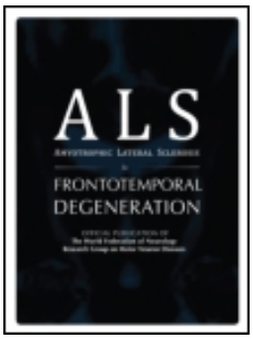

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

ISSN: 2167-8421 (Print) 2167-9223 (Online) Journal homepage: informahealthcare.com/journals/iafd20

THEME 11 THERAPEUTIC STRATEGIES

To cite this article: (2014) THEME 11 THERAPEUTIC STRATEGIES, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 15:sup1, 197-211, DOI: 10.3109/21678421.2014.960188

To link to this article: https://doi.org/10.3109/21678421.2014.960188

Published online: 10 Nov 2014.

THEME 11 THERAPEUTIC STRATEGIES

P264 GENE THERAPY FOR SPORADIC ALS USING AN INTRAVENOUS INJECTION OF AAV VECTOR

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Keywords: adeno-associated virus (AAV), AMPA receptor, ADAR2

Background: Amyotrophic lateral sclerosis (ALS) affects the middle-aged and elderly and is characterized by progressive muscular weakness resulting from degeneration of motor neurons in the spinal cord and the brain stem. There is no known cure and the patients die from respiratory muscle failure within a few years after onset. Downregulation of the RNA editing enzyme ADAR2 is involved in the death of motor neurons of sporadic ALS patients, which accounts for the great majority of cases of the disease. Therefore, normalization of ADAR2 activity in motor neurons is a likely therapeutic strategy for ALS.

Objectives: We developed an adeno-associated virus serotype 9 (AAV9) vector that would enable gene delivery only to the neurons of the brain and spinal cord via intravenous injection. We investigated whether delivery of the ADAR2 gene to motor neurons using the AAV9 vector would prevent the progression of symptoms of the disease and the degeneration of motor neurons in mechanistic model mice of sporadic ALS (conditional ADAR2 knockout mice or AR2 mice).

Results: Expression of the ADAR2 gene in motor neurons stopped the process leading to cell death and symptoms due to effective prevention of cell death without any apparent side-effects, even when administered after the emergence of symptoms,. Through the use of the AAV9 vector to trigger gene expression only in neurons, we could overcome the difficulty to introduce genetic material into the brain and spinal cord by intravenous injection, and demonstrate that one intravenous injection alone was sufficient to bring about long-lasting expression of an effective quantity of the ADAR2 gene.

Discussion and conclusion: While this result was achieved with a model mouse, it is thought that a similar molecular mechanism underlies sporadic ALS in human patients, and as the human ADAR2 gene had a therapeutic effect in the model mouse, it is anticipated that a similar form of gene therapy will be effective in treating human ALS as well. Further, the AAV9 vector is known to be safe, and after confirming the safety of the improved AAV9 vector and determining optimal dosage, it is hoped that this research will open a new route to the treatment of ALS. Currently gene therapy has a strong image as a replacement therapy for rare genetic disorders, but this research is unique in that it shows that gene therapy is possible even in sporadic cases if the molecular pathology of the disease is understood.

Acknowledgements: JST, MEXT, MHLW

DOI: 10.3109/21678421.2014.960188/264

P265 ADENOVIRAL TARGETING OF THE MOTOR END PLATE REGION FOR INCREASED TRANSDUCTION OF MOTOR NEURONS AND SKELETAL MYOFIBRES

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Keywords: gene therapy, motor neurons, adeno-associated virus (AAV)

Background: Gene therapy is an exciting technique that has the capability to introduce novel therapeutic genes that can either maintain or re-establish functional connectivity in a deficient system. Various approaches previously used for the treatment of rodent models of ALS have included the delivery of trophic factors (eg, insulin-like growth factor, etc.) or have used siRNA to knockdown mutant defective genes (eg, SOD1, etc.). In a gene therapy scenario, intramuscular injections and the subsequent retrograde transport of a viral vector is a minimally invasive way to transduce both the innervating motor neuron and the skeletal muscle. We have previously shown that targeting the entire length of muscles' motor end plate (MEP) region significantly increase the uptake of a retrograde tracer into corresponding motor neurons (1,2,3). However, using the MEPs as a target to deliver viruses into the spinal cord motor neurons has not yet been explored.

Objectives: The aim of this study was to determine if targeting the entire MEP region of a muscle with adenovirus would, as is the case with retrograde tracers, significantly increase expression of the transgene within spinal cord motor neurons in the wild-type mouse.

Methods: Recombinant adenovirus serotype 5 driven by the CMV promoter and encoding the reporter tag-GFP (Ad-GFP) was obtained through the UPenn vector core. Using our recently published MEP map as a guide (2), Ad-GFP (5.3×10^{12} pfu/ml) was injected along the entire MEP region along various muscles. Mice were subsequently intra-cardially perfused and the spinal cord, and injected skeletal muscles were dissected out, sectioned and the tissue were analysed under epifluorescence.

Results: This analysis showed that targeting the MEPs with Ad-GFP produced significant expression of GFP within spinal cord motor neurons and the targeted skeletal muscles. Moreover, GFP expression was also present within ventral roots, dorsal roots and dorsal root ganglia.

Discussion and conclusion: This study suggests that targeting muscles' MEP regions with an adenovirus is an effective and minimally invasive way to retrogradely deliver therapeutic

ISSN 2167-8421 print/ISSN 2167-9223 online © 2014 Informa Healthcare
DOI: 10.3109/21678421.2014.960188

genes into spinal cord motor neurons and skeletal myofibres. These data have implications for gene therapies aiming to maintain synaptic health between skeletal muscle fibres and the innervating spinal cord motor neurons.

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DOI: 10.3109/21678421.2014.960188/265

P266 SPECIFIC GENE DELIVERY TO CORTICOSPINAL MOTOR NEURONS BY AAV

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Keywords: corticospinal motor neurons, adeno-associated virus (AAV), motor neuron circuitry

Background: Corticospinal motor neurons (CSMN) are limited in numbers and are embedded among hundreds of different neuron types in the heterogeneous structure of the cerebral cortex. They are important for the initiation and modulation of voluntary movement as they receive, integrate, translate and transmit the cerebral cortex input towards spinal cord targets. CSMN degeneration is a hallmark for many neurodegenerative diseases and their genetic modulation is required for cellular therapies. The application of adeno-associated virus (AAV) in the central nervous system (CNS) has multiple advantages (1). AAV-IGF increased the lifespan of the well-characterized hSOD1^{G93A} ALS mouse model (2) and since then multiple therapeutic and translational studies have been proposed for motor neuron diseases. AAV serotypes are extremely malleable and combinations of promoters and capsid engineering have improved the transduction efficiency towards specific cell types. In ALS, targeting vulnerable neuron populations without affecting other neuron types within the cerebral cortex represents a major obstacle to establish new therapeutic strategies. We have recently reported specific transduction of CSMN after injection into the corticospinal tract (3). In this report, CSMN degeneration is observed associated with a distinct pattern of vacuolization that has not been reported before in the hSOD1^{G93A} ALS mouse model.

Objectives: To develop approaches that allow selective and specific gene delivery to CSMN using AAV.

Methods: We used seven AAV serotypes (AAV2-1, AAV2-2, AAV2-5, AAV2-6, AAV2-7, AAV2-8, and AAV2-9) that harbour the eGFP gene under control of the CMV promoter. AAVs were injected directly into the motor cortex in conjunction with retrograde labelling with red fluorescent microspheres to marks CSMN in the motor cortex and to investigate specific tropism for CSMN. In addition to co-labelling with red fluorescent microspheres, Ctip2 co-localization was used to confirm CSMN transduction.

Results: All AAVs tested showed varied tropism for neurons and glial cells including astrocytes. Our results clearly demonstrate the superiority of AAV2-2 for specific CSMN transduction, which is highly improved upon utilization of CBA promoter. Our results also indicated that diseased CSMN could be transduced effectively.

Discussion and conclusion: Our results suggest that the choice of the promoter is critically important to enhance selectivity on gene expression in CSMN. Identification of AAV serotypes that transduce only a select set of neuron populations, even upon direct cortical injection is critically important to develop effective and long-term gene therapy approaches in the cerebral cortex.

Acknowledgments: Northwestern Weinberg Grant (MJS), The Milton Safenowitz Post-Doctoral Fellowship from the ALS (JHJ). NUCATS Translational Innovation award, Les Turner ALS Foundation, and Wenske Foundation (PHO).

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DOI: 10.3109/21678421.2014.960188/266

P267 TARGETED NON-VIRAL GENE DELIVERY TO MOTOR NEURONS

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Keywords: gene therapy, targeted, p75NTR

Background: Targeted gene therapy for motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS) using non-viral nanoconstructs has huge potential for treatment, but so far, has underachieved. Our group has been developing non-viral gene delivery nanocarriers called 'immunogenes'. An immunogene is comprised of an internalizing antibody to a cell surface receptor that is conjugated to a polycationic carrier, which can bind and condense DNA/ RNA (1). Extensive research has revealed that the common neurotrophin receptor p75 (p75NTR) is naturally expressed in embryonic and neonatal motor neurons, as well as in adult motor neurons that are damaged or diseased, including MND/ALS. p75NTR is retrogradely trafficked in motor neuron signalling endosomes, thus we hypothesize this receptor is an ideal target for therapeutic gene delivery. Our current non-viral gene delivery agent consists of an antibody to p75NTR (MLR2) conjugated to a PEGylated polyethylenimine (PEI-PEG12) creating an immunoporter, which upon binding to a plasmid forms the immunogene. We aimed to characterise the ability of this carrier to transfect motor neurons *in vitro* and *in vivo*.

Methods: MLR2-PEI-PEG12 (immunoporter) was constructed and tested for its ability to bind/condense plasmid DNA (pVIVO2 that can express GFP) to form the immunogene and neutralise charge using a gel-retardation assay. Embryonic motor neurons were isolated from C57BL/6J (B6) mice and GFP expression was checked after application of MLR2-PEI-PEG12-pVIVO2. Finally, neonatal B6 mice were

injected intraperitoneally with MLR2-PEI-PEG12-pVIVO2 and then spinal motor neurons positive for ChAT and p75NTR were checked for GFP expression.

Results: MLR2-PEI-PEG12 was able to bind and condense pVIVO2 DNA and become charge neutral. MLR2-PEI-PEG12 successfully delivered pVIVO2 plasmid DNA specifically to primary motor neuron cultures isolated from neonatal mice and GFP was expressed. Approximately 25.4% of lumbar motor neurons in neonatal B6 mice (n = 5) identified by ChAT also expressed GFP after intraperitoneal injections of MLR2-PEI-PEG12 (150μg) carrying pVIVO2 (116μg). The GFP expressing motor neurons were also identified as expressing p75NTR.

Discussion and conclusion: We have shown that the immunogene comprising MLR2-PEI-PEG12-pVIVO2 has the ability to specifically transfect p75NTR expressing motor neurons. Further work will be needed to apply this technique to mice with MND and eventually as targeted therapy for MND/ALS.

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DOI: 10.3109/21678421.2014.960188/267

P268 LIPOSOME-ENCAPSULATED H-FERRITIN IMPROVES SURVIVAL IN AN SOD1 MUTANT MOUSE MODEL OF ALS

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Keywords: iron, lipopolysaccharide, infusion

Background: The misregulation of iron and subsequent oxidative stress are consistent features shared between humans with ALS and animal models of the disease. The iron sequestration protein H-ferritin has ferroxidase activity and limits the toxic potential of iron, making it an attractive therapy to pursue in ALS. One of the disadvantages of most systemically-delivered treatments for neurological diseases is that they exert their biological effects not only at their target sites but also at peripheral tissue and cells. This often results in dilution of the agent below therapeutic levels to the target tissue; a way to reduce the amount of agent administered and thus to potentially reduce toxicity is to utilize liposomal drug carriers.

Objective: To determine if infusion of liposome-encapsulated iron-poor H-ferritin has neurorescue properties in a murine model of ALS.

Methods: At 90 days of age, mice with the $SOD1^{G93A}$ mutation underwent surgery to permit continuous infusion into the lateral ventricle. There were a total of three groups: animals that received infusion of liposome-encapsulated H-ferritin that was targeted to microglia by the presence of lipopolysaccharide (LPS) on the surface of the liposome (n = 6), animals that received infusion of non-targeted liposome-encapsulated H-ferritin (n = 10), and a No Surgery (control) group (n = 20). Disease onset was assessed by performance on the rotarod apparatus, and endpoint was determined by the inability of the animal to right itself.

Results: Treatment with H-ferritin encapsulated by non-targeted liposomes resulted in a median lifespan of 136.5 days, as compared to 128.5 and 126 days for the LPS-targeted liposome and No Surgery groups, respectively. Histological examination of lumbar spinal cord sections indicated less extensive microglial activation at end-stage in the non-targeted liposome group as compared to the LPS-directed liposome treated group. Furthermore, the motor neurons that remained at end-state in the non-targeted liposomal group had thick, extensively branched projections, which were features not seen in the LPS-targeted group.

Discussion and conclusion: We propose that the limited benefit of LPS-directed delivery of H-ferritin is due to over-stimulation of microglia by accessing them through the TLR-4 receptor. Therefore, a plausible explanation as to why the non-directed liposomes are effective is that microglia are not further activated by our therapy, yet as the main phagocytic cell type in the CNS, they readily uptake the liposomes. Our intervention in the animal model is of particular relevance to the clinical population because our intervention occurs at a stage of the disease at which individuals with ALS would begin to notice symptoms and seek treatment. Therefore, liposomal delivery of H-ferritin may be of greater clinical benefit than those compounds tested while animals are pre-symptomatic.

Acknowledgement: This work was supported by a Department of Defense Therapeutic Idea Award.

DOI: 10.3109/21678421.2014.960188/268

P269 OPTIMAL CONDITIONS FOR TRANSPLANTATION OF MESENCHYMAL STEM CELLS IN THE ALS MOUSE MODEL

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Keywords: mesenchymal stem cells (MSC), neurotrophic factor, transplantation

Background: Stem cell therapy is a promising therapeutic approach for the treatment of amyotrophic lateral sclerosis (ALS). Mesenchymal stem cells (MSCs) are one of the best cell sources in such an application. We previously established an MSC clone (MSC3-31) that simultaneously overexpressed glial cell-derived neurotrophic factor, hepatocyte growth factor and insulin-like growth factor-1. This cell line provides the opportunity for stable transplantation and thorough analysis.

Objectives: The aim of this study was to optimize transplantation conditions, focusing in particular on the transplantation route as well as mouse recipient age. We compared the efficacies of transplantation using the fourth cerebral ventricle (CV) vs. intravenous (IV) injection, using mice that were 60 or 100 days old.

Methods: (1) High copy SOD^{G93A} transgenic (ALS) mice were given an oral immunosuppressive agent from one week before the transplantation until death. (2) MSC3-31 cells were transplanted into the ALS mice (60 or 100 days of age) via the CV or via IV (through a surgically exposed jugular vein). Phosphate buffered saline was given to the ALS mice (60 or 100 days) via CV or IV as control groups. (3) Clinical evaluations (body weight, hind limb extension reflex score, etc.) were performed to assess treated and control groups. (4) Immunohistochemical observations were performed on the spinal cords of both groups.

Results: In the groups transplanted via CV, there was an encouraging trend resulting in delayed death in the treated mice compared to the controls in mice transplanted at 60 days. Further, in transplants of MSC3-31 cells via CV at 100 days, ALS model mice showed a longer life span than did the control group (treated group vs. control group: 147.7 ± 2.5 days vs 140.8 ± 1.3, $p < 0.005$). For the IV groups, significant differences were not found in the 100-day-transplanted group regarding onset time or life span. Although the 60-day-treated group showed a tendency for a delayed onset time, it was not significantly different compared to the controls.

Discussion and conclusion: Encouraging trends were observed following transplantation via CV as well as IV at 60 days. Nevertheless, we conclude that optimal transplantation parameters are via CV transplantation at 100 days of age in ALS mice.

Acknowledgements: We thank Dr. Toguchida J at Kyoto University for providing hiMSC. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (WY), the 21st Century Center of Excellence program from Japan Society for the Promotion of Science (OM, KY and WY) and by a Grant from the Research Committee of CNS Degenerative Diseases, Ministry of Health, Labour and Welfare of Japan (NK).

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DOI: 10.3109/21678421.2014.960188/269

P270 A DOSE ESCALATION SAFETY TRIAL ON INTRATHECAL DELIVERY OF AUTOLOGOUS ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mesenchymal stromal cells, stem cells, clinical trials

Background: Mesenchymal stromal cells (MSCs) hold promise as a treatment for neurodegenerative diseases such as ALS due to their known paracrine effects on the CNS and immune system.

Objectives: We report interim results from our dose-escalation safety trial using intrathecal autologous adipose-derived MSCs (clinicaltrials.gov #NCT01609283).

Methods: Fifteen patients with ALS symptoms for 1-2 years were treated with 1-2 doses of 10, 50 or 100×10^6 MSCs via lumbar puncture. Patients were monitored for adverse events via symptom diary, clinical visits, blood, ALSFRS-R, CSF and MRI.

Results: Intrathecal MSC treatment was well tolerated, with reported mild adverse events unlikely related to treatment. At the 50×10^6 dose, most patients developed mild CSF pleocytosis (< 18 cells/uL), and one patient exhibited asymptomatic MRI lumbar nerve root thickening. No patients developed worsening weakness (more than expected with ALS), paresthesias/pain, or bowel/bladder dysfunction on follow-up (median 129 days, range 27-402 days).

Discussion and conclusion: Intrathecal autologous adipose-derived MSCs appear safe in ALS patients at the doses tested, and further clinical trials to assess efficacy should be considered.

Acknowledgements: This work was supported by a grant from the NIH (UL1 TR000135 and K08 169443), the Mayo Clinic Center for Regenerative Medicine, the Judith and Jean Pape Adams Charitable Foundation, and the Schmidt, Shannon and Mayo Foundations.

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DOI: 10.3109/21678421.2014.960188/270

P271 SLOWING DISEASE PROGRESSION IN THE SOD1 MOUSE MODEL OF ALS BY BLOCKING NEUREGULIN

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Keywords: neuregulin1 antagonist, therapeutic target, disease progression

Background: Neuregulin1 (NRG1) is a gliotrophic factor that regulates glial development and survival, synaptogenesis, axoglial interactions, and microglial activation. We recently found that NRG1 receptors are activated on microglia in the ventral horn of both ALS patients and in ALS-SOD1 mice (1). NRG1 signalling is also activated on microglia in the corticospinal tracts in ALS patients with predominant upper motor neuron signs (2), suggesting a common pathological mechanism (1, 2). We have developed a targeted NRG1 antagonist called HBD-S-H4 that when given intrathecally, reduced microglia activation in a rat chronic spinal cord pain model. Therefore, here we hypothesize that blocking NRG1 with HBD-S-H4 could be a new potential therapeutic treatment to slow microglial activation and disease progression in patients with ALS.

Objectives: To determine whether blocking NRG1 in the central nervous system (CNS) slows disease progression and prolongs survival in the ALS-SOD1 mouse model.

Methods: To determine if blocking NRG1 signalling would provide therapeutic benefit in the ALS-SOD1 mouse model, we used two different methods to deliver HBD-S-H4 to the CNS. In one approach we generated triple transgenic (Tg)

mice to express HBD-S-H4 in the CNS of SOD1 mice (GFAP-tTA:tetO-HBD-S-H4:SOD1^{G93A}). In an alternate approach, we injected HBD-S-H4 weekly through an implanted intracerebroventricular (icv) cannula for 9 or more weeks. Body weight, disease onset and progression, animal survival as well as pathological changes were measured in the triple Tg mice, HBD-S-H4 treated SOD1 mice and compared with their respective control groups.

Results: Our data shows that the expression of HBD-S-H4 in the CNS delays disease onset and prolongs survival in GFAP-tTA:tetO-HBD-S-H4:SOD1 mice compared with GFAP-tTA:SOD1 as well as SOD1 mice. Consistent with this therapeutic effect of transgenic expression of HBD-S-4, we found that high levels of HBD-S-H4 expression correlate with longer survival. Weekly icv treatment of recombinant HBD-S-H4 for 9 weeks had no toxic effects and was found to delay disease onset and prolong survival in the SOD1 mice. Measurements of the cellular pathology in GFAP-tTA:tetO-HBD-S-H4:SOD1 Tg mice and HBD-S-H4 icv-treated SOD1 mice are currently underway.

Discussion and conclusion: We have identified a common therapeutic target of NRG1 receptor activation on activated microglia in both ALS patients and the ALS-SOD1 mouse model. We are currently testing whether our NRG1 antagonist functions by blocking the communication between neuron and glia in the SOD1 mouse model and whether this would be a potential therapeutic treatment for patients with ALS.

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DOI: 10.3109/21678421.2014.960188/271

P272 ADMINISTRATION OF ANTIBODIES FOR MISFOLDED SOD1 PROLONG SURVIVAL IN SOD1 MOUSE MODELS

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Keywords: SOD1, misfolded, antibodies

Background: Since the identification of mutations in the superoxide dismutase 1 (SOD1) gene as a cause of amyotrophic lateral sclerosis (ALS), substantial efforts have been made to understand how mutations in SOD1 trigger motor neuron degeneration. Recent work has focused on the connection between toxicity and the propensity of mutant SOD1 protein to misfold. Mice expressing mutant SOD1 recapitulate many of the pathological and clinical features of ALS. Several reports have shown that targeting SOD1 by active or passive immunization can prolong survival.

Objective: We sought to compare the performance of antibodies with varying affinity for forms of SOD1 across two different mouse models. We used SOD1^{G93A} high copy mice and SOD1^{G37R} mice.

Methods: Congenic C57Bl6 mice expressing either SOD1^{G93A} or SOD1^{G37R} were obtained from Jackson Labs. Mice expressing G93A were dosed once a week starting at 50 days old at 30 mg/kg. Mice expressing G37R were aged to 6 months prior to dosing. Animals were regularly tested for motor performance by a rotarod analysis. Body weights and clinical scores were routinely assessed. Antibodies tested in these studies include B8H10, 3H1, and MB591-37.

Results: Disease onset as assessed by time to lose 10% of peak body weight was significantly delayed in both models. Disease onset as assessed by clinical observation was delayed in the G93A model, but not the G37R model. In contrast, rotarod performance was not altered in the G93A model, but was significantly improved in the G37R mice. Antibodies improved survival in both mouse models. The absolute change was much greater in the G37R mice; however, the relative change as calculated by a percent increase in life compared to control IgG dosed mice, was very similar for both models.

Discussion and conclusion: These data are consistent with the hypothesis that misfolded SOD1 represents a toxic form of SOD1. Consistent with prior reports, administration of antibodies directed against misfolded SOD1 confers increased survival in mutant SOD1 expressing mice. While some differential effects were seen across the models, in general the performance was very comparable. The G37R mice showed greater sensitivity in the motor performance assays, but this may reflect the slower disease course as opposed to a fundamental difference in disease pathobiology.

In conclusion, treatment of SOD1^{G93A} mice with antibodies specific for misfolded SOD1 improves survival, but additional work to understand the mechanism of action is needed.

Acknowledgements: Antibodies were licensed from AviTix Inc. and Amorfix Life Sciences and obtained through an MTA with MassBiologics. All authors were full-time employees of Biogen Idec.

DOI: 10.3109/21678421.2014.960188/272

P273 CHARACTERIZATION OF ANTIBODIES TO MISFOLDED SOD1

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Keywords: antibodies, misfolded, SOD1

Background: Mutations in the Cu/Zn superoxide dismutase (SOD1) gene cause about 20% of familial or 1–2% of all ALS cases. The mutations are thought to cause a gain of function, making SOD1 more prone to aggregation, and ultimately leading to motor neuron cell death. It has been proposed that misfolded SOD1 also plays a role in sporadic ALS and that misfolded wild-type SOD1 can propagate from cell to cell in a prion-like manner. In order to target extracellular misfolded SOD1, several groups have generated antibodies to misfolded SOD1 and used them to treat mutant SOD1 transgenic mice.

Objective: To characterize a large number of antibodies generated to different misfolded or mutant SOD1 antigens, in order to select the best candidates for *in vivo* studies and to use them to develop an assay to measure misfolded SOD1.

Methods: The apparent affinities of the antibodies for guanidine-denatured, oxidized or native SOD1 were determined. Mutant SOD1 recognition was assessed by immunoprecipitation from cell lysates containing various SOD1 mutants. Epitope mapping was done using deletion or point mutants of SOD1 and with a peptide array. An ELISA to specifically quantify misfolded SOD1 using one of the misfolded Abs was developed in addition to a native SOD1 ELISA. The ELISA was applied to soluble spinal cord extracts and western blots were used to examine insoluble SOD1 prepared from longitudinal samples from 3 strains of SOD1 transgenic mice. Immunohistochemistry on G93A transgenic or control spinal cord tissue was carried out using 3 of the Abs.

Results: The antibodies were ranked with respect to their affinity and selectivity to mutant, denatured and oxidized SOD1. Epitope mapping determined that one region of exon 3 in SOD1 was recognized by several high affinity antibodies. Based on our *in vitro* experiments, 6 antibodies were selected for *in vivo* studies. ELISA results showed that soluble misfolded SOD1 levels rose throughout the majority of the lifetime of the animal before decreasing at end stage, while native levels also increased and remained elevated. Higher molecular weight SOD1 species were apparent after disease onset, which correlated with an increased level of ubiquitination in the extracts. Three antibodies recognized misfolded SOD1 in $SOD1^{G93A}$ mice, but not in non tg mice, by IHC and are now being used to stain ALS patient samples.

Discussion and conclusion: Antibodies were identified that bind with high selectivity to denatured, oxidized, and mutant SOD1 proteins. Antibodies were also used to establish ELISAs to measure misfolded SOD1 in transgenic spinal cord tissue extracts and to visualize misfolded SOD1 in transgenic spinal cord sections by immunohistochemistry.

Acknowledgements: Antibodies were licensed from AviTx Inc., Amorfix Life Sciences and via an MTA from Mass Biologics.

DOI: 10.3109/21678421.2014.960188/273

P274 THE MND-ATTENUATING COMPOUND CUII(ATSM) ACTIVATES THE ANTIOXIDANT NRF2 PATHWAY IN CULTURED ASTROCYTES

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Keywords: therapeutic, Nrf2, astrocyte

Background: We have demonstrated that diacetylbis (4-methylthiosemicarbazonato) Cu^{II} (Cu^{II}(atsm)) significantly delays symptom onset and extends lifespan in multiple transgenic mouse models of motor neuron disease (1, 2, 3).

Objectives: This study seeks to elucidate the mechanism by which Cu^{II}(atsm) induces its potent neuroprotective effects. Nrf2 is an important transcription factor regulating a suite of antioxidant genes and its activation is impaired in MND spinal cord and brain (4). Induction of Nrf2 in MND model mice is protective and astrocyte-dependent (5). Therefore, the protective effects of Cu^{II}(atsm) may involve stimulation of the Nrf2 pathway in astrocytes. In line with this, Cu^{II}(atsm) attenuates oxidative damage and astrocyte activation in MND model mice (2, 3).

Methods: Primary astrocytes cultured from the brains of newborn mice were treated with Cu^{II}(atsm) for up to 24h ($n = 3$-4 independent cultures). Nrf2 activation was assessed by nuclear accumulation of Nrf2 and induction of its targets including heme oxygenase-1 and glutamate-cysteine ligase. The latter controls the synthesis of the critical antioxidant glutathione. Accordingly, cellular and exported glutathione content were also determined. Stimulation of glutathione content and export by Cu^{II}(atsm) was further assessed in astrocytes derived from human neural progenitor cells and in cultured neurons. To exclude non-specific pathways, induction of glutathione was assessed in astrocytes cultured from Nrf2-deficient mice. Increased bioavailability of Cu by Cu^{II}(atsm) was assessed by co-administration with the metal chelator TPEN.

Results: Cu^{II}(atsm) induced nuclear accumulation of Nrf2, increased HO1 expression and GCL activity, and increased glutathione content and export from astrocytes ($p < 0.05$). These effects were replicated in cells of human origin ($p < 0.05$). Cu^{II}(atsm) did not increase the glutathione content or export of cells deficient in Nrf2 ($p > 0.05$), nor in cultured neurons ($p > 0.05$). Co-administration of Cu^{II}(atsm) with the metal chelator TPEN blocked induction of the Nrf2 pathway, including nuclear accumulation of Nrf2, HO1 expression and glutathione content ($p > 0.05$).

Discussion and conclusion: These results demonstrate that Cu^{II}(atsm) activates the antioxidant Nrf2 pathway in cultured astrocytes. These effects appear to translate into human cells, to be limited to astrocytes, and are dependent upon the presence of Nrf2 and increased bioavailable Cu. These actions may contribute to the neuroprotective and disease-attenuating activity of Cu^{II}(atsm) observed *in vivo*, and indicates that Nrf2 may be a valuable therapeutic target for the treatment of motor neuron disease.

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DOI: 10.3109/21678421.2014.960188/274

P275 CORRECTING DEFECTIVE ENDOPLASMIC RETICULUM-MITOCHONDRIA INTERACTIONS AS A NEW THERAPEUTIC TARGET FOR ALS: CHARACTERISATION OF NOVEL DRUG SCREENS

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Keywords: TDP-43, ER-mitochondria associations, VAPB-PTPIP51 interaction

Background: Mitochondria and the ER form close associations and these regulate a number of fundamental physiological processes including energy and phospholipid metabolism, Ca^{2+} homeostasis, mitochondrial biogenesis and transport, ER stress, autophagy and apoptosis. Disruption of ER-mitochondria contacts has been described in ALS. To dissect the pathological processes involving the ER-mitochondria axis the identification of the molecular tethers that connect regions of ER with mitochondria is essential. Recently, we identified the integral ER protein VAPB and the outer mitochondrial membrane protein PTPIP51 as interacting proteins functioning as tethering scaffolds (1, 2 ,3). Moreover, we showed that expression of wild-type and ALS/FTD mutant TDP-43 disrupts both ER-mitochondria associations and the VAPB-PTPIP51 interaction 1. The VAPB-PTPIP51 interaction thus represents a new therapeutic target for ALS/FTD. Here, we describe a fast and reliable cellular assay for monitoring the VAPB-PTPIP51 interaction which allows screening for small molecules that might correct defective ER-mitochondria and VAPB-PTPIP51 associations in disease.

Methods: We created plasmids in which the cytoplasmic domain of VAPB was fused to the DNA binding domain of the yeast transcription factor GAL4, and the cytoplasmic domain of PTPIP51 was fused to the viral DNA-transactivator domain VP16. HeLa cells were transfected with each of these plasmids either alone or in combination and with a GAL4-UAS luciferase reporter plasmid. Other positive and negative controls were included. We also treated cells with selected kinase inhibitors and monitored their effect on the VAPB-PTPIP51 interaction.

Results: We obtained robust luciferase signals in VAPB-GAL4 (DNA binding domain) and PTPIP51-VP16 co-transfected cells but not in negative control transfected cells. Using the assay and appropriate kinase inhibitors, we identified signalling pathways that impact on the VAPB-PTPIP51 interaction.

Conclusion: We have identified the VAPB-PTPIP51 interaction and ER-mitochondria associations as new molecular targets for the treatment of ALS. We have designed a robust high-throughput screen for identifying small molecules that might correct defective VAPB-PTPIP51 and ER-mitochondria associations in ALS.

Acknowledgements: This work was supported by the Motor Neurone Disease Association, ARUK, MRC, Wellcome Trust.

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DOI: 10.3109/21678421.2014.960188/275

P276 IMPROVEMENTS IN MOTOR FUNCTION, CA2 + CLEARANCE AND MARKERS OF ENDOPLASMIC RETICULUM STRESS WITH 6-GINGEROL TREATMENT IN SOD1^{G93A} ALS MICE

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Keywords: SERCA function, ER stress, gingerol

Background: We recently identified impairments in intracellular Ca^{2+} clearance, reductions the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) protein expression (SERCA1 and SERCA2) and increased markers of endoplasmic reticulum (ER) stress in skeletal muscle of the SOD1^{G93A} mouse model of ALS. In mouse models of muscular dystrophy there is a rescue of the muscle wasting pathology with overexpression of the fast fibre-specific isoform SERCA1 in skeletal muscle (1). Thus, altered intracellular Ca^{2+} homeostasis may be a common final pathway for mediating cellular death and skeletal muscle atrophy in various neuromuscular diseases.

Objectives: The purpose of this study was to obtain proof of concept for a SERCA activator in improving functional outcomes and rescuing the ER stress associated with skeletal muscle dysfunction in SOD1^{G93A} mice.

Methods: We identified a small molecule, 6-gingerol, which has been shown to increase SERCA1 activity (2). At 35d, mice were assigned to treatment groups: i) wild-type control treated with vehicle (WT-Veh; n = 4; 3 female (F) and 1 male (M)); ii) SOD1^{G93A} treated with vehicle (ALS-Tg Veh; n = 4; 3 F and 1 M; iii) SOD1^{G93A} mice treated with 6-gingerol (ALS-Tg Gin; n = 4; 3 F and 1 M). ALS-Tg Gin mice received 6-gingerol (5 mg/kg, ip) daily for 10 wks; Veh mice received 0.4% ethanol in PBS ip daily.

Results: At 115d, grip function was reduced in ALS-Tg Veh to 17% of WT-Veh level ($p < 0.05$) and showed improvement in ALS-Tg Gin (to 42% of WT-Veh; $p = 0.08$). Intracellular Ca^{2+} regulation was assessed in isolated single muscle fibres using Fura-2. Consistent with our previous studies, resting Fura-2 ratio was significantly increased in ALS-Tg Veh vs. WT-Veh and was lower in ALS-Tg Gin vs. ALS-Tg Veh ($p = 0.13$). SR Ca^{2+} pump function was determined by the time taken for Fura-2 ratio to return to 25% of its baseline level. This Ca^{2+} decay time was measured following 50 and 100 Hz tetani. There was a significant increase in Ca^{2+} decay time in ALS-Tg Veh compared to WT-Veh ($p < 0.05$) and an improvement in ALS-Gin vs. ALS-Veh ($p = 0.11$). Treatment with 6-gingerol rescued the increase in the ER stress-induced cell death marker CHOP and also attenuated the decrease in SERCA1 expression.

Discussion and conclusion: Overall, these data support the premise that 6-gingerol increases intracellular Ca^{2+} clearance by activating the Ca^{2+} pumping function of SERCA, and is associated with reductions of ER stress markers in ALS-Tg mice. These changes are associated with an improvement in neuromuscular function.

Conclusion: These preliminary data provide proof of concept for the use of a SERCA agonist in improving motor function and attenuating the cellular damage that occurs with denervation and muscle atrophy in ALS.

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DOI: 10.3109/21678421.2014.960188/276

P277 CNS102 IMPROVES SURVIVAL AND MOTOR BEHAVIOR IN SOD1 MICE AND PROTECTS AGAINST EXCITOTOXICITY THROUGH MULTIPLE SIGNALING PATHWAYS

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Keywords: SOD1, heat shock protein, excitotoxicity

Background: CNS102 is the purified all-trans isomer of geranylgeranylacetone (GGA). The isomeric mixture of GGA (teprenone) is a pharmaceutical drug for gastric ulcers in Japan and it has been widely reported to induce the expression of heat shock protein 70 (HSP70). HSP70 is protective in degenerating neurons, and if teprenone is given at an oral dose of 600mg/kg or larger it is neuroprotective in rodent models of neurodegeneration.

Objective: To show that CNS102 is effective against neurodegeneration in rodents at a dose low enough to be feasible for development into a therapeutic treatment of amyotrophic lateral sclerosis (ALS), and to elucidate the pathways through which CNS102 has neuroprotective activity.

Methods: Two rodent models were tested for CNS102 efficacy. $SOD1^{G93A}$ mice (n = 16) were orally administered CNS102 (12 mg/kg), riluzole (8 mg/kg) or vehicle daily starting at P39 and assessed for motor deficits (neurological scores catwalk, grip test, tail suspension test), body weight and survival. Excitotoxic cell death was induced by kainic acid (KA) in Sprague-Dawley rats (n = 9-11) following six daily oral administrations of 100mg/kg CNS102 or PBS. Neuronal cell death was quantified by histology, and HSP expression by ELISA and qPCR. In N2A cells the HSP response to CNS102 was characterized with an i-HSP70 5′-UTR driven luciferase reporter assay, western blot, ELISA and qPCR. Prenylation of the small GTPases Rap1A and RhoA was quantified by western blot and morphological effects were determined with a neurite outgrowth assay.

Results: CNS102-treated SOD1 mice performed better on the cat walk demonstrating improved stride length and running speed. Grip strength and hind leg extension by tail hang assay was improved, and neuroscore analysis showed that the rate of increase was 33% greater in vehicle- vs. CNS102-treated SOD1-mice. Physiologically, CNS102 reduced the rate of loss of body weight by over 40% compared to vehicle-treated SOD1 mice. Median survival was prolonged by 8 days ($p < 0.0007$) and 6.5 days ($p < 0.015$) in CNS102 and riluzole treated SOD1 mice respectively, compared to vehicle treated SOD1 mice. In rats CNS102 reduced excitotoxicity induced neuron loss by 33% and increased HSP70, GRP78 and HSP27 expression. In N2A cells we found a dose response to CNS 102 of HSF1, HSP70, HSP40 and HSP90, increased prenylation of Rap1A and RhoA and stronger growth of neurites.

Discussion and conclusion: We demonstrate efficacy of CNS102 for improvements in survival and motor deficits in SOD1 mice, and protection against excitotoxic neurodegeneration in rats. Our data suggests that the neuroprotective effect of CNS102 might be mediated through modulation of the HSP response and prenylation of small GTPases. The low effective dose in the SOD1 model makes CNS102 a feasible candidate for development as a therapeutic for ALS.

DOI: 10.3109/21678421.2014.960188/277

P278 INTRATHECAL BACLOFEN FOR SPASTICITY IN MOTOR NEURON DISEASE (MND): LONG-TERM EXPERIENCES

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Keywords: intrathecal baclofen (ITB), spasticity, long-term-follow-up

Background: Management of severe spasticity in MND is often unsatisfactory due to intolerance or inefficacy of oral medications. In patients with primary lateral sclerosis (PLS) and upper-motor neuron predominant ALS, intrathecal baclofen (ITB) therapy can be an option. However, little is known about long-term outcome in these patients.

Objectives: To report on long-term experiences with ITB for severe spasticity in MND patients in Switzerland.

Methods: A total of 16 patients, referred by ALS clinics for evaluation of ITB therapy, were examined by a neurologist, an occupational, a speech, and a physiotherapist at baseline. In all patients, ITB was administered by a probatory external pump (connected with a subcutaneous intrathecal catheter about 40–60 cm above L3/L4 puncture level), the dosage was increased according to clinical signs and oral antispastic medication tapered off and stopped. ALS Functional Rating Scale (ALSFRS-R), Functional Independence Measure scores, speech, swallowing, and spasticity (modified Ashworth scale) were evaluated before and under ITB therapy. Only in case of clear benefit, was a permanent ITB pump implanted. All patients were followed in ALS clinics.

Results: From 2/2007 to 5/2014, sixteen patients (12 men, 4 women), mean age 48.5 years, were treated with ITB via probatory external pump. Four patients were diagnosed with PLS, 12 with ALS. At baseline, mean disease duration was 55 months, ALSFRS-R 29.2. In all patients spasticity was reduced, no side effects occurred. Four patients did not go on a permanent ITB pump because symptoms did not improve or deteriorated. A permanent pump (Synchromed II, Medtronic) was implanted in 12 patients, mean ITB starting dosage 50 ug/d. All patients, followed in ALS clinics (one lost to follow-up), continued ITB therapy. Seven patients died of respiratory failure due to progression of MND. In this group, mean duration of ITB treatment was 18.1 months, compared to 29.5 months in the 4 patients who are still alive. At the last evaluation, mean ALSFRS-R was 11, and 27.5, ITB dosage 70 ug/d, and 135 ug/d respectively.

Discussion and conclusion: In MND patients, the pattern of muscle tone and strength varies substantially and individually. Severe spasticity might require ITB therapy, but progression of atrophic paresis has to be considered. In our patients, escalation of ITB dosage in the course of the disease was often needed.

Conclusion: ITB can safely and effectively reduce spasticity in long-term course of selected patients with MND.

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DOI: 10.3109/21678421.2014.960188/278

P279 PSYCHOLOGICAL AND PSYCHOTHERAPEUTIC APPROACHES FOR PEOPLE WITH MND: A QUALITATIVE STUDY

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Keywords: psychological, psychotherapeutic, palliative care

Background: People with MND are often dissatisfied with the level of care provided, as they move their focus from physical health concerns to emotional support, as the disease progresses (1). However there seems to be little or no research on the range and effectiveness of psychotherapeutic interventions that could support the person with MND.

Objective: The aim was to review current psychotherapeutic approaches used by counsellors, psychologists and psychotherapists in Ireland and gain an insight into approaches used elsewhere.

Methods: A qualitative study was used using semi-structured interviews. Participants include 8 Irish therapists representing public and private service and 2 therapists from UK and Italy representing public service. Data was analysed using principles of grounded theory (2) to generate principal categories that best describe the therapists' approaches. The interview schedule was designed in collaboration with a person with a terminal neurological disorder.

Results: From this study five principal categories were identified that outline the therapists' approaches as follows: Therapy pre-requisites (creating a therapeutic space in an appropriate timeframe and location); Experience and awareness of MND (understanding the physical and possible cognitive impacts of MND); Relationship context (embracing the emotional affect for the person with MND by managing own emotional affect);Theoretical model and interventions (offering a combination of supportive and empowering approaches); Perceptions of outcome (providing space to talk and express feelings, self-direct and ease them on their journey).

Discussion and conclusion: There is no consensus about a specific approach; due to the complexity of the disease and variety of presentations. Common approaches included supporting the person in the "here and now" by providing a "fine focus" on what they can still do, re-affirming their ability to self-direct and supporting emotional exploration. The findings indicate therapists should have an experience of MND, the limitations in mobility, communication and cognitive processing, to be able to provide a safe place to talk.

Therapists use various approaches that provide different perceived outcomes. Different approaches may be required during disease progression. Therapists need to understand the pre-requisites and have experience of MND. Therapists' awareness and management of their own emotional affect enhances their therapeutic ability.

Acknowledgements: The participants. Professor Orla Hardiman, Bernie Corr and Irish Motor Neurone Disease Association (IMNDA) for support in recruitment. Irish Hospice Foundation for sponsoring a development grant for psychosocial training based in part on this research.

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DOI: 10.3109/21678421.2014.960188/279

P280 GENDER DIFFERENCES IN EMERGING BEHAVIORAL CHANGE IN ALS SUGGEST A NEUROENDOCRINE MODEL FOR TREATMENT

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Keywords: gender, behaviour, neuroendocrine

Background: ALS is associated with frontotemporal lobar degeneration (FTLD) in ~50% of patients, in the absence of dementia, characterized by primary progressive aphasia and/or behavioural decline. Our published work demonstrated gender differences in ALS with behavioural impairment (ALSbi) that included significantly more males with the Disinhibited subtype, including impulsivity, jocularity, and loss of insight. The Apathy subtype was equivalent between genders, while a significantly greater proportion of women evidenced Personal Neglect. From these findings we generated a theory of FTLD emergence involving midbrain dominance and motivation circuitry. In conjunction with this, we hypothesize a neuroendocrine model of neuroprotection whereby oestrogen replacement may forestall disease progression in peri-menopausal female ALS patients with emerging signs of FTLD.

Objectives: We investigated gender differences in pattern of emergence of behavioural change in ALS, and their relationship to oestrogen levels in females.

Methods: Behavioural assessment was pursued by evaluation of patient executive functioning and caregiver interview with the structured Frontal Behavioural Inventory (n = 171). To evaluate executive functioning, we assessed patients with the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes.

Results: Consistent with our previous findings, we found a significantly greater number of males with the Disinhibition subtype ($p = 0.014$). Males also showed a greater proportion of the Stereopathy behavioural subtype ($p = 0.048$). Apathy was again equivalent between genders, while females again evidenced a greater incidence rate of moderate-severe Personal Neglect (7.0%) in comparison to males (4.5%). Medication records review of female estrogen status for patients aged 31-74, including oestrogen replacement in peri-menopausal and menopausal patients, showed a strong relationship to both higher executive functioning capacities (similarities ($p = 0.005$), judgment ($p = 0.018$), letter fluency ($p = 0.004$)), and attenuation of Apathy ($p = 0.022$), the latter unrelated to age ($p = 0.076$).

Discussion and conclusion: Gender differences are present in emerging ALSbi, and relate to oestrogen status. These findings evidence the potential of oestrogen as a therapeutic agent to attenuate executive functioning and behavioural decline in emerging ALS-FTLD. Given the overlap in genes associated with ALS, FTLD and breast cancer (1), as well as the conflicting findings in literature on the benefit and risks of oestrogen replacement, gonadal steroidal hormones likely serve as immuno-modulatory agents. Their action may range from inhibitory to stimulatory in a concentration dependent manner, influenced as well by the nature of the target tissue. Oestrogen analogs are needed to attenuate neurodegeneration while inhibiting over-activation in the breast and uterus, akin to the selective oestrogen receptor modulators currently applied as therapeutics in the treatment of breast cancer (2).

Acknowledgements: We thank our patients for their dedication and commitment.

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DOI: 10.3109/21678421.2014.960188/280

P281 IMPACT OF EXPIRATORY MUSCLE STRENGTH TRAINING ON BULBAR FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS: UPDATES FROM A RANDOMIZED SHAM-CONTROLLED CLINICAL TRIAL

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Keywords: exercise, treatment, expiratory muscle strength training (EMST)

Background: The role of exercise in individuals with ALS is controversial. We have recently reported that expiratory muscle strength training (EMST) is feasible, safe and lead to improvements in expiratory force generating pressures, swallowing kinematics, cough spirometry and airway protection during swallowing in a pilot study of 25 ALS patients. Further work is needed to validate these preliminary findings and to further elucidate the potential role of exercise in this patient population.

Objective: Determine the efficacy of a targeted bulbar strength training program (EMST) on maximum expiratory pressure (MEP), swallow kinematics, cough spirometry, quality of life and disease progression in ALS patients with mild to moderately severe symptoms.

Methods: This is a randomized blinded sham-controlled clinical trial enrolling 48 patients with mild-moderate ALS (possible, probable or definite Revised El-Escorial Criteria). Patients will undergo eight-weeks of daily training with an active ($n = 24$) or sham ($n = 24$) device. The primary outcome variable is MEP (cmH_2O). Secondary measures include: kinematic and temporal swallowing indices; cough spirometry measures; and the Penetration Aspiration Scale score (an index of airway safety during swallowing). Tertiary outcomes include patient-reported measures of: swallow-related quality of life; dysphagia severity; and functional oral intake using validated scales (SWAL-QOL, EAT-10, FOIS respectively). Finally, the impact of EMST on disease progression over time will be investigated via the ALSFRS-R. Statistical analysis performed on interim data constituted a 2×2 (time $\times$ group) mixed model ANOVA with alpha set at 0.05.

Discussion and conclusion: At time of abstract submission, 28 individuals have been enrolled in this RCT with 20 individuals completing the trial. Interim data indicate a significant time by group interaction for the primary outcome variable, Maximum Expiratory Pressure ($F(1) = 9.10$, $p = 0.01$). Post-hoc analysis revealed a significant increase in MEPs for ALS patients in the active EMST group ($p = 0.03$, mean difference $37.35cmH_2O$) and a significant between groups difference (active vs. sham) at the post-treatment time point ($p = 0.02$, mean difference $78.45cmH_2O$). A significant group by condition interaction was also revealed for ALSFRS-R scores. Post-hoc analysis revealed a significant reduction for the sham group pre vs. post-treatment ($p = 0.02$) but not for the active group ($p = 0.47$). No significant differences were revealed for patient-reported swallowing severity data (EAT-10), however those in the active group had a mean group improvement of 42.02% while those in the sham group demonstrated a 1.36% improvement. Cough spirometry and kinematic swallow physiology measures are currently being analyzed. These data and those of currently active patients enrolled in this trial will be presented at the 25th International Symposium on ALS/MND.

Current interim data from this RCT confirm our previous findings and suggest that strength training of bulbar musculature may be beneficial for improving and maintaining expiratory generating pressures and may impact measures of global disease progression.

DOI: 10.3109/21678421.2014.960188/281

P282 MECHANICAL INSUFFLATION/EXSUFFLATION WITH HIGH FREQUENCY CHEST WALL OSCILLATION: RESULTS OF A CLINICAL TRIAL

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Keywords: respiratory management, therapy, standards of care

Background: Secretion management is an essential aspect of respiratory care in patients with motor neuron disease. Poor or compromised muscle strength can eventually lead to atelectasis, and infection, especially in the context of impaired lung volumes. Mechanical Insufflation/Exsufflation (I/E) has been highly effective in assisting with mobilization of secre-

tions by mimicking a cough reflex and in the delivery of high inspiratory volumes. Cycling between insufflation and exsufflation can either be performed manually or automatically on the coffalator unit.

Patients with poor mucocilliary action may develop excessive secretions blocking the smaller airways that may not be easily mobilized with mechanical I/E therapy. With the smaller airways, The Vest or Respirtech therapy unit is based upon a technology called high-frequency chest wall oscillation (HFCWO). HFCWO therapy is administered by a device consisting of an inflatable vest connected by hoses to an air-pulse generator. The generator rapidly inflates and deflates the vest, gently compressing and releasing the chest wall to create airflow within the lungs. Evenly distributed oscillating forces applied externally to the chest wall generate cough-like shear forces within the airways that dislodge mucus from the bronchial walls, increase mobilization, and move it along towards central airways. This action also works to thin thick secretions, making them easier to clear. Once the mucus has advanced from smaller to larger airways, it can be easily removed by coughing and expectoration or by suctioning.

We have recently completed a clinical trial, comparing I/E therapy with the combination of I/E therapy and HFCWO.

Methods: Patients completed a battery of pulmonary function studies, body plethysmography, CT scan of the chest and respiratory function questionnaires. Lung volume, pre and post treatments will be compared as well as the benefit to patient's ventilation and respiration.

Results: Results of our final analysis will be presented. Currently, the last of the patients enrolled are completing the study and all data will be available by the time of the presentation.

DOI: 10.3109/21678421.2014.960188/282

P283 RELATIONSHIPS BETWEEN RILUZOLE AND TIRASEMTIV LEVELS ON OUTCOMES IN THE BENEFIT-ALS TRIAL

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Keywords: clinical trials, skeletal muscle activation, pharmacodynamics

Objectives: The BENEFIT-ALS Trial evaluated the effects of Tirasemtiv, a fast skeletal muscle activator, in 711 patients with ALS randomized either to placebo or to an escalating dose of Tirasemtiv up to 500 mg per day. After 3 months, statistically significant differences favouring Tirasemtiv were found in extremity strength and in slow vital capacity (SVC), although there was no significant difference between groups in ALSFRS-R. In this report, we examine the relationship between plasma Tirasemtiv levels and riluzole levels on patient efficacy outcomes and adverse events.

Methods: 711 patients enrolled in the study; 106 patients dropped out prior to randomization and 156 patients were removed from the analysis due to a drug dispensing error that occurred mid-study. All other patients who had at least 1 efficacy assessment are included in this report. At each study visit, both plasma riluzole and Tirasemtiv levels were obtained. The relationship of adverse event frequency to both riluzole and Tirasemtiv levels was determined combining all visits. For all efficacy measures (ALSFRS-R, SVC, Maximum Voluntary Ventilation (MVV), Sniff Nasal Inspiratory Pressure (SNIP), Muscle Strength assessed via Hand Held Dynamometry (HHD)), patients were divided into concentration quartiles for both riluzole and Tirasemtiv, and analyses based on slope of percent change from baseline were performed.

Results: Although riluzole levels in the Tirasemtiv group were approximately 40% higher than in the placebo group, there was no indication that this difference was related to frequency or intensity of adverse events. The relationship between treatment emergent adverse events and Tirasemtiv levels were evaluated. Efficacy measures were evaluated both as a function of maximum tolerated Tirasemtiv dose and serum Tirasemtiv concentration. For SVC, there was a positive relationship between serum concentration and SVC benefit. Initial modelling suggests that the relationships between Tirasemtiv concentration and other efficacy measures were not linear. With respect to efficacy, riluzole levels had no impact on any measure.

Discussion and conclusion: In the BENEFIT-ALS trial, riluzole seemed to not have an impact either on Tirasemtiv efficacy or tolerability, suggesting that the strategy of dose lowering in patients on Tirasemtiv had the intended effect. Adverse events were evaluated with respect to Tirasemtiv levels; the relationship of Tirasemtiv to measures of efficacy was complex. Multiple models relating Tirasemtiv concentration to efficacy measures will be explored and presented. These results will help inform further development of this agent.

DOI: 10.3109/21678421.2014.960188/283

P284 EFFECT OF RILUZOLE TREATMENT IN PATIENTS FROM EMILIA ROMAGNA, ITALY: A POPULATION BASED STUDY

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Keywords: riluzole, population based registry, survival

Background: Riluzole is the only drug approved for ALS treatment. Although, a number of concerns about effectiveness still persist, mainly due to the slight increase in survival in front of a relatively high cost of the drug. On the other hand we do not have other pharmacological therapeutic strategies, so the drug is approved in many western countries, and largely used in Europe and in Italy.

Methods: This study was performed in 9 provinces and 11 local health units of Emilia Romagna (population 4.4 million inhabitants), with the involvement of 17 neurological departments. From 2009 onwards, a prospective registry has been collecting all cases of incident ALS among residents in Emilia Romagna region. For each patient, the main demographic and clinical information were collected by the caring physicians. In addiction a follow up case report form has been completed

during each patient follow up reporting data on support procedures, death, and information on treatments and their interruption.

Results: From 1.1.2009 to 31.12.2013 in Emilia Romagna, 566 patients (54.9% M, 45.1% F) received a new diagnosis of ALS. Mean time from onset to diagnosis was 12.9 months. Mean age at onset was 66.4 years. 470 patients (83%) were treated with riluzole, whereas 96 patients were not. Median overall tracheostomy-free survival was 50 months (from onset). Patients who did not take Riluzole were older (mean years at onset: 69.8 years vs 65.7 years, $p < 0.01$), were more frequently bulbar ($p = 0.01$) and more frequently demented ($p = 0.02$). Moreover, patients who did not take riluzole were more frequently classified as possible ALS according to El-Escorial Diagnostic criteria (EEC) at diagnosis.

Overall, riluzole treatment did not influence the rate of tracheostomy-free survival of patients. This was confirmed at stratified analysis, which showed that riluzole treatment did not prolong survival in any of the examined subgroup (bulbar, younger patients, definite or probable ALS according to EEC).

Discussion and conclusion: This is an observational study on the use of riluzole in ALS patients from an Italian registry. When the first RCT on riluzole showed benefit from the treatment many concerns were raised, mainly due to the greater benefit in bulbar patients. This led to further RCTs, among which one, including also advanced ALS, did not demonstrate an effect of the drug on survival. The same results were reported after a fourth trial carried out in Japan. Our study has many limitations due to its observational nature, but shows that riluzole treatment does not change survival significantly in a population based setting.

DOI: 10.3109/21678421.2014.960188/284

P285 DISCONTINUOUS RILUZOLE TREATMENT MAY PROVIDE A BETTER THERAPEUTIC ALTERNATIVE FOR ALS PATIENTS

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Keywords: riluzole, glial cells, neurotrophic factors

Background: Riluzole is the only FDA approved drug for the treatment of amyotrophic lateral sclerosis. The mechanism by which riluzole affords its protection is unknown. We have previously shown that riluzole stimulates astrocytes to produce trophic factors for motor neurons.

Objective: We hypothesize that the protective effect of riluzole is due to stimulation of trophic factors by motor neuron associated cells.

Methods: *In vitro* studies*:* Astrocytes were treated with 1μM riluzole for 24 hours and 6 days. Schwann cells were treated with 1μM Riluzole for 24 hours and 3 days. Riluzole was removed before allowing the cells to condition new media for 24 hours. Purified motor neurons were cultured for three days in a 1:10 dilution of the conditioned media. I*n vivo* studies*:* Nontransgenic mice were treated with 100mg/mL riluzole in the drinking water for 3, 6, 15 and 30 days. Animals were then sacrificed and muscle, brain, spinal cord and sciatic nerve were removed. Trophic factor levels were quantified using ELISA and qPCR.

Results: Conditioned media from astrocytes treated with riluzole for 24 hours increased motor neuron survival when compared to untreated conditioned media. Neutralizing antibodies against cardiotrophin-1 (CT-1) partially blocked the trophic factor support following 24 hours riluzole treatment ($p < 0.005$). Similar results were obtained using Schwann cells treated with riluzole for 24 hours ($p < 0.005$). Conditioned media from cells with chronic riluzole treatment did not increase motor neuron survival in comparison to untreated controls. CT-1 expression in Schwann cells was induced following 24 hours riluzole treatment but decreased significantly at 6 days ($p > 0.05$). The MAP kinase p38, a critical regulator of Schwann cell differentiation and myelination, is inhibited following 24 hours riluzole treatment. Cardiotrophin-1 expression in Schwann cells was induced by inhibition of p38. In mice treated with riluzole, there is an increase in the production of GDNF, BDNF, and CT-1 in the spinal cord during the first 15 days, but at 30 days the level of these trophic factors was reduced significantly. The same effect was observed in muscle at 15 days. CT-1 production in the sciatic nerve increased by 6 days, but decreased significantly at 15 days ($p < 0.05$). Expression levels of GDNF, BDNF and CT-1 mRNA followed similar trends than protein levels as determined by qPCR.

Discussion and conclusion: The results reveal that chronic riluzole treatment reduces trophic factor production by glial cells. These results suggest that riluzole has opposite acute and chronic effects on the production of trophic factors, which can explain the small protective effect on ALS patient survival.

Adjusting the dosing regimen for ALS patients may improve drug efficacy and patient survival.

DOI: 10.3109/21678421.2014.960188/285

P286 FAST SKELETAL MUSCLE TROPONIN ACTIVATOR TIRASEMTIV INCREASES MUSCLE FUNCTION AND PERFORMANCE IN MOUSE MODELS OF SPINAL MUSCULAR ATROPHY

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Keywords: SMA, muscle atrophy, fast skeletal troponin activator

Background: The small molecule *Tirasemtiv* is a specific fast skeletal muscle troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force *in situ* in response to sub-maximal rates of nerve stimulation and decreased fatigability.

Objectives: The objective of this study was to investigate the effect of *Tirasemtiv* on skeletal muscle function in two SMA mouse models with mild and moderate levels of muscle dysfunction and weakness.

Methods: Two SMA mouse models were evaluated: a model corresponding to intermediate SMA and a less severe model corresponding to adult-onset SMA. Both models were evaluated *in situ* for plantarflexor isometric muscle force in response

to sciatic nerve stimulation, *in situ* muscle fatigability, and *in vivo* forelimb grip strength and inverted grid hang time.

Results: Intermediate and adult onset SMA mice had lower compound muscle action potentials (CMAP), motor unit number estimation (MUNE) numbers, and hindlimb muscle atrophy. Compared to sibling controls (CON), isometric muscle force *in situ* was significantly lower in both SMA mouse models at all submaximal and tetanic rates of nerve stimulation (10 to 200Hz) ($n = 10$-15/group, $p < 0.0001$, CON vs. SMA). In the intermediate SMA mice, *Tirasemtiv* (10 mg/kg, IP) significantly increased isometric force in response to submaximal (20Hz) nerve stimulation in both female (Vehicle:37 ± 4.7mN vs. *Tirasemtiv*:62 ± 7.2mN, mean $\pm$ S.E.M., $n = 6$/group, $p < 0.05$) and male (Vehicle:24 ± 4 mN vs. *Tirasemtiv*:47 ± 7.9 mN, $n = 4$-5/group, $p < 0.05$) SMA mice. In adult onset SMA mice, *Tirasemtiv* (10 mg/kg, IP) significantly increased submaximal isometric force in response to nerve stimulations between 10-60 Hz ($n = 7$-8/group, $p < 0.001$). In both mouse models, *Tirasemtiv*-treated SMA mice had higher muscle force under fatiguing conditions induced by repeated nerve stimulation. *Tirasemtiv* (10 mg/kg, PO) significantly increased forelimb grip strength *in vivo* in intermediate SMA mice compared to vehicle (43 ± 3.8g vs. 52 ± 4.4g, $n = 9$/group, $p < 0.05$). Adult-onset SMA mice had significantly lower hang time *in vivo* compared to CON mice (CON:197 ± 23 sec, $n = 17$ vs. SMA:138 ± 18 sec, $n = 25$, $p < 0.05$). *Tirasemtiv*(10 mg/kg, PO) significantly increased inverted grid hang time in SMA mice (138 ± 18 vs. 192 ± 34 sec, $n = 25$, $p < 0.05$).

Discussion and conclusion: Intermediate and adult-onset SMA mice exhibited nerve dysfunction, muscle atrophy, and weakness. Single doses of *Tirasemtiv* significantly increased submaximal force and fatigue resistance *in situ*, and grip strength and grid hang time *in vivo* in SMA mice. These results suggest that *Tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving muscle function in spinal muscular atrophy.

Acknowledgements: Generation of the SMA mice was funded by NIH and Families of Spinal Muscular Atrophy (FSMA). Conduct of the studies with *Tirasemtiv* was funded by FSMA and Cytokinetics, Inc. DTH, LK, FIM, and JRJ are currently employees of Cytokinetics, Inc. and were compensated financially for their work.

DOI: 10.3109/21678421.2014.960188/286

P287 DIPALS: PATIENT AND CARER EXPERIENCES OF DIAPHRAGM PACING IN MOTOR NEURONE DISEASE

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Keywords: diaphragm pacing, respiratory support, patient views

Background: Diaphragm movement is an essential element of adequate respiration. There has been considerable interest therefore in using methods of diaphragm stimulation to maintain respiratory function in patients with motor neurone disease (1). Evidence of its effectiveness is limited however, and there have been suggestions that some patients may find it difficult to tolerate (2).

Objectives: The qualitative work reported here forms part of a large-scale randomised controlled trial of the NeuRx RA/4 Diaphragm Pacing System in patients with motor neurone disease (ISRCTN53817913). Objectives of this component are: i) to evaluate the acceptability of the pacing device; ii) to explore the impact of having the system fitted on everyday living.

Methods: Data were collected at two time points, at one month following surgery and six months later. Semi-structured interviews were carried out with patients and carers in their homes.

Results: Fourteen patients took part in the qualitative element of the study. Nine were able to be interviewed at both time points. Participants described the journey from initial invitation to take part in the study, expectations of the procedure versus the reality, post-operative care, views and operation of the equipment, impact on everyday activities, perceptions of the effect, to reflections in hindsight on having the pacer fitted. Patient experience of the surgery varied considerably, with a number having post-surgical complications and longer than expected length of stay, whereas others described it as a minor procedure. Operation of the equipment was generally described as uncomplicated, although some had found the positioning of the socket to be inconvenient, and the fragility of the wiring was reported to be a concern. There was considerable variation in patient ability to tolerate the sensation of the pacer working. At follow up, all patients still had the system in operation with usage varying from two hours to 24 hours per day. The system was rated positively compared to non-invasive ventilation (NIV) however participants described uncertainty regarding any perceived benefit from using the device.

Conclusion: Patient experiences of trialling diaphragm pacing varied considerably. While some experienced pain using the device, others reported feeling only a minor sensation. The device was generally acceptable to patients in terms of ease of operation and impact on life, and while not perceiving immediate gains compared to NIV, patients described their hopes for a beneficial effect in the long term.

Acknowledgements: This project was funded by the National Institute for Health Research (NIHR) Health Technology and Assessment programme.

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DOI: 10.3109/21678421.2014.960188/287

P288 PRO-ACT: EARLY RESULTS FROM THE LARGEST ALS CLINICAL TRIALS DATABASE

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Keywords: disease progression, patients stratification, PRO-ACT

Background and objectives: Large datasets are critical for identifying statistically significant and emphasize biologically relevant observations, especially in a rare disease like ALS. The heterogeneity of the ALS patient population presents a substantial barrier to understanding disease mechanisms and to the planning and interpretation of ALS clinical trials, leading to large, expensive, and potentially unbalanced trials. Therefore, pooling together information from completed ALS clinical trials - into the Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform provides an unprecedented opportunity to increase our understanding of the ALS patient population and further ALS research. In this presentation we will outline results already gained from analyses performed on the PRO-ACT database.

Methods and results: The PRO-ACT platform contains the records of over 8500 ALS patients who participated in 17 completed clinical trials. Data include demographics, family history, vital signs, clinical assessments, lab data, medication and survival information, as well as newly added information about concomitant medication use and adverse events. Additional data are expected to be introduced to the database in 2015. The database was made open-access to researchers worldwide in December, 2012, and since then has attracted the attention of over 330 researchers from 42 countries, including 26 pharmaceutical companies and over 100 academic institutions and hospitals.

The PRO-ACT database provides the unique and unprecedented opportunity to gain deeper understanding of the natural history of the disease, to estimate various traits including individual disease progression and survival, to allow patient stratification and help identify potential diagnostic markers for disease progression. Indeed, analysis of the PRO-ACT database has revealed several novel and important findings, including: identification of several baseline variables that significantly correlate with ALSFRS slope in a multivariate analysis (controlling for age, gender, time from onset and baseline functional measures); Development of novel methods to stratify patients into slow and fast progressors; Identification of several novel predictors of disease progression and of survival and of factors differentiating specifically the very fast and very slow patients.

Discussion and conclusion: These results demonstrate some recent advances in our understanding of ALS clinical information, as well as the power of the PRO-ACT database in addressing important yet unaddressed questions. They demonstrate the importance of pooling together large amount of information for developing a better understanding of ALS natural history, prognostic factors and disease variables and patient stratification. These finding, and future findings to come, can foster our understanding of ALS, and be of help in the design of future clinical trials.

DOI: 10.3109/21678421.2014.960188/288

P289 PHYSICIANS, RESEARCHERS AND PATIENTS AS WILLING PARTICIPANTS IN CLINICAL RESEARCH ENTERPRISE: INCENTIVES AND TECHNOLOGY

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Keywords: collaborations, incentives, prognosis

Background: It is beneficial for medical, research and patient communities to aggregate and cross-link clinical and research information from clinical encounters, clinical studies, health records, and self-reported patient outcomes, while connecting to biospecimen and image repositories. Absence of incentives and desire to share and collaborate slows progress. Patient empowerment is a new paradigm.

Objective: To introduce system of incentives and supporting technologies for standardized yet flexible approach to secure collaboration, integration, harmonization and sharing of clinical and research information by all clinical research enterprise participants.

Methods: Patients, patient advocacy groups, clinicians and researchers were interviewed on foundations that incentivize them to collaborate and share information (physicians/researchers) and participate in research (patients/caregivers/advocacies). Results of surveys and recommendations from PCORI taskforces are analyzed and applied to NeuroBANKTM as truly collaborative platform for disease-specific clinical research. NIH-developed Global Unique ID (GUID) technology allows linking various data sources into coherent distributed meta-dataset, while maintaining regulatory compliance. Disease-area-specific central authority for generating GUIDs is set up by Neurological Clinical Research Institute to assist the neurodegenerative diseases clinical and research community with collecting patients' data and linking it to other information. Informed Consent Metalayer tracks and matches information with its requestor.

Results: For clinicians/researchers, the major obstacles quoted were absence of time, of funding to support data entry process, and too many obligations to fulfil. Some of these issues could be resolved with a carrot/stick approach: additional funding ("carrot", Canadian ALS Association), threat to withdraw existing funding ("stick", MDA, NIMH) or combination (MDA). Technology help with a single point-of-entry approach, as data are captured once and system distributes cleaned data further according to physicians' obligations (EHR, disease registries, hospital repositories, etc.) in predefined formats. Additional incentives are generated by the system patients' summary reports and post-patient-encounter reports. Utilization of existing records with ability to enter new information is paramount. Virtual biobanking approach with centralized clinical data repository proved to be successful and sensible.

From patients' perspective, incentives to participate in research vary with patients' age and disease progression. While

survival concerns prevail, quality of life is more important in the adult population. Current trends suggest shift of power in clinical research enterprise towards patients, especially in data sharing, patient-reported outcomes and patients' control of research directions. Patients may refuse to participate in research projects if information is not shared freely.

Conclusion: NeuroBANKTM platform allows aggregation of existing datasets and direct data capture. Integration of the GUID technology with NeuroBANKTM provides mechanism of linking same-patients' data from multiple sources. GUID technology is uniquely suitable for use with de-identified datasets. It may link biospecimen collections and images with clinical and research data and electronic health records. GUID approach facilitates international collaboration, even when without affiliation with NeuroBANKTM and other collaborative efforts.

Acknowledgment: ALS Association

DOI: 10.3109/21678421.2014.960188/289