamers resistant to ribonucleases so that these aptamers can be tested in vivo. This is because, unmodified, RNA aptamers have limited stability in vivo due to their inherent sensitivity to ribonucleases, the enzymes that catalyze their degradation. Therefore, the aptamers we discovered represent water-soluble, highly potent and selective templates for design of better inhibitors as drug candidates for a potential new ALS therapy.

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P4.56
Alleration of a cacostatic stress response slows disease progression in a mouse model of familial ALS
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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron cell loss, muscular atrophy and a shortened lifespan. Patient survival is highly variable as some individuals die within months while others live for many years. Epidemiological evidence suggests that either stress or a non-optimal homeostatic adjustment (i.e. stress response) to disease might account for some of this variability. We show here that recurrent exposure of SOD1G93A mice to stress adversely influences disease progression. Moreover, during normal disease course, ALS mice enter a state of cacostasis by developing an aberrant glucocorticoid circadian rhythm. We also demonstrate that a heightenened glucocorticoid level in ALS mice negatively correlates with lifespan. Importantly, when the glucocorticoid levels are lowered, survival is significantly improved. These results suggest that a cacostatic stress response to disease catalyzes further deterioration and that strategies to modulate glucocorticoid metabolism might be a viable therapeutic approach for ALS.

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P4.57
Effects of air stacking on peak cough flows and forced vital capacity in patients with muscular dystrophy and spinal muscular atrophy
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Respiratory muscle weakness and decreased cough capacity are the main causes of pulmonary complications that result in morbidity and mortality in patients with neuromuscular disorders. The progressive loss of respiratory muscle strength leads to restrictive lung disease and decreased lung volumes. However, the loss of the expiratory muscle strength results in inefficient cough and the accumulation of secretion in common viral respiratory infections. To analyze the effect of manual insufflations on peak cough flows (PCFs) and forced vital capacity (FVC) of patients with spinal muscular atrophy (SMA) type II and III, congenital and limb-girdle muscular dystrophy. We evaluated 34 patients with muscular dystrophy and SMA, 11 patients with SMA type II, 5 patients with SMA type III, 13 patients with congenital muscular dystrophy and 5 with limb-girdle muscular dystrophy. All the patients had never done air stacking before and none of them ever used noninvasive ventilation. The PCF, FVC and maximum insufflation capacity (MIC) of the patients were measured in a sitting position using a portable spirometer. Unassisted peak cough flow and manual assisted technique of assisted PCF were evaluated. The results were compared using Pearson’s correlation test and ANOVA with repeated measures, followed by Tukey’s post hoc test (p < 0.05). The mean age of the patients was 13 ± 6 years. The mean value of MICs (1800 ± 950 mL) was higher than FVC (1560 ± 630 mL) (p < 0.001). There was increase in the mean value of PCF 3.6 ± 2.3 L/s (p < 0.05). There was an important increase in MIC despite the fact the patients were never trained air stacking before.

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P4.58
Muscle fiber type-predominant promoter activity in lentiviral-mediated transgenic mouse
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Variations in gene promoter/enhancer activity in different muscle fiber types after gene transduction was noticed previously, but poorly analyzed. The murine stem cell virus (MSCV) promoter drives strong, stable gene expression in hematopoietic stem cells and several other cells, including cerebellar Purkinje cells, but it has not been studied in muscle. We injected a lentiviral vector carrying an MSCV-EGFP cassette (LvMSCV-EGFP) into tibialis anterior muscles and observed strong EGFP expression in muscle fibers. We also generated lentiviral-mediated transgenic mice carrying the MSCV-EGFP cassette and detected transgene expression in striated muscles. MSCV promoter activity in skeletal muscle is fiber-type-dependent when delivered directly by lentiviral infection as well as in transgenic mice generated by lentiviral infection. Further analysis of this promoter may have the potential to achieve certain gene expression in severely affected muscles of mdx mice. The Lv-mediated transgenic mouse may prove a useful tool for assessing the enhancer/promoter activities of a variety of different regulatory cassettes.

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P4.59
Standard operating procedures for preclinical efficacy studies in animal models of neuromuscular disease
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Disease-specific animal models offer the possibility to test the efficacy of potential therapeutic interventions for neuromuscular diseases. Much data is generated every year reporting important effects and strategies. However, the lack of a consensus on the appropriate animal models and efficacy readouts and the lack of standardized protocols present a major hurdle to compare data from different laboratories. Given the restricted patient population available for clinical trials and the need to avoid duplicating efforts, it is important to create standards that help reducing the risk of false results and accelerate the translation of promising treatments to clinical development. TREAT-NMD aims at overcoming these hurdles by developing standard procedures and guidelines to conduct adequately planned efficacy studies in selected animal models. With the support of patient organizations and research institutes, experts were involved in workshops to reach a consensus on appropriate animal models and readouts. So far, recommendations for preclinical efficacy studies have been developed for Duchenne Muscular Dystrophy (DMD), Spinal Muscular Atrophy and Congenital Muscular Dystrophy. A total of 30 protocols, compiled and approved by several laboratories, are available online under www.treat-nmd.eu, meant to support researchers in assessing a range of histological, physiological, behavioral, biochemical and electrophysiological readouts. Also, a consensus paper on the choice of appropriate animal models for DMD was published 2009 and another one is underway that proposes guidelines for efficacy studies in the mdx mouse model. In summary, the current effort to develop standardized procedures is an important step to overcome limitations of preclinical assessment of possible interventions for neuromuscular diseases. The use of available protocols will certainly facilitate comparison of data and prioritization of emerging therapy strategies in preparation for clinical trials.

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REGISTRIES: POSTER PRESENTATIONS

P4.60
New horizons in the DuchenneConnect registry
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Therapeutic advances and a shift in the role of advocacy foundations reinforce the important role for Duchenne/Becker muscular dystrophy (DBMD) registries. DuchenneConnect is a self-report patient registry, developed to educate and connect patients, providers and researchers and facilitate clinical trials. The Registry is developing a Clinic Services Resource survey (launch April 2011) to report on clinical care in the United States. DuchenneConnect features web-based surveys stored in a HIPAA-compliant database. The Profile survey captures the patient’s clinical presentation and genotype, designed and curated following the TREAT-NMD Neuromuscular Network registry guidelines. The Clinic Services Resource survey, designed in partnership with CARE-NMD, captures the therapeutic services (as recommended in the Care Considerations) reported by clinics and families. Of ~2100 patient participants from 78 countries, ~75% completed the Profile survey. Of those, 83% had genetic testing, and 50% submitted results for curation while 25% described the result. ~50% (~31%) participants are non-ambulatory and ~48% take corticosteroids. 265 professionals have registered. The Clinic Services Resource will be available to ~1400 patient and 198 US professionals. Data is regularly queried to target sub-populations for planning and dissemination of study announcements. Additional DuchenneConnect and preliminary Clinic Resource data will be reported. Numerous novel and traditional therapeutic approaches are under development for DBMD. Care guidelines allow evaluation of clinical care in a standardized way. The improved characterization of patients in DuchenneConnect will continue to provide critical data for researchers and identify gaps in care. By organizing the community, DuchenneConnect enhances disease understanding, reduces fragmentation, and shares key resources (human subjects, patient data, infrastructure, etc.).

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P4.61
The creation of a network after an international conference
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In June 2010, it was held in Brazil an International Conference on Neuromuscular Diseases. Lecturers from several European countries, from the United States and Argentina were present. Brazilian neurologists and other health professionals working on the neuromuscular field attended the meeting. We had the support of TREAT-NMD and of muscular dystrophy and spinal atrophy associations. The Brazilian physicians were moved by the cosmopolitan atmosphere. In August of the same year, it was held the Brazilian Congress of Neurology in Rio de Janeiro. Once again physicians from all over the country interested in the attention of neuromuscular diseases patients met. They agreed with the creation of a national network. The main purposes of that association would be to train professionals to create regional medical centers according to international standards of care and to keep patients clinical data. Those measures will allow the selection of subjects for multicentric clinical trials. The network is called REDE NEUROMUSCULAR BRASILEIRA DE PESQUISA CLINICA-RENBRAPEC (Brazilian network for clinical research on neuromuscular diseases). The first participants are Acary Oliveira, Alexandra Araujo, Arnaldo Godoy, Claudia Sobreira, Edmar Zanoteli, Gloria Penteke, Juliana Giannetti, Luiz Duro, Marcia Cruz, Marco Albuquerque, Maria B. Resende, Suely Marie and Umbertina Reed, from the Southeastern region; Carlo Marone, Francisco Rotta, Rosana Scola and Salmo Raskin, from the Southern region; Carolina Cunha, Francoise Cunha, Mario Donzaleigh and Vanessa van der Linden, from the Northeastern region; Helio van der Linden Jr., from the Central region and Regina Duarte, from the Northern region. Health professionals from different