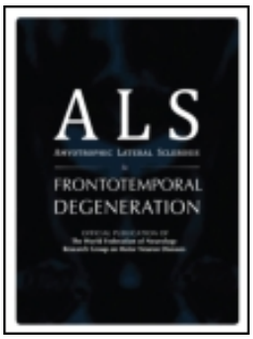

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THEME 11 THERAPEUTIC STRATEGIES

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THEME 11 THERAPEUTIC STRATEGIES

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P287 CLINICAL EFFICACY OF STEM CELLS IN ALS: CORRELATION WITH TROPHIC SUPPORT AND EXPERIMENTAL *IN VIVO* STUDY

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Keywords: mesenchymal stem cells, neuroprotection, trophic factor

Background: Bone marrow mesenchymal stem cell (MSCs) have the potential to modify the disease progression and show neuroprotective and paracrine capabilities in models of amyotrophic lateral sclerosis (ALS).

Objectives: We examined the clinical effects of autologous MSCs in ALS patients and conducted two experimental studies: the first study evaluated the trophic support by MSCs isolated from patients compared with clinical response; study 2 confirmed their efficacy through an *in vivo* model of ALS.

Methods: We enrolled 37 patients for stem cells therapy. MSCs were injected twice intrathecally at an interval of 1 month. Remnant MSCs after therapy were analysed to measure the protein levels of bFGF-2, SDF-1α, VEGF, IGF-1, BDNF, ANG, IL-4, IL-10 and TGF-b in culture supernatants. The individual levels were compared with the clinical response (responder vs non-responder), which were grouped after the closure of clinical trial. For the *in vivo* study, MSCs isolated from one of each group were transplated into cisterna magna in SOD1 mice.

Results: There were no serious adverse events related to therapy in ALS patients. In the responder group, the levels of VEGF, ANG and TGF-b were higher, suggesting different trophic support between responder and non-responder groups. MSC-treated mice showed significant delayed symptom onset and decreased motor neuron loss compared to PBS-treated mice. Moreover, the mice group that received MSCs isolated from responder patient showed prolonged survival and attenuation of the functional decline of motor performance using rotarod test.

Discussion and conclusion: Although further study is needed, our research shows the possibility of selecting candidate patients for autologous MSCs therapy based on the specific characteristics of stem cells, especially trophic factors secreting capacity.

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P288 AMYOTROPHIC LATERAL SCLEROSIS: NEW THERAPEUTIC PERSPECTIVES OFFERED BY IPSC-DERIVED NEURAL STEM CELLS

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Keywords: neural stem cell, transplantation, iPSCs

Objectives: The primary aim of this project was to investigate the therapeutic potential of induced pluripotent stem cell (iPSC)-derived neural stem cell (NSC) transplantation in an established model of amyotrophic lateral sclerosis (ALS), $SOD1^{G93A}$ mice.

Background: ALS is a fatal, incurable neurological disorder with a high social burden. Recently, the scientific community has paid increasing attention to iPSCs as a source of NSCs that can be used for both modeling and therapeutic transplantation strategies.

Methods: We generated iPSCs from healthy human skin fibroblasts through the overexpression of pluripotent-reprogramming factors after transient, non-viral transfection of episomal vectors. We differentiated iPSCs using an established protocol to promote neuronal fate. By FACS selection, we isolated a primitive NSC subpopulation, based on its high ALDH activity and low side scatter (ADLHhiSSClo).

This population was selected due to its capacity to proliferate and differentiate into the three neuroectodermal lineages, and to its high potential ability to reach the central nervous system (CNS). The phenotype of these cells was defined by morphological gene expression and protein profile analysis. iPSC-purified NSCs were administered by intrathecal or systemic intravenous injections into ALS mice, and neuropathological assays and functional tests were performed.

Results: We investigated ADLHhiSSClo NSCs ability to migrate to the CNS after minimally invasive injection and to engraft into the host spinal cord. We demonstrated that iPSC-derived NSCs transplantation significantly prolonged the lifespan of SOD1G93A mice. This approach also improved the disease phenotype in treated animals. Specifically, we observed a better survival of motor neurons, preservation of neuromuscular junctions, and improvement of motor capabilities, coordination and movement after transplantation.

Discussion and conclusions: These data suggest that iPSC-derived NSC transplantation represents a promising opportunity for effective cell-based therapy for ALS and other motor neuron diseases, highly suitable for clinical applications.

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P289 ISOLATION OF PURE IPSC-DERIVED HUMAN MOTOR NEURONS BY A NOVEL P75/HB9 DOUBLE-SELECTION FACS PROCEDURE

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Keywords: IPS cells, FACS, qPCR

Background: The targeted differentiation of human-induced pluripotent stem cells (iPSCs) into ALS-relevant cell types offers unique opportunities for ALS disease modelling, drug testing and eventually cell replacement. Yet, the study of ALS-related phenotypes in iPS-derived cultures is hampered by high inter- and intra-individual variability of differentiating cultures.

Method: To prepare pure motor neuronal cultures, we generated iPS clones from ALS patients and healthy control subjects, optimized the differentiation protocol and developed a novel FACS selection procedure.

Results: The iPS-derived cultures contained motor neurons (HB9+: 14.6 ± 5.2 % of neurons) expressing markers of median motor neuron subtype (LHX3) or lateral motor neuron subtype (FOXP1). To purify these motor neurons from unwanted neural precursor and interneuron populations, we used a lentiviral reporter vector expressing RFP under control of a 3.6 kb-long minimal HB9 promoter in combination with a monoclonal antibody directed against a surface epitope of the low-affinity neurotrophin receptor p75. Using FACS, Hb9 (RFP)/p75 double-positive cells were isolated on a routine basis at a ratio of 0.16 ± 0.02 % of total. The FACS-isolated cells re-attached on culture dishes and developed large cell bodies with prominent neurites after several days *in vitro*. The bona fide motor neurons were exquisitely pure as judged from QPCR and immunofluorescence analyses.

Discussion: We anticipate that pure cultures of IPS-derived human motor neurons will pave the way to indepth studies of ALS-relevant disease phenotypes and experimental therapies.

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P290 ENDOGENOUS STEM CELL MOBILIZATION IN A MOUSE MODEL OF ALS

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Keywords: hematopoietic stem cell, Neulasta, stem cell therapy

Background: One of the most common motor neuron diseases is the well-known disease ALS, which nowadays lacks an appropriate therapy. The challenge is the search for new therapeutic approaches. Recently, cell therapy has risen as a potential candidate for the treatment of neurodegenerative diseases (1). In this study, we propose the use of Pegfilgramstin, a pegylated form of the Granulocyte Colony Stimulation Factor pegylated analog Filgrastim, which is commercially available from Amgen with the name of Neulasta® and is usually used in human clinics.

Objectives: Our main aim is to study the effect of Pegfilgramstin (Neulasta®) in transgenic $SOD1^{G93A}$ mice to analyze the possible hematopoietic stem cell (HSC) mobilization response.

Methods: Transgenic $SOD1^{G93A}$ mice were treated subcutaneously with Pegfilgramstin, once at week beginning at the age of 70 days. At 75, 90, 105 days and endpoint stage, blood samples were extracted from tail vein and HSC, myeloid precursor cells (MPC), lymphoid precursor cells (LPC), monocytes and lymphocyte cells were quantified by flow cytometry and identified according to antigenic expression pattern. Behavioural tests and survival of the animals were monitored. Transcriptional expression of neurodegeneration markers was analyzed using real-time PCR.

Results: Animals treated with Neulasta showed a significant increase in survival rate. Furthermore, a slower decline of motor functions was observed in mice treated with Neulasta. Flow cytometry revealed that HSC, LPC and MPC were mobilized by Neulasta, and this effect was prolonged over time. At the endpoint stage, a significant reduction in the relative number of HSC was observed in treated animals. The transcriptional level of *Impa1* and *Nnt* showed a down-regulation. Additionally, the transcript levels of *Col19a1* and *Mef2c* were down-regulated. No statistically significant differences were observed, under treatment, in the transcriptional levels of Pax7 and myogenic regulatory factors, although a robust down-regulation of *Chrma1* and *RRad* transcriptional expression was observed. Previous studies support these results (2,3).

Discussion: Mobilization of endogenous HSC by Neulasta demonstrated a beneficial effect on locomotor performance and prolongs the survival of transgenic SOD1^{G93A} mice. Over time, Neulasta treatments increased HSCs inperipheral blood. Moreover, under Neulasta treatment, the NMJ stabilization and muscle metabolic restoration can be improved in the animals. These results suggested that mobilizing stem cells, facilitating their transformation and assisting in their recruitment by damaged tissue could be a potential therapeutic approach. Further studies will be needed to determinate its molecular mechanism.

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P291 SCF-ACTIVATED BONE MARROW TRANSPLANTATION IN ALS MODEL MICE

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Keywords: stem cell factor, bone marrow-derived cell, microglia

Background: Transplantation of bone marrow derived-cells or hematopoietic stem cells from wild-type mice was effective in an ALS mouse model. In addition, growth or differentiation factors were reported to protect motor neurons. However, clinical trial of bone marrow transplantation (BMT) or supplement of differentiation factors has shown no successful gain. Additional development is required for the treatment of ALS patients. We hypothesis that a combination therapy of BMT and differentiation factors has the possibility to enhance therapeutic effect.

Objectives: To clarify that the stimulation of stem cell factor (SCF) or FMS-like tyrosine kinase 3(flt3) enhances the therapeutic effect of BMT in hSOD1^{G93A} transgenic mice.

Methods: We gave BMT to 8-week hSOD1^{G93A} transgenic mouse after pre-incubation of bone marrow cells from wild-type mouse with SCF, flt3 or nothing for 12 h. Following transplantation, rotarod motor function test was performed and survival rate was checked, every week. In addition, we analyzed the appearance and the character of bone marrow-derived cell in spinal cord using bone marrow cells from GFP transgenic mice.

Results: Motor function and survival rate were ameliorated in the SCF-stimulated BMT group (SCF-BMT) more than in the flt3-stimulated BMT group (flt3-BMT) and in non-stimulated BMT group (WT-BMT). During this time, many bone marrow-derived cells migrated in spinal cord; this number was remarkably increased in SCF-BMT compared to that in flt3-BMT and in WT-BMT. The majority of bone marrow-derived cells in spinal cord expressed Iba1 known as a microglia marker. Furthermore in the SCF-BMT group, cells expressed glutamate transporter 1(GLT-1).

Discussion: The combination therapy of SCF pre-incubation and bone marrow transplantation improves the therapeutic effect of BMT. The effect is likely caused by the change of migrated bone marrow-derived microglia to neuroprotective cells. This combination therapy is superior to simple BMT treatment, and has a high potential of a new therapy for ALS patients.

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P292 INTRAMUSCULAR TRANSPLANTION OF MUSCLE PROGENITOR CELLS THAT SECRET NEUROTROPHIC FACTORS SIGNIFICANTLY DELAY THE SYMPTOMS AND INCREASE THE LIFESPAN OF MSOD1 MICE

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Keywords: neurotrophic factors, muscle progenitor cells, retrograde transport

Introduction: Neurotrophic factors (NTFs) preserve and protect motor neuron in ALS models. However, all of the clinical studies with administration of NTFs in ALS patients failed. We have developed muscle progenitor cell (MPCs) populations expressing BDNF, GDNF, VEGF or IGF-1, (MPC-NTFs). Combined conditioned media collected from the cells rescued motor neurons cell line (NSC-34) from various insults. Furthermore, MPC-NTF transplantation enhanced the regeneration of rat sciatic nerves after injury. Here we examined the retrograde transport of NTFs along motor neuron axons and tested whether MPC-NTFs transplanted into muscles can improve the symptoms and survival of SOD1 mice.

Results: A mixture of MPC populations each expressing one of the four NTFs was transplanted into the hind legs of SOD1 mice on days 90, 104 and 118 of life. We found a significant delay of the symptoms (up to 30 days in the male) and extension of lifespan (12 days in the males and 18 days in females). However, transplantation of MPC alone or MPC overexpressing just GDNF did not elicit any improvement. The results suggest a synergistic effect of the transplantation of MPCs expression several NTFs. In order to study the possible synergistic effect of the mixture of MPC-NTFs on a signal transduction pathway; we focused on the PI3K-AKT motor neuron survival pathway. We found that the supernatant of a mixture of condition media from MPC populations expressing NTFs increases the phosphorylated AKT by 6- to 8-fold compare to MPC expressing a single NTF.

Discussion and conclusion: Here, we have built a novel powerful strategy enabling a stable, long-term administration of four NTFs factors cocktail. Since intramuscular inoculated muscle progenitor cells participate in the formation of post-mitotic multinucleated fibers, this route of administration of genetically manipulated MPCs results in a stable, long-term expression of the four NTFs. The constant and continuous

releases of the critical NTFs from the muscle fibers through the neuromuscular junction into the motor neuron system, and the retrograde transport to the cell bodies in spinal cords probably inhibit cell death pathways. We hope that our study will lead to a novel strategy to slow the progress and alleviate the symptoms of ALS, and extend the life expectancy and quality of affected patients.

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P293 PERSONALIZING ALS TREATMENT: DIRECT AND SIMULTANEOUS DRUG HIGH-CONTENT ANALYSIS SCREENING ON CELL-BASED MODEL FROM SEVERAL SALS PATIENTS

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Keywords: biomarkers, HCA, drug screening

Background: The causes for most cases of ALS (sporadic ALS (sALS)) are unknown, and the clinical course is highly variable, suggesting that multiple factors underlie the disease mechanism and therefore personalized ALS platforms for drug treatment need to be developed. To this end, we have isolated specific biomarkers found in non-neuronal samples of sALS patients and identified disease-related phenotypic signatures.

Results: We have recently found by Q-RTPCR analysis four novel ALS potential biomarkers in non-neural tissues from sporadic ALS patients in bone marrow mesenchymal stem cells (hMSC) and peripheral blood leukocytes that may have direct diagnostic and pathological implications in the disease (1, 2). Moreover, we have solid evidence that hMSC are ideal tools for image-based high-content analysis (HCA) screening assays aimed for drug high-throughput screening (HTS), since these cells can be kept at defined culture conditions without serum (3, 4).

In fact ALS-hMSCs show significant differences in various cell phenotypic signatures that are relevant to HCA assay development. The sALS cells respond differently to stress induction as compared to non-ALS controls. Recently we have discovered that these four potential ALS biomarkers are differentially expressed also in neuronal and non-neuronal tissues of the transgenic mouse ALS model SOD-1$^{(693A)}$ as compared with wild-type littermates at different times within the 120 days of disease progression (2). Importantly, these results support the physiopathological, diagnostic and prognostic relevance of the ALS biomarkers detected in hMSC and blood samples of sALS patients.

Discussion and conclusion: We propose a new strategy for personalized drug screening that will be based on ALS-associated biological cell phenotypes and molecular biomarkers expression in cell samples of ALS patients. We hope that from this screening, we will be able to identify personalized drugs or drug combinations for future treatment of the ALS patients involved in the study.

Acknowledgments: This work was supported by the Chief Scientist Office of the Ministry of Health (grant 3/6056) and by The Legacy Heritage Biomedical Program of the Israel Science Foundation (grant No. 429/09)

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P294 CHROMOSOMALLY MODIFIED MESENCHYMAL STEM CELLS SECRETING GDNF, IGF-1, AND HGF ATTENUATE DISEASE PROGRESSION IN AN ALS ANIMAL MODEL

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Keywords: trophic factor, stem cell, transplantation

Background: Stem cell transplantation and the injection of neuronal trophic factors are not necessarily mutually exclusive. We might therefore expect to obtain synergistic effects when combining both therapies for the treatment of a neurodegenerative disorder such as amyotrophic lateral sclerosis (ALS). Using a human artificial chromosome (HAC) system, we previously established mesenchymal stem cells (MSCs) that simultaneously express glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-1), and hepatocyte growth factor (HGF). Following transplantation of these cells into an ALS mice model, we also confirmed the efficacy of the transplantation on the ALS disease phenotypes.

The aims of the present experiments were to determine the best parameters for effective transplantation, such as when the transplantation should be conducted and how many cells should be administered for therapy.

Methods: The chromosomally modified MSCs were transplanted into 60-, 80-, 100-, or 120-day-old, high-copy SOD1^{G93A} transgenic mice via the fourth ventricle, from which the cells distributed throughout the spinal cord. Littermate-, age- and sex-matched mice received sham operations or were transplanted with MSCs devoid of the chromosomal modification. The ALS mice model used in the experiments demonstrated ALS symptoms around 120 days and mice expired around 150 days of age. From 1 week prior to the transplantation until death occurred, body weight and the hind limb extension reflex score were measured once a week. FK-506, an immunosuppressive agent, was administered orally to prevent MSC rejection.

Results: In the mice transplanted at 80 and 120 days of age, there were no beneficial results in terms of the age of onset, death, or disease duration compared to control mice. In the mice transplanted at 60 days of age, there were encouraging results with delayed death and increased duration of disease in the treated mice compared to the controls. Where mice were transplanted at 100 days of age, there was statistical significance in terms of the age of death and disease duration, this effect was not seen in the mice transplanted with MSCs without chromosomal modification. When comparing the number of cells used for transplantation, transplantation with

2 million cells showed a greater benefit than that observed with 6 million cells.

Conclusions: The chromosomally modified MSCs delayed the onset of death and increased the duration of disease in the ALS models with mice treated at around 100 days of age, with 2 million cells demonstrating the best transplantation outcome. *In vivo* cell tracing, biochemical and histological investigations are now underway.

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P295 NOVEL FEATURES OF ADULT RAT FACIAL MOTONEURONE RESCUE BY A MUSCLE-DERIVED ISOFORM OF IGF-1

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Keywords: avulsion, facial nerve, MGF

Background: We previously found that gene transfer of an isoform of insulin-like growth factor-1 (IGF-1) that is expressed by active muscle (termed Mechano-Growth Factor, MGF) rescued 80% of adult rat motoneurones following facial nerve avulsion when 80% would have died (1).

Objectives: In this study, we tested the possibilities that (i) the 24aa C-terminal MGF peptide (MGF24) delivered at the time of nerve injury is also neuroprotective; (ii) MGF24 acts via the IGF-1 receptor; (2) and (iii) MGF24 acts through activation of protein kinase C (3).

Methods: The right facial nerve was avulsed as it emerged from the stylomastoid foramen in groups of 5–6 anaesthetised adult (3m) Sprague-Dawley rats (4). In one group, 10µl of 1µg/µl MGF24 in saline was injected into the foramen immediately after avulsion. In two other groups, MGF24 was co-injected with 1µg/µl of either (i) an antibody to the rat IGF-1 receptor or (ii) the protein kinase C inhibitor GF109203X. Control groups received avulsion only or 10µl of either (i) saline, (ii) 1µg/µl liver-type IGF-1 or (iii) 1µg/µl glial-cell derived neurotrophic factor (GDNF). Anaesthetised rats were perfused with fixative 1 month later and numbers of motoneurones were determined stereologically (4).

Results: One month following avulsion only and avulsion plus saline, 80% of motoneurones were lost ipsilaterally. This loss was reduced to 50% by IGF-1 and MGF24 and 20% by GDNF ($p < 0.05$ vs. avulsion only). Co-injection of MGF24 with the IGF-1 receptor antibody or the Protein Kinase C inhibitor resulted in 53% and 60% loss of motoneurones, respectively ($p < 0.05$ vs. avulsion only).

Discussion and conclusion: Our results concur with several published reports that neuronal rescue by MGF is independent of the IGF-1 receptor, but they also indicate that adult motoneuronal rescue by MGF24 does not require protein kinase C activation. Whether this is a novel feature of MGF per se, or whether it is specific to our experimental model, remains to be determined.

Acknowledgement: The study was funded by The University of Adelaide HDR Scholarship Programme.

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P296 THE BIOLOGICAL RELEVANCE OF CORTICOSPINAL MOTOR NEURONS TO MOTOR NEURON DISEASES

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Keywords: upper motor neurons, cortical connectivity, selective vulnerability

Background: Corticospinal motor neurons (CSMNs) have a unique executive function in the brain. They receive, integrate, translate and transmit cerebral cortex inputs to spinal targets, acting as the spokes person for the cerebral cortex especially for motor neuron circuitry. Even though it is the pre-motor neurons that determine CSMN function, it is CSMN that connects the brain and the spinal cord for the execution and modulation of most of the voluntary movement in humans. Therefore, the health and connectivity of CSMN is centrally important for our movement, and CSMN degeneration leads to many motor neuron diseases contributing to paralysis in patients. Understanding mechanisms that underlie selective vulnerability and progressive degeneration of CSMN is critically important for building effective treatment strategies for motor neuron diseases, in which voluntary movement is impaired. Studying the details of CSMN biology has been limited due to the complexity of the cerebral cortex and the limited numbers of CSMN. However, recent developments allow their visualization, isolation and detailed investigation at different stages of the disease.

Results: We found that CSMN show varied transduction efficiency to different AAV serotypes, and that it can be retrogradely transduced by AAV2-2 in the most effective way. Most importantly, introduction of eGFP gene into constructs exposed the cytoarchitectural details of CSMN and revealed that apical dendrite degeneration is an early event in CSMN degeneration and lack of spines especially on apical dendrites suggest early cortical modulation defects in ALS.

Generation of a novel reporter line, the UCHL1-eGFP mice, which express eGFP under the UCHL1 promoter genetically labels CSMN and allows their detailed cellular analysis *in vivo*. Such studies uncovered autophagy as an intrinsic mechanism involved in CSMN apical dendrite degeneration, and begins to reveal the intrinsic mechanims that are involved for their vulnerability.

Discussion: Our ongoing studies shed light onto upper motor neurons as they reveal the intrinsic and extrinsic factors that are involved in CSMN vulnerability and progressive degeneration. We believe that further investigation into CSMN biology will ultimately help to develop effective

treatment strategies for ALS and related motor neuron diseases.

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P297 CHRONIC TREATMENT WITH LITHIUM DOES NOT IMPROVE NEUROMUSCULAR PHENOTYPE IN A MOUSE MODEL OF SEVERE SPINAL MUSCULAR ATROPHY

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Keywords: lithium, spinal muscular atrophy, SMNdelta7 mouse

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by defective levels of the survival motor neuron (SMN) protein. SMA causes spinal motoneuron (MN) loss and progressive muscle paralysis. There is no effective therapy for this disease. Although different strategies focused on increasing the expression of functional SMN protein have been assayed, numerous SMN-independent therapeutic approaches have been demonstrated to have potential effectiveness in improving SMA phenotype in mouse models and clinical trials (1). Recent works have shown that compounds which inhibit GSK-3β activity are effective in promoting MN survival and ameliorating lifespan in models of MN diseases including SMA (2).

Objectives: Taking into account the reported neuroprotective actions of lithium (Li) through the inhibition of GSK-3β in different studies (3), and the controversial effects found in ALS (4), we tested its potential efficiency as a therapeutic agent in a mouse model of SMA.

Methods: CD1 non-transgenic animals, and $Smn^{-/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$ (SMNΔ7) mice and their WT littermates were used in this study. Mice were daily treated with subcutaneous injections of different doses of LiCl starting on P1. Li plasma concentration was determined using atomic absorption spectrophotometry. Doses of LiCl were administered to achieve a plasma concentration of Li similar to the therapeutic range in humans (0.6–1.5 mEq/L). Motor behavioural tests, and histolopathological and western blot analysis in spinal cord and skeletal muscles were performed.

Results: Chronic treatment with Li, initiated before the appearance of disease symptoms, although inhibited GSK-3β, did not improve the median survival, motor behavior, and spinal MN loss linked to SMA. Li administration did not either ameliorate the microglial and astroglial reaction in the spinal cord or the depletion of glutamatergic synapses on MNs observed in SMNΔ7 animals. Moreover, Li treatment did not mitigate muscle atrophy or calcitonin gene-related peptide (CGRP) downregulation in the neuromuscular junctions linked to the disease (5). However, a significant reduction in apoptotic cell death found in the skeletal muscle of SMA mice (5) was observed after Li treatment.

Discussion and conclusion: These results demonstrate that despite its inhibitory action on GSK-3β, chronically administered Li has no beneficial effect on lifespan or neuromuscular dysfunction of SMN 7 mice.

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P298 GHRELIN ATTENUATES DISEASE PROGRESSION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ghrelin, SOD1^{G93A} mouse, food intake

Background: Ghrelin is a stomach-derived peptide hormone which is identified as an endogenous ligand for growth hormone secretagogue receptor 1a (GHS-R1a), and stimulates growth hormone (GH) secretion from the anterior pituitary gland. Ghrelin is also known to regulate energy homeostasis by stimulating food intake and promoting adiposity via a GH-independent mechanism. Recently, growing interest has centred on the role of energy metabolism in amyotrophic lateral sclerosis (ALS).

Objectives: The aim of the current study was to examine the effects of ghrelin in a mouse model of ALS (SOD1^{G93A} mice).

Methods: In study 1, Ad libitum fed SOD1^{G93A} mice were treated with Ghrelin (50 μg/day, n = 15) or vehicle (saline, n = 15) starting at 10 weeks of age. Food intake, body weight change, forelimb grip strength and survival period were analyzed. In study 2, Ad libitum fed SOD1^{G93A} mice were treated with Ghrelin (50 μg/day, n = 8) or vehicle (saline, n = 7) and wild-type (WT) mice were treated with vehicle (saline, n = 8), starting at 10 weeks of age. Gene expression levels in skeletal muscle and number of motor neurons in spinal cord were analyzed. In study 3, WT mice were treated with vehicle under ad libitum fed condition (n = 9) or food-restricted conditions (n = 8) and food-restricted SOD1^{G93A} mice were treated with Ghrelin (50 μg/day, n = 15) or vehicle (saline, n = 13) starting at 10 weeks of age. Food-restricted mice were provided with approximately 90% of the mean amount of food consumed by ad libitum fed SOD1^{G93A} mice. All of the food-restricted mice consumed all food provided during the experiment. Body weight change, lean mass change, forelimb grip strength, gene expression levels in skeletal muscle and number of motor neurons in spinal cord were analyzed.

Results: Ghrelin treatment significantly extended survival period, increased food intake and body weight change,

suppressed forelimb grip strength reduction and prevented motor neuron loss in spinal cord compared with vehicle group in ad libitum fed condition. On the other hand, ghrelin treatment did not suppress the reduction of forelimb grip strength and did not prevent motor neuron loss in food restricted condition. Even in this condition, ghrelin treatment suppressed body weight and lean mass loss and suppressed gene expression which is related to muscle atrophy.

Conclusion: These results demonstrate for the first time that ghrelin significantly attenuates disease progression in SOD1^{G93A} mice mainly through its orexigenic effect.

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P299 INHIBITION OF EXTRACELLULAR CYCLOPHILIN A AS A POSSIBLE THERAPEUTIC TARGET FOR ALS

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Keywords: SOD1, cyclosporin A, inflammation

Background: Cyclophilin A (CypA) is an abundant and ubiquitously expressed multifunctional protein. Its best characterized property is the peptidyl-prolyl *cis-trans* isomerase activity. It is also secreted extracellularly where it has a proinflammatory cytokine-like behaviour. We found that CypA is a translational biomarker of ALS (1), is upregulated during disease progression, is sequestered in insoluble aggregates (2) and is aberrantly secreted extracellularly (eCypA), as demonstrated by high levels of the protein in the cerebrospinal fluid in both ALS patients and SOD1^{G93A} animal models. eCypA plays major roles in inflammatory mechanisms, and these functions are mediated by the interaction with its receptor, CD147. Preliminary data suggested that eCypA was specifically toxic for motor neurons. We hypothesized that astrocytes expressing mutant SOD1 secrete high levels of CypA that exerts a specific toxic effect on motor neurons. The recent discovery of a cyclosporine A (CsA) derivate (MM-218) able to selectively inhibit eCypA allowed us to develop a possible pharmacological approach based on modulation of eCypA function.

Objectives: The aim of this study is to test novel therapeutic approaches based on the modulation of the extracellular function of CypA, in both *in vitro* and *in vivo* models of ALS.

Methods: We used an *in vitro* model consisting of astrocyte-spinal neuron co-cultures, expressing or not SOD1^{G93A}. In this system, we demonstrated that expression of mutant SOD1 in both cell populations causes selective and spontaneous loss of large motor neurons.

Results: We confirmed that conditioned media from astrocyte-spinal neuron co-cultures expressing SOD1^{G93A} present high levels of eCypA. To study the specificity and mechanism of the toxic effect of CypA, we investigated CD147 expression in the SOD1^{G93A} mouse model of ALS. We observed increased expression of CD147 in motor neurons in the presence of the SOD1 mutation. We then tested the effect of MM-218 and four additional CsA analogs on the astrocyte-spinal neuron co-cultures expressing mutant SOD1. We observed that chronic treatments with eCypA inhibitors were not toxic for motor neurons and could rescue motor neurons in a specific range of concentrations.

Discussion and conclusion: The eCypA toxic activities on motor neurons are likely mediated by the interaction with CD147. SOD1^{G93A} motor neurons *in vivo* are possibly more vulnerable to eCypA because of a higher expression of CD147. We demonstrated that inhibiting extracellular CypA with MM-218 rescued SOD1^{G93A} motor neurons. Thus, we confirmed that modulating extracellular activity of eCypA is a potential novel pharmacological approach. Starting from these preliminary data, we are now developing a therapeutic approach in the SOD1^{G93A} mouse model.

Acknowledgements: AriSLA Foundation

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P300 APO-H-FERRITIN INFUSION AS A THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: iron, SOD1 G93A, mouse

Background: Iron misregulation and deposition are consistent features in humans with amyotrophic lateral sclerosis (ALS) and in animal models of the disease. This aberrant iron homeostasis can induce oxidative stress and has been implicated in disease pathogenesis. A recent study by Jeong et al. (1) demonstrated that chelation of iron with a synthetic compound favourably impacts disease progression in the murine model of ALS. Therefore, it has become increasingly appreciated that appropriate management of iron is disrupted in ALS and restoring iron regulation may be an attractive therapeutic strategy.

Intracellular iron homeostasis is maintained by a number of proteins working in synergy; of particular note is H-ferritin. The ferroxidase activity of this protein limits the formation of reactive oxygen species, and both over-expression and exogenous application of ferritin have been shown to be neuroprotective. We propose that H-ferritin is advantageous over a chemical chelator to bind iron because this protein is a naturally occurring ionophore that can redistribute bound iron using pathways already present in the body, and the biological fate of the recycled iron is not detrimental.

Objectives: The objective of this work is to determine whether exogenous application of iron-poor H-ferritin (apo-H-ferritin) into the brain is a means to provide neuroprotection. Primary outcome measures are to evaluate whether infusion of apo-H-ferritin into the lateral ventricles delays disease onset and extends lifespan in the murine model of ALS.

Methods: At 70 days of age, mice with the $SOD1^{G93A}$ mutation underwent surgery to implant a mini-osmotic pump and cannula to deliver continuous infusion of apo-H-ferritin into the lateral ventricle. Pumps were replaced 28 days after the initial surgery to maintain the H-ferritin levels. Onset of disease was behaviourally assessed by performance on the rotarod apparatus, and endpoint was determined by the inability of the animal to right itself within 30 seconds of being placed on its side.

Results: Mice that received infusion of apo-H-ferritin at a concentration of 2.0 mg/ml demonstrated a delay in disease onset and a modest extension of lifespan. Infusion of apo-H-ferrtitin at 4.0 mg/ml was of limited benefit but did not accelerate onset or endpoint as compared to the no surgery control group.

Discussion and conclusion: The data from these experiments suggest that iron chelation using the natural ionophore H-ferritin favorably impacts disease progression the $SOD1^{G93A}$ mouse model of the disease. Our data indicate that further exploration and assessment of apo-H-ferritin as a novel strategy to treat ALS is warranted.

Acknowledgements: This work was supported by a Department of Defense Therapeutic Idea Award and the Robert Luongo ALS Fund.

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P301 ADMINISTRATION OF ANTIBODIES FOR MISFOLDED SOD1 PROLONG SURVIVAL IN THE SOD1-G93A MOUSE

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Keywords: misfolded, SOD1, in vivo

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 rank a panel of antibodies obtained from AviTix and Amorfix to misfolded SOD1 in terms of their affinity and selectivity for mutant, denatured and oxidized SOD1. The top candidates were evaluated for their therapeutic potential in the SOD1-G93A mouse model.

Methods: Antibodies specific to misfolded SOD1 were profiled for their binding affinities for native or misfolded SOD1 and their abilities to immunoprecipitate mutant SOD1. One highly specific antibody was used to develop an ELISA for misfolded SOD1. Antibodies were chosen for *in vivo* assessment in the $SOD1^{G93A}$ mice using chronic and acute dosing paradigms, where SOD1 levels were quantified in the spinal cord. Two survival studies ($n = 14$ and $n = 20$) were run in female B6 $SOD1^{G93A}$ mice dosed weekly with 30 mg/kg intraperitoneally starting around day 50 until day 156. Mice received either a control antibody or one of 2 anti-misfolded SOD1 antibodies. Rotarod performance, body weight, onset and survival were measured. Drug levels in the spinal cord and blood plasma were also measured.

Results: Initial characterization led to the selection of two antibodies, B8H10 and 3H1, for survival studies in the B6 $SOD1^{G93A}$ mouse model. Both antibodies delayed loss in body weight and increased survival when dosed chronically. An 8- to10-day improvement in median survival was observed. No changes in rotarod testing were observed, nor were any significant changes detected in native or misfolded SOD1.

Discussion: Antibodies with good selectivity for misfolded SOD1 were identified. Treatment of female B6 SOD1-G93A mice expressing mutant SOD1 with these antibodies improved survival, but did not reduce levels of misfolded SOD1 in the spinal cord or improve motor function as assessed by rotarod. This discrepancy warrants further investigation, such as assessment of dose-responsiveness of the survival effect and demonstration of central target engagement.

Conclusion: Treatment of $SOD1^{G93A}$ mice with antibodies specific to misfolded SOD1 improves survival, but additional work to understand the mechanism of action is needed.

Acknowledgements: Antibodies were licensed from AviTix and Amorfix. All authors were full-time employees of Biogen Idec.

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P302 NEUROPROTECTIVE AND IMMUNOMODULATORY EFFECTS OF THE SIGMA-1 RECEPTOR (S1R) AGONIST PRE-084, IN A MOUSE MODEL OF MOTOR NEURON DISEASE NOT LINKED TO SOD1 MUTATION

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Keywords: Sigma-1 receptor, neuroprotection, neuroinflammation

Background: *Sigma-1 receptors* (S1Rs) reside at the mitochondrion-associated ER membrane, where they exert

chaperone-like activity and act as inter-organelle modulators of Ca^{2+}-homeostasis, ER-stress and cell survival. S1R-gene mutations have been associated with familial forms of FTLD-MND (1) and of juvenile ALS (2). Knockout of S1R in $SOD1^{G93A}$ mice accelerates disease progression (3), whereas treatment with PRE-084 improves locomotor function and motor neuron (MN) survival, supporting the hypothesis that S1R may represent a new therapeutic target for ALS (4).

Objectives: We aimed at validating S1R as therapeutic target for MND pathologies unrelated to SOD1 mutation such as sporadic ALS. The L967Q-Vps54 mutant Wobbler (wr) mouse was used as a model of MND not linked to SOD1 mutations (5,6).

Methods: Wr mice were treated with PRE-084 (0.25 mg/kg i.p.) from 4 to 12 weeks of age. Locomotor behaviour was assessed twice a week. Analyses of MN survival, astrocyte, oligodendrocyte and microglial (CD11b) markers, S1R protein levels and cellular distribution were performed at the end of treatment.

Results: In healthy mice, S1R was mainly present in spinal cord MN, co-localizing with ER-markers and was detectable in oligodendrocytes and myelin sheaths. In symptomatic mice, S1R increased in the surviving MN and it was detectable in some hypertrophic astrocytes and reactive microglia. PRE-084 significantly improved MN survival (26.5%) and motor performance after the week 4 of treatment. PRE-084 reduced astrogliosis and increased $CD11b^+$ cells without changing the average density of CD11b labelling. Evaluation of microglial phenotypes highlighted a significant increase in the number of $CD68^+$ cells in the white matter of PRE-084-treated mice. The majority of $CD68^+$ cells were also $CD206^+$ and localized close to radial glial cells.

Discussion: The increase in S1R in MN of symptomatic and PRE-084-treated wr mice, together with the beneficial effects exerted by PRE-084 on neuroprotection and motor symptoms, supports S1R as a molecular player crucially involved in the endogenous reaction to pro-degenerative stressors. Since behavioural effects are observed after 4 weeks from the beginning of treatment, sustained stimulation of S1R may be necessary to activate pathways associated with cellular plasticity. Microglia reactivity affects disease progression in ALS. Thus, a shift from an inflammatory M1 to a M2 phenotype ($CD206^+$) involved in tissue restoration may be part of the pro-regenerative response, induced by the S1R agonist.

Conclusions: Our results support pharmacological manipulation of S1R as a promising strategy to cure MND pathologies unrelated to SOD1 gene mutation. An immunomodulatory effect may be part of the mechanism of MN protection mediated by S1R.

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P303 CHANGES IN ENDOCANNABINOID RECEPTORS AND ENZYMES AND BENEFICIAL EFFECTS OF A SATIVEX®-LIKE COMBINATION OF PHYTOCANNABINOIDS IN AN EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: endocannabinoid system, Sativex, neuroprotection

Background: Different cannabinoid compounds, ie Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabinol, selective CB_2 receptor agonists or fatty acid amide hydrolase (FAAH) inhibitors, afforded neuroprotection in the experimental model of amyotrophic lateral sclerosis (ALS) generated by overexpression of a mutated form of superoxide dismutase-1 (SOD-1) (1). By contrast, these mice have been poorly studied to determine the alterations caused by the disease in those elements of the endocannabinoid system targeted by the above treatments.

Objectives: In the present study, we addressed two objectives: (i) to analyze the changes in endocannabinoid receptors and enzymes in the spinal cord of SOD-1 transgenic mice at an advanced phase in the disease progression (17- to 18-weeks old); (ii) to evaluate the cannabis-based medicine SativexÒ, which is a combination of botanical extracts enriched in both Δ^9-THC and cannabidiol (CBD), as a disease-modifying therapy in this experimental ALS model, based on the potentiality of this combination to act through different mechanisms frequently activated by cannabinoid compounds.

Methods: i) To analyze the changes in endocannabinoid system components, we use RT-PCR analyses of untreated animals at several disease stages. ii) Pharmacological experiments consisted of a daily administration of Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts, at a dose of 40 mg/kg (equivalent to 20 mg/kg for each phytocannabinoid), starting when both wild-type and SOD-1 transgenic mice were 10 weeks old (the first symptoms in these animals typically appear at this age).

Results: Our biochemical studies proved a significant increase in CB_2 receptors and NAPE enzyme in SOD-1 transgenic males, and only CB_2 receptors in SOD-1 transgenic females. Moreover, trends toward an increase were also found for MAGL and DAGL enzymes in both genders, but not in CB_1 receptors and FAAH enzyme.

Our results demonstrated that the treatment of SOD-1 transgenic mice with the Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts: (i) partially attenuated the weight loss typical of these animals, but this positive effect was only found in males not in females; (ii) delayed the progression of neurological deficits, in particular in females; and (iii) slightly increased animal survival, an effect observed in both genders.

Discussion and conclusion: In summary, our results provide support to the possibility that Sativex® may serve as a novel disease-modifying therapy in ALS, a disorder with a poor therapeutic outcome at present with only one medicine already approved, Rilutek®, but with a modest efficacy on disease progression. More preclinical studies in additional models of ALS, that is, TDP-43 transgenic mice, will be necessary before clinical evaluation of SativexÒ in ALS patients.

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P304 EFFECTS OF DEXPRAMIPEXOLE ON WHITE BLOOD CELLS IN A MINI-PIG TOXICOLOGY STUDY AND FROM TWO CLINICAL TRIALS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: eosinophilic-associated diseases, dexpramipexole, hematology

Background: Dexpramipexole is a small, orally bioavailable molecule with demonstrated cytoprotective properties in *in vitro* and *in vivo* preclinical studies. Dexpramipexole is in development for the treatment of ALS. The potential for dexpramipexole in the treatment of eosinophilic-associated diseases (EAD) became apparent from a 39-week toxicology study in mini-pigs, and from Phase 2 and Phase 3 clinical trial results.

Objectives: To determine whether treatment with dexpramipexole in ALS subjects resulted in levels of granulocyte reduction similar to that noted in pre-clinical toxicology studies.

Methods: The Phase II, 2-part study randomized 102 subjects and the phase III study randomized 943 subjects in a double-blind, placebo-controlled trial to assess the safety and efficacy of dexpramipexole in ALS, respectively. Subjects were randomized to 25mg, 75mg, or 150mg dexpramipexole twice daily, or placebo for up to 9 months (Phase II), or 150mg dexpramipexole twice daily, or placebo for up to 18 months (Phase III). Monthly CBCs were obtained in both studies. The chronic minipig study was performed to evaluate the potential toxicity of dexpramipexole after administration for up to 39 weeks. Three treatment groups of Gottingen Minipigs® were administered dexpramipexole, at respective dose levels of 7.5, 25, and 75/50 mg/kg/day.

Results: In a chronic toxicology study in Gottingen minipigs, a time- and dose-dependent decrease in eosinophils was observed. In CL201, part 1 of this study, eosinophils were reduced at 12 weeks in the 150- and 300-mg groups. In part 2 of this study, the 4-week washout showed a partial return to baseline. Patients were then re-randomized to 50 or 300 mg/day for 28 weeks, and the 300-mg group demonstrated a clear reduction in eosinophils. In the phase 3 EMPOWER trial, 82% of dexpramipexole-treated ALS patients experienced a 50% or greater decrease in blood eosinophil counts compared to baseline values with additional small reductions in neutrophils, basophils, monocytes, and lymphocytes. However, there were no clinically significant changes in monocytes and lymphocytes. Neutropenia (ANC, $<1.5 \times 109$/L) was observed in 29 dexpramipexole-treated patients (6.1%) and 8 (1.7%) patients receiving placebo, and was reversible upon withdrawal of treatment.

Discussion and conclusion: These empirical observations in essentially three human studies and a large animal toxicology study demonstrate a very robust effect of dexpramipexole on blood eosinophil counts at doses that appear to be well tolerated. The slowly developing and sustained reduction in eosinophils, as well as other cells arising from a multipotential hemapoietic stem cell, suggests that dexpramipexole might diminish hematopoiesis, accelerate apoptosis, or promote migration of eosinophils into tissue, or perhaps a combination of several of these effects. As dexpramipexole is well tolerated in humans following exposures up to 18 months, it may represent a novel therapeutic approach for the treatment of EAD.

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P305 DETERMINING THE SAFETY OF L-SERINE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) AT VARIED DOSES

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Keywords: BMAA, L-Serine, environmental causes

Background: Previous studies into the Guamian ALS–Parkinson's dementia complex have identified β-methylamino-L-alanine (BMAA), as the potential neurotoxin responsible for ALS. BMAA is a non-essential amino acid produced by cyanobacteria. The hypothesis has been that some individuals are vulnerable to BMAA deposition into their central nervous system where it is incorporated into proteins which can then serve as a reservoir for this neurotoxin. Recent evidence suggests that BMAA may be mis-incorporated in the place of the amino acid serine in brain proteins. It has been demonstrated in mammalian neuronal cell cultures that exogenous L-serine could prevent the BMAA neurotoxin from being mis-incorporated into proteins, thereby preventing cell death. Other studies have demonstrated that very high doses of L-serine may compete with the transport of a number of non-essential amino acids across the blood–brain barrier via the y^+ transporter. These findings lead us to believe that high doses of L-serine could possibly stop the mis-incorporation of BMAA into brain proteins which in turn would slow or even abate the progression of ALS.

Objective: To determine the safety and tolerability of L-serine given at 0.5 g twice daily (BID), 2.5g BID, 7.5g BID

or 15g BID for six months in patients with sporadic ALS, and to measure levels of BMAA in CSF, blood and urine pre- and post treatment.

Methods: Twenty patients diagnosed with sporadic ALS on stable doses of riluzole were enrolled. Eligible patients were randomized in a double-blinded fashion to four separate doses for 6 months. CSF, blood and urine were collected and shipped to the Institute for Ethnomedicine for BMAA analysis at baseline and at month 6. ALS-FRS-R and safety laboratories were measured monthly. FVC and the quality of life visual analogue scale (QOLVAS) were measured at baseline and at months 3 and 6.

Results: To date, seven patients have been randomized and 4 patients have completed 3 months of therapy. The average of participants is 64-years of age, and average time of diagnosis to screening is 11 months. No drug-related side effects have been reported from any of the subjects. The rate of ALS progression as measured by monthly change in ALSFRS-R scores in the four patients who have completed 3 months of therapy is 0.5.

Conclusion: L-serine at different doses seem to be well tolerated in patients with ALS

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P306 INHIBITION OF NOX 2 NADPH OXIDASES AS A POTENTIAL TREATMENT FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: NADPH oxidase, neuroinflammation, ROS

Background: Neuroinflammation and oxidative stress are common features of multiple neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS). Chronic activation of microglia and production of proinflammatory and cytotoxic factors including reactive oxygen species (ROS) contribute to progressive degeneration of neurons. In a transgenic model of ALS, NADPH oxidase 2 (NOX2) expression is strongly increased and NOX2 represents a major source of ROS generation during the progression of the disease. Thus inhibition of NOX2 may represent a new promising strategy for the treatment of neurodegenerative disorders. However, no specific and potent NOX2 inhibitor is currently available.

Objectives: To identify novel NOX inhibitors; to evaluate the effect of NOX inhibitors on ROS production in an *in vitro* model of neuroinflammation; to develop new readouts to evaluate oxidative stress *in vivo*; and to evaluate the effect of NOX inhibitors on survival, motor function and oxidative stress in an *in vivo* model of ALS.

Methods: In order to identify small molecule NOX2 inhibitors, we performed a screen of a NINDS library using PMA-activated neutrophils and luminol-enhanced luminescence as a read-out. We tested the effect of selected compounds on ROS production in mouse microglial RA2 cells stably expressing $SOD1^{G93A}$ and activated with LPS. We used LC/MS to quantify formation of superoxide anion in spinal cord of SOD1 G93A mice. We performed *in vivo* tests of selected compounds in $SOD1^{G93A}$ mouse model of ALS.

Results: In search of NOX inhibitors, we identified a group of compounds of the phenothiazine family. Based on their potency of NOX2 inhibition, we selected the following compounds: prochlorperazine ($IC50 = 2.4 \pm 0.4$ μM), promazine ($IC50 = 7.8 \pm 2.2$ μM), perphenazine ($IC50 = 3.9 \pm 0.7$ μM), thioridazine ($IC50 = 2.2 \pm 0.2$ μM). We showed that thioridazine and perphenazine decrease production of O_2- and H_2O_2 in RA2 $SOD1^{G93A}$ cells activated with LPS and this decrease is concentration dependent. We found that O_2- production is increased in the spinal cords of $SOD1^{G93A}$ mice as compared to WT littermates. *In vivo* experiments suggest that thioridazine has a modest protective effect in $SOD1^{G93A}$ mice by increasing their survival by 7 days ($p = 0.028$).

Discussion and conclusion: In conclusion, our preliminary results demonstrate that inhibition of NOX2 is a promising strategy for the treatment of neuroinflammation in neurodegenerative disorders such as ALS. Experiments evaluating the effect of these compounds on ROS production and oxidative stress *in vivo* are ongoing.

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P307 CLINICAL TRIAL OF EDARAVONE IN AMYOTROPHIC LATERAL SCLEROSIS/ PARKINSONISM–DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

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Keywords: Kii ALS/PDC, Clinical trial, Edaravone

Background: Amyotrophic lateral sclerosis/parkinsonism–dementia complex of the Kii peninsula of Japan (Kii ALS/PDC) is a unique ALS with fronto-temporal dementia. The purpose of this study is to reveal clinical effects of Edaravone, a free radical scavenger, on the patients with Kii ALS/PDC.

Objective: Five patients with Kii ALS/PDC (ratio of men to women, 4:1; average age, 68.4 years; average duration of the illness, 9.4 years) were submitted for the study.

Method: Edaravone (30 mg per one time) was administered twice a week, as an intravenous drip infusion to the patients

with Kii ALS/PDC for 16 weeks. Vitamin C (2000 mg/day) and vitamin E (300 mg/day) were used concomitantly. The effect of Edaravone was evaluated using mini mental state examination (MMSE), ALS functional rating scale revised (ALSFRS-R), unified Parkinson's disease rating scale (UPDRS), apathy scale, frontal assessment battery (FAB) and clinical assessment for spontaneity (CAS). This study was approved with the ethical committee of Nansei town hospital.

Result: Two out of the five patients, who had relatively mild symptoms, showed improvement of spontaneity, and mental and physical activity. The mildest patient showed marked improvement in UPDRS, apathy scale and CAS. The other one showed no apparent change. The residual two patients, who were bed-ridden, showed irritability, violence and sexual deviation: Edaravone was given up for 2 months.

Discussion: On the presupposition that oxidative stress plays an important role on the pathomechanism of Kii ALS/PDC, we performed a clinical trial of the free radical scavenger, Edaravone, in five patients with Kii ALS/PDC. Edaravone may be effective in patients at an early stage of the disease. A further large-scale trial will be needed.

Conclusion: Edaravone was effective to Kii ALS/PDC in the early stages of disease.

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P308 SAFETY OF CENTRAL VENOUS CATHETER IN SELF-ADMINISTRATION OF CEFTRIAXONE IN ALS

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Keywords: ceftriaxone, central venous catheter, infection

Background: ALS is a fatal motor neuron disease with only one disease-modifying FDA-approved treatment. There is a dire medical need for new disease modifying treatments. The clinical trial of ceftriaxone was initiated to address this need (1). Ceftriaxone requires twice daily intravenous administration; for this trial central venous catheter (CVC) and home administration by patient and/or caregiver were used, posing a novel challenge in neurological clinical trials. CVCs are associated with risk of infection especially in debilitated patients and have typically required licensed personnel to manage. Trial participants underwent standardized teaching and regular re-evaluation of subjects and caregivers on CVC line care.

Objectives: To compare the incidence of CVC infection in people enrolled in a trial of ceftriaxone with those reported in a retrospective analysis of Strategic Healthcare Program (SHP) database of home infusions using intravenous catheters (2).

Methods: The study of ceftriaxone for ALS is a multicenter, adaptive design clinical trial of ceftriaxone for ALS involving a total of 513 subjects (66 in Stage 2 and 447 in Stage 3) (1). Incidence of CVC-associated infection was collected from the study database for both Stages 2 and 3. Comparison data were obtained from SHP.

Results: In the 513 subjects in the ceftriaxone study, the incidence of CVC infection included line infection (0.23 per 1000 catheter days), exit site infection (0.12 per 1000 catheter days), for an overall CVC infection rate of 0.35 per 1000 catheter days. Overall SHP infection rates reported for tunnelled catheters were 0.70 per 1000 catheter days.

Discussion and conclusion: We found a lower incidence of CVC-associated infections in our study population compared to the rates reported by SHP. The intensive standardized teaching and regular re-evaluation of subjects and caregivers knowledge of CVC safety provided by licensed personnel, and the prohibition of blood draws from CVCs in the ceftriaxone trial are likely contributors.

CVC infection rates from the trial of ceftriaxone for ALS suggest that with specialized training, patients and caregivers can self-administer long-term IV medications via Hickman catheter safely. This opens the possibility for testing other IV drugs for neurodegenerative diseases such as ALS.

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P309 HEAT SHOCK FACTOR-1 (HSF-1) CONTROLS PATHOLOGICAL LESION DISTRIBUTION OF POLYGLUTAMINE-INDUCED MOTOR NEURON DISEASE

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Keywords: Heat shock factor-1, polyglutamine-induced motor neuron disease, pathological lesion selectivity

Background: Adult-onset motor neuron diseases including amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA) share a common feature that disease-causing proteins selectively accumulate in specific regions despite a broad expression. Heat shock factor-1 (Hsf-1) regulates the expression level of Hsps, such as Hsp70, Hsp105 and Hsp40, which are molecular chaperones that play protective roles in the neurodegenerative process by refolding and solubilizing pathogenic proteins. To elucidate the role of Hsf-1 in the pathological lesion selectivity of motor neuron diseases, here we investigate the effect of this molecule on the pathogenesis of the SBMA mouse model. SBMA is a late-onset motor neuron disease caused by the expansion of a CAG repeats in the gene-coding androgen receptor (AR). This

disease affects susceptible regions, such as spinal anterior horn, brainstem, and pancreas, whereas the causative protein is ubiquitously expressed.

Methods: We performed immunohistochemistry and Western blotting of various tissues from wild-type; AR-97Q (SBMA model mouse: 97Q $^{+/-}$, Hsf-1 $^{+/+}$); and AR-97Q *Hsf-1*$^{+/-}$ (heterozygous Hsf-1 knockout SBMA mouse: 97Q $^{+/-}$, Hsf-1 $^{+/-}$) mice using anti-Hsf-1, anti-Hsp70 and 1C2 antibodies. Moreover, we analyzed the effect of lentiviral over-expression of HSF-1 in this mouse model.

Results: Hsf-1 expression levels are associated with the accumulation of pathogenic AR in each region of SBMA mouse. For example, in the cerebellum of AR-97Q mice, there was a scarce accumulation of pathogenic AR in Purkinje cells, where Hsf-1 was expressed at a high level. Conversely, there were abundant polyglutamine-positive cells in the cerebellar granular cell layer, which showed poor immunoreactivity for Hsf-1. In heterozygous Hsf-1 knockout SBMA mice, abnormal AR accumulates in the cerebral visual cortex, liver, and pituitary, which are not affected in their genetically unmodified counterparts. In the spinal anterior horn and other parts of central nervous system of AR-97Q *Hsf-1* $^{+/-}$ mice, the accumulation of mutant AR was substantially increased through Hsp70 down-regulation.

Furthermore, the frequency of pathogenic AR accumulation around the lentiviral vector-injected area of the motor cortex and striatum where HSF-1 was highly expressed was decreased in comparison with that in the contralateral side without treatment. In addition, the neuron sizes of the motor cortex and striatum were significantly increased by the HSF-1 injection.

Discussion: These findings suggest that Hsf-1 contributes to the determination of the pathological lesion selectivity in SBMA.

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P310 PROTEIN DISULPHIDE ISOMERASE IS PROTECTIVE AGAINST MUTANT SOD1, TDP-43 AND FUS PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: PDI, SOD1, ER stress

Background: Superoxide dismutase (SOD1), fused in Sarcoma (FUS) and Tar-DNA-binding protein-43 (TDP-43) are key proteins linked to amyotrophic lateral sclerosis (ALS) pathology. Whilst neurodegenerative mechanisms are not fully defined in ALS, dysfunction to the endoplasmic reticulum (ER) is increasingly implicated in the pathology. Protein disulphide isomerase (PDI) is a chaperone which also functions as disulphide isomerase in the formation and reduction of protein disulphide bonds. It is primarily located in the ER, but it is also found in other cellular locations. Our laboratory previously showed that over-expression of PDI is protective against mutant SOD1. PDI has also been shown to co-localise with FUS and TDP-43-positive inclusions in ALS patients.

Objectives: Here we examined whether over-expression of PDI is also protective against FUS and TDP-43 cellular pathology, ER stress and mis-translocation in the cytoplasm. Furthermore, we examined the mechanism by which PDI is protective. A small molecule mimic of the PDI active site was examined *in-vivo* in SOD1 mice.

Methods: Wild-type and mutant TDP-43 and FUS constructs were co-transfected with PDI in neuronal cell lines; cellular pathological hallmarks, including translocation to the cytoplasm, ER stress and ER-Golgi trafficking were examined using confocal microscopy, immunocytochemistry and immunoprecipitation. Immunohistochemistry was performed using ALS patient tissues.

Results: Over-expression of PDI was protective in neuronal cell lines expressing either mutant TDP-43 or FUS against (i) translocation from nucleus to the cytoplasm; (ii) ER stress ;(iii) inhibition of ER-Golgi transport. Mutations of key residues in the PDI active site demonstrated that the disulphide interchange activity rather than the chaperone activity was necessary for its protective ability. Furthermore, we demonstrated that PDI in the cytoplasm, rather than the ER, is responsible for the neuroprotective activity. Moreover, the small molecular mimic of PDI which mimics its disulphide interchange activity rescued the loss of motor neurons and reduced ER stress in SOD1^{G93A} mice. PDI also co-localised with C9orf72-positive inclusions in ALS patient's motor neurons consistent with the notion that it has broad protective activity against multiple misfolded proteins in ALS

Conclusion: PDI is protective against the major misfolded proteins linked to ALS. The molecular mimic may therefore be a novel therapeutic target in multiple forms of ALS.

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P311 POSSIBLE MITOCHONDRIAL TARGET ENGAGEMENT IN AN OPEN-LABEL TRIAL OF RASAGILINE FOR ALS

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Keywords: pharmacology, biomarkers, therapy

Background: Oxidative stress, mitochondrial dysfunction and apoptosis have been proposed as the cause of motor neu-

ron death in ALS. Rasagiline is FDA-approved for the symptomatic treatment of Parkinson's disease and has demonstrated neuroprotective activities against neurotoxins in neuronal cell cultures and in the SOD mouse model of ALS. *In vitro* experiments indicate that rasagiline stabilizes mitochondria under stress.

Objectives: The specific aim of this open-label screening study is to determine whether rasagiline is safe in this patient population. The secondary aims are to determine whether mitochondrial function is affected by rasagiline by comparing mitochondrial biomarker levels before and after drug treatment, and to obtain preliminary data on disease progression using the ALSFRS-R.

Method: This is a phase II multi-center open-label study in El Escorial probable or definite ALS who met our criteria. Subjects were treated with rasagiline 2 mg daily for 12 months. Biomarkers were obtained before treatment and at 6 and 12 months and included lymphocyte mitochondrial membrane potentials (two methods, JC-1 and Mitotracker); oxygen radical antioxidant capacity (ORAC) assay to measure antioxidative stress; BCL-2/BAX protein ratios using Western blot; and lymphocyte Annexin levels for apoptosis.

Results: Thirty-six patients enrolled at nine centers of the Western ALS study group and 23 patients completed 12 months of treatment. ALSFRS-R declined at a rate of 1.19 per month and was not significantly different from historical controls. Biomarker assays showed increased mitochondrial hyperpolarization (JC-1, fluorescence ratio at baseline = 0.54, 6 months = 0.62, 12 months = 2.43; $p = 0.05$; Mitotracker, percent fluorescence at baseline = 18.05%, 6 months = 28.18%, 12 months = 67.67%; $p < 0.05$). Annexin showed a decrease in cell apoptosis (percent fluorescence at baseline = 29.4%, 6 months = 27.95%, 12 months = 22.72%; $p < 0.05$), Bcl/Bax ratio showed anti-apoptotic cell conditions (baseline = 0.03, 6 months = 0.05, 12 months = 0.27; $p < 0.05$), ORAC showed antioxidant protection against oxidative stress (baseline = 4834.92µmol, 6 months = 6575.45µmol, 12 months = 5700.19µmol; $p < 0.05$).

Conclusion: We may have evidence of mitochondrial target engagement. Other explanations for the biomarker data also include: disease progression that may have changed the assay measurements, or technical components (equipment, experience, processing of samples). Inclusion of a placebo-treated group could help resolve this or, alternatively, re-measuring biomarker parameters after a drug wash-out period. This drug needs to be studied further in ALS and a Phase II placebo-controlled trial is underway.

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P312 REGULATION OF IP3-RECEPTOR-MEDIATED CALCIUM SIGNALING AND CELL DEATH BY THE BH4 DOMAIN OF BCL-XL IN ALS ASTROCYTES

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Keywords: astrocytes, cell death, cell-penetrating peptides

Background: A major constraint to the comprehension of ALS pathogenesis has been long represented by the assumption that this disorder selectively affects motor neurons in a cell-autonomous manner. Yet, the increased knowledge of the complex cellular interactions that exist within the CNS has recently moved the focus of the investigations towards non-neuronal cells, particularly astrocytes. Astrocytes can damage motor neurons by secreting toxic factors, but they can play a deleterious role also by losing functions that are neurosupportive. Recently, we reported that a subpopulation of spinal cord astrocytes degenerates in the microenvironment of motor neurons in the hSOD1^{G93A} mouse model of ALS. Mechanistic studies *in vitro* identified a role for the transmitter glutamate in the gliodegenerative process via the activation of its IP_3-generating metabotropic receptor 5 (mGluR5) (1).

Objectives: The aims of the present project are threefold: i) to study the mechanism(s) underlying astrocyte degeneration in ALS, downstream mGluR5; ii) to investigate the impact of a biologically active peptide, consisting of the BH4 domain of Bcl-X_L fused to the protein transduction domain of the HIV TAT protein (TAT-BH4), towards mGluR5-driven calcium (Ca^{2+}) signaling; and iii) to explore the therapeutic potential of TAT-BH4 *in vivo*, in ALS mice.

Methods: *Cell Cultures:* Primary astroglial cultures were prepared from spinal cord of newborn mice, as previously described (1, 2). *Ca^{2+} imaging:* astrocytes were plated on glass coverslips and loaded with Fluo4-AM. *In vivo* treatment*:* hSOD1^{G93A} mice were administrated daily 5 mg/kg TAT-BH4 peptide, intraperitoneally, starting at the age of 40 days.

Results: Much evidence indicates that non-physiological formation of IP_3 can prompt IP_3 receptor (IP_3R)-mediated Ca^{2+} release from the intracellular stores and trigger various forms of cell death. Based on this, here we investigated the intracellular Ca^{2+} signaling that occurs downstream of mGluR5 in hSOD1^{G93A}-expressing astrocytes. Contrary to wild-type cells, we found that stimulation of mGluR5 causes aberrant and persistent elevations of intracellular $^{Ca2+}$concentrations in the absence of spontaneous oscillations. The interaction of IP_3Rs with the anti-apoptotic protein Bcl-X_L was previously described to prevent cell death by modulating intracellular Ca^{2+} signals. In mutant SOD1-expressing astrocytes, we found that the sole BH4 domain of Bcl-X_L, fused to TAT, is sufficient to restore sustained Ca^{2+} oscillations and cell death resistance. Furthermore, chronic treatment of hSOD1^{G93A} mice with the TAT-BH4 peptide reduces astrocyte degeneration, slightly delays disease onset, and improves both motor performance and survival (2).

Discussion and conclusion: Our results highlight the glioprotective potential of TAT-BH4 and indicate this peptide as a novel therapeutic for the treatment of ALS.

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P313 DELETING EPHRIN-B2 FROM REACTIVE ASTROCYTES IS BENEFICIAL IN ALS

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Keywords: ephrinb2, astrocytes, rodents

Background: Recently it has been shown that the EphA4 receptor is a modifier of ALS. Genetic and pharmacological inhibition of EphA4 rescues the phenotype in the zebrafish model of ALS and increases survival in ALS rodent models. In ALS patients, an inverse correlation was found between EphA4 expression and disease onset. However, what the mechanism of action is has not yet been fully elucidated. Remarkably it is known that EphA4 interacts with both ephrin-a and ephrin-b ligands, which are also bound to the cell membrane by a GPI-anchor or a transmembrane domain, respectively. Several of the EphA4 interaction partners have been shown to be expressed on reactive astrocytes, microglia and oligodendrocytes. These cells play an important role in the pathogenesis of ALS and surround motor neurons which abundantly express EphA4.

A promising candidate for the Eph 4 bind partner is ephrin-b2 as this has been shown to be highly expressed by reactive astrocytes after spinal cord injury. In the spinal cord of WT SOD1 mice, ephrin-b2 was highly expressed in motor neurons while only faint expression could be detected in astrocytes. At symptomatic stages, the expression pattern changes and high immunoreactivity could be detected in astrocytes while the neuronal expression diminished. Similar results were obtained in spinal cords from ALS patients and controls. Although the expression pattern of ephrin-b2 changes during disease progression, the overall expression stays the same as checked by RT-PCR analysis.

Results: We hypothesised that deleting ephrin-b2 from reactive astrocytes might have a beneficial effect on ALS. For this purpose we crossed the conditional ephrin-b2 knockout mouse with a GFAP-specific Cre-line and the $SOD1^{G93A}$ ALS model. Even though the GFAP-Cre promoter shows leaky expression, we find delayed disease onset and prolonged disease duration. These results suggest that ephrin-b2 might play a role in modifying amyotrophic lateral sclerosis, but it will need further investigation.

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P314 INTERLEUKIN-1 RECEPTOR ANTAGONIST TREATMENT OF ALS PATIENTS WITH PREDOMINANT LOWER MOTOR NEURON INVOLVEMENT

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Keywords: Anakinra, IL-1 receptor antagonist, neuroinflammation

Background: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease of adult onset. Neuroinflammation contributes to ALS disease progression. The familial ALS-linked mutant superoxide dismutase 1 (SOD1) activates the inflammasome and the secretion of IL-1b in microglia. Inflammasome-deficiencies or treatment with recombinant IL-1 receptor antagonist (IL-1RA, Anakinra) extended the lifespan of G93A-SOD1 transgenic mice and attenuated the inflammatory pathology (1).

Objective: The aim of this open-label phase-IIb-study was to evaluate the safety and tolerability of Anakinra in 20 ALS patients with a predominant presentation of lower motor neuron dysfunction PMA.

Methods: In an open-label phase-IIb-study (NCT01277315), we included 20 ALS patients (18 male, 2 female; ALS-FRSr = 40.7/48) with either a predominant (N = 9) or pure lower motor neuron degeneration (progressive muscular atrophy; PMA; n = 11). The patients were treated daily for 52 weeks with subcutaneous Anakinra (100 mg/day) in combination with oral riluzol. Serum levels of IL-1RA, IL-6 and IL-1ß were analysed, and the expression levels of the most common ALS-genes (SOD1 und C9orf72) were determined.

Results: As expected, there were very frequent skin reactions (58%) at the beginning of the therapy, which decreased in intensity and frequency during the treatment. Light to moderate headache was reported at least once by less than half of the patients (47%). We did not observe any serious side effects, but three patients dropped out during the trial (two due to ALS-related hypoventilation syndrome and one withdrew from the trial). Between 6 and 12 months of treatment, we found a statistically non-significant reduction of the intraindividual progression rate in patients with PMA compared to those with signs of both upper and lower motor neuron degeneration.

Discussion: Anakinra was well tolerated by ALS patients. In a subgroup of ALS patients presenting with PMA, there was a trend towards slower disease progression. This exploratory trial is limited in proof of efficacy by the small number of patients. Based on the safety and tolerability results of this study, a phase-III-study is justified.

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P315 IS IVIG TREATMENT WARRANTED IN PATIENTS WITH PROGRESSIVE ASYMMETRIC LOWER MOTOR NEURON LIMB WEAKNESS WITHOUT CONDUCTION BLOCK? A PROSPECTIVE, COHORT STUDY

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Keywords: Intravenous immunoglobulin, multifocal motor neuropathy, progressive muscular atrophy

Background: Treatable progressive lower motor neuron syndromes (PLMNS), such as multifocal motor neuropathy (MMN), remain difficult to distinguish from lower motor neuron (LMN) predominant amyotrophic lateral sclerosis (ALS) early in the course of disease. Small case studies demonstrating successful treatment of selected patients, with asymmetric limb weakness that does not meet the diagnostic criteria of MMN, with conduction block (CB) have prompted clinicians to recommend a trial of intravenous immunoglobulin (IVIg) for patients with progressive and asymmetric distal LMN limb weakness without electrodiagnostic features of MMN. However, this treatment approach produces a significant burden on health care resources.

Objectives: To prospectively evaluate the likelihood of response to IVIg by patients presenting with progressive, asymmetric, pure LMN limb weakness, and to determine the clinical phenotype of those who respond.

Methods: The study prospectively recruited 31 consecutive patients with progressive, focal-onset LMN limb weakness, without evidence of clinical upper motor neuron signs, sensory, respiratory or bulbar involvement, or evidence of motor nerve conduction block on electrodiagnostic studies. Each patient underwent treatment with IVIg (2g/kg) for a minimum of 3 months. Electrodiagnostic studies, a neuromuscular symptom score and expanded Medical Research Council sum score were documented before and after IVIg treatment. The final diagnosis was determined after prolonged clinical follow-up.

Results: Only 3 out of 31 patients (10%) responded to IVIg. Of the remaining 28 patients, 43% developed UMN signs and were diagnosed with ALS, 32% developed bulbar and respiratory symptoms and were diagnosed with progressive muscular atrophy (PMA) and 25% developed progressive spreading LMN limb weakness and were diagnosed with probable PMA. All responders demonstrated distal upper limb (UL) onset weakness, EMG abnormalities confined to the clinically weak muscles, and a normal CK. This set of features was also identified in 31% of non-responders presenting with distal UL weakness. Gender, age at onset, number of involved limb regions and the duration of symptoms prior to treatment were not significantly different between groups. Significant side effects from IVIg therapy were reported by 39% of patients.

Discussion: The rate of response to IVIg in this series was considerably lower than previously published studies (response rates, 40–74%), possibly due to previous studies reporting selected groups of patients, and including patients with some electrophysiological features of demyelination, hence increasing the likelihood of an underlying inflammatory etiology. If IVIg treatment was limited to patients in this series who demonstrated the clinical and laboratory features found in the responders, a rate of response of 50% would have been achieved.

Conclusion: The findings of the present study do not support uniform use of IVIg in patients presenting with progressive, asymmetric LMN limb weakness without conduction block, but rather appropriate patient selection based on clinical and laboratory findings.

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P316 2B3-201, GLUTATHIONE PEGYLATED LIPOSOMAL METHYLPREDNISOLONE, ENHANCES BRAIN DELIVERY OF METHYLPREDNISOLONE AND REDUCES PATHOLOGY IN A MOUSE MODEL OF ALS

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Keywords: blood-brain barrier, drug delivery, neuroinflammation

Background: Study of ALS pathophysiology has revealed neuro-inflammation to be a prominent feature across a range of research techniques. Both in ALS patients and in transgenic mice, over-expressing mutant superoxide dismutase-1 ($SOD1^{G93A}$), activation or proliferation of microglia and astrocytes have been observed (1). The anti-inflammatory glutathione pegylated liposomal methylprednisolone (2B3-201) demonstrated superior efficacy and reduced side effects compared to free methylprednisolone (MP) in rodent models of neuro-inflammation (2).

Objective: To investigate the pharmacokinetics, brain delivery, and safety of 2B3-201, and its efficacy in reducing the neuro-inflammatory pathology associated with the transgenic *SOD1* mouse model of ALS.

Methods: 2B3-201 was investigated in a pharmacokinetic and biodistribution studies in both rats and in *SOD1* mice and compared to free MP. In addition, CNS behavioural and repeat-dose toxicology studies were carried out in rats at several dose levels. Next, the efficacy of 2B3-201 was investigated in *SOD1* mice, in which 60-day oldanimals received 8 weekly intravenous injections with either 2B3-201 or free MP (both at 10 mg/kg). *SOD1* and wild-type (WT) animals both receiving saline were used as controls. Efficacy measurements included motor function (rotor-rod), and at the endpoint (116-day-old mice), T2-weighted MRI was used to detect signal intensity in brainstem nuclei (V, VII and XII) and correlated with immunohistochemistry for astrocytes (GFAP) and microglia (Iba1).

Results: 2B3-201 showed an enhanced plasma circulation in rats (half-life of approximately 7 h versus several minutes for free MP), and higher sustained levels of 2B3-201 in brain and spinal cord of *SOD1* mice. 2B3-201 did not lead to the psychotic-like behavioural effects observed in rats with free MP treatment. Repeated weekly administrations of 2B3-201

were well tolerated in rats, while the same weekly doses of free MP caused side effects, such as urine retention. Compared to WT mice, all *SOD1* groups showed a significant decrease in motor performance from 100 d, without any significant treatment effects being observed. All *SOD1* mice showed a significant increase in signal intensity on T2-weighted MR images compared to WT mice ($p < 0.001$), which may reflect the combination of neuronal vacuolation and glial activation in these motor nuclei. Treatment with 2B3-201 reduced T2 hyperintensity, to a greater extent than free MP ($p < 0.01$).

Discussion and conclusion: It is concluded that the higher sustained CNS levels of 2B3-201 compared to free MP contributed to the increased efficacy in attenuating the MRI measures of neuro-inflammation. This was also in the context of an improved safety profile, as a result of drug encapsulation. The CNS-targeted anti-inflammatory agent 2B3-201 has therapeutic potential in human ALS.

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P317 INHIBITION OF PROTEIN AGGREGATION IN ALS

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Keywords: SOD-1, transgenic mice, stem cell-derived motor neurons, therapeutic

Background: To test the role of protein aggregation in motor neuron degeneration and cell death linked to ALS, we have evaluated a novel inhibitor of the aggregation of mutant superoxide dismutase (SOD-1). This compound, named molecular tweezer, or tweezer is a lysine-specific inhibitor of aggregation.

Methods: Stem-cell-derived motor neurons in cell culture were treated with the molecular tweezer CLR01 and survival and formation of protein aggregation in the cytosol were evaluated. In parallel, we treated mutant G93 SOD-1 transgenic mice with the tweezer and are following motor function, animal survival and motor neuron degeneration in these mice.

Results: Using stem-cell-derived motor neurons that express mutant SOD-1 as a disease model, we show that the molecular tweezer prolongs survival in this model system. Transgenic mice expressing mutant G93A exhibit preserved motor function and strength throughout their lifespan. At the time of this initial submission, we do not have sufficient data to analyze mouse survival rates.

Discussion: In summary, the molecular tweezer shows promise as a protein inhibitor in models of familial ALS linked to SOD-1 and may be useful as a research tool that helps understand disease mechanisms in ALS.

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P318 TARGETED GENOME EDITING FOR DEVELOPING NOVEL THERAPEUTIC APPROACHES FOR SMA

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Keywords: SMA, iPSCs, genome editing

Background: Spinal muscular atrophy (SMA) is a severe autosomal recessive genetic motor neuron disease and the leading genetic cause of infant mortality. A possible strategy for treating patients with SMA consists of using genetically corrected induced pluripotent stem cells (iPSCs) for autologous cell therapy. The genetic correction can be achieved by direct genome editing using different strategies including site-specific nucleases (TALENs).

Objectives: To describe the genetic correction of human SMA-induced pluripotent stem cells (iPSCs) using site-specific nucleases TALEN.

Methods: Using a non-viral process, we produced and characterized iPSCs from human SMA fibroblasts and healthy subjects. As a first strategy, we employed a SMN2 sequence-specific oligodeoxynucleotides to direct the exchange of a T to C at position + 6 of exon 7 in iPSC. This allows for the modification of SMN2 to a more SMN1-like sequence. In the second approach, we design a pair of TALENs for SMN2 genomic loci spanning the region flanking the same nucleotide. This method allows for the production of a double-strand break in the region. To complete the genetic correction process, we created a Piggy Back donor plasmid to correct the mutation by homologous recombination.

Results: Using defined methods, we successfully isolated SMA and WT iPSC subclones that were free from vectors and exogenous sequences. We were able to correct iPSC lines through a targeted gene correction approach with single-stranded oligonucleotides demonstrating the ability to isolate iPSC clones in which SMN2 functions as a SMN1-like gene. While motor neurons from uncorrected SMA-iPSCs reproduced disease-specific features, genetically corrected motor neurons showed phenotypic improvement. We synthesize a TALEN pair, and we were able to demonstrate its ability to cut into the target region a SMN2 plasmid. The co-trasfection of TALEN pair and the donor plasmids in SMA iPSC is ongoing.

Discussion and conclusion: Our results suggest that creating genetically corrected SMA-iPSCs could represent a viable cell source for therapeutic transplantation in SMA patients.

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P319 NEW SYNERGISTIC GENETIC TREATMENT EXTENDS SIGNIFICANTLY DELAYS SYMPTOM ONSET AND PROLONGED SURVIVAL IN ALS MICE

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Keywords: gene therapy, glutamate uptake, NRF2

Background: Astrocyte activation occurs in response to central nervous system (CNS) insult and is considered a double-edged sword in many pathological conditions. Furthermore, we have previously described a reduced astrocytic glutamatergic and trophic response to activation. Here, we selected three pathways severally affected in ALS. For each pathway, we selected a single key gene that could affect the entire pathway. EAAT2, the major astrocytic glutamate uptake transporter, can reduce the synaptic glutamate availability, GDH2 converts glutamate into α keto glutarate in the metabolic pathway, thus depleting glutamate bio-availability, and NRF-2 is a major transcription factor in the cellular anti-oxidant response.

Results: In a mouse model of ALS ($SOD1^{G93A}$), intracisternal and intra-muscular injections of three lenti-viral constructs delayed body weight loss, preserved reflex score and motor performance, significantly delays symptom onset and prolonged survival by 120% and 136% from symptom onset in male and female ALS mice, respectively. Treatment of ALS mice with each of the genes individually had little effect.

Conclusions: Our approach to increase the anti-oxidant response in combination with reducing the glutamate excitotoxic levels in the central nervous system as well as with depleting the systemic glutamate bio-availability has proven to be a very effective therapeutic strategy in the ALS mouse model. We hope that our study might provide a novel strategy to slow disease progression and alleviate symptoms of patients suffering from ALS.

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P320 THE ROLE OF STABILIZED NEUROPEPTIDES DERIVED FROM HYPERIMMUNE CAPRINE SERA (HICS) IN MOTOR NEURON DISEASE – IMPLICATIONS FOR A NOVEL THERAPEUTIC STRATEGY IN ALS PATIENTS

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Keywords: human, neuropeptide, biomarker

Objectives: The objective of the study was to determine whether targeting of the hypothalmo-pituitary-adrenal axis at a specific site using novel stabilized neuropeptides could elicit measurable efficacy in patients from a multi-center open-label prospective ALS study conducted up to 12 months in duration.

Recently, HCIS has exhibited evidence of neuroprotection, neuroregeneration and abrogation of pro-inflammatory responses in several in-vivo animal models of neurodegenerative disease and in two separate human phase II double-blind placebo clinical trial in secondary progressive multiple sclerosis and diffuse systemic sclerosis respectively.

Method: A single-arm multi-center open-label study up to 12 months was conducted in 21 subjects with definite ALS (according to the E1 Escorial criteria) received a daily s.c. dose of 1ml (4.5mg/ml) of HICS. The primary intention-to-treat analyses were ALSFRS-R and survival. Secondary outcomes were ALSAQ-40, Jablecki score, FVC, muscle strength, BMI, safety and tolerability. The M:F ratio was 9:1 (10% bulbar: 90% limb onset). In summary, improvement or stabilization in the ALSRFS-R was noted in the group of patients studied, with the majority having been treated for >6 months. Patients showed a significant improvement in ALSFRS-R, ALSAQ-40, and in ALS scores of Jablecki (8.2%, $p<0.05$), muscle power and lung function (FVC) during the study period were also noted. No adverse events were recorded during the entire duration of the study using HICS.

Results: The mechanism of action was investigated in the ALS cohort from serum taken from the patients pre- and post-treatment with HICS. Multiple micro-RNAs previously implicated in the pathogenesis of ALS, together with several candidate neuropeptides and pNFH change, were determined and related to clinical outcome. The results were compared to normal, no neurological disease, controls ($n=10$) and patients from a phase II secondary progressive MS double-blind clinical trial ($n=20$) who were treated with HICS. The groups acted as controls to elucidate whether a definable prognostic marker could be identified specific to the ALS cohort. These studies together with results from two independent $SOD1^{G93A}$ mouse studies using HICS have allowed progress in the development of a rationale for further work in cadaveric human brain tissue taken from ALS patients where the various studies have allowed us to focus on mechanisms of neurotoxicity in ALS. The results of which will be available in Q4 of this year.

Discussion: HICS showed efficacy in humans with ALS with no safety concerns or adverse event recorded. This confirmed the safety profile of the drug once again as seen in two separate phase II clinical trials recently completed. The results of the open-label study in ALS are certainly encouraging but will need to be confirmed in a randomised placebo-control trial in the future.

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