TST-01: A Therapeutic Device to remove toxic proteins from the cerebrospinal fluid (CSF) known to cause ALS disease progression

Dr Marcie Glicksman[1], William Siopes[1], Kevin Kalish[1], Gianna Riccardi[1], Dr. Josh Vose[1], Anthony DePasqua[1]
[1]*Enclear Therapies, Newburyport, United States*

Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
EnClear Therapies is a device-based, biotech company developing a novel therapy to remove the toxic proteins that accumulate in the cerebrospinal fluid (CSF) during ALS. Evidence that CSF contains toxic proteins that are causal to the neurodegenerative effects of the disease has been demonstrated in multiple cell and animal models. The addition of ALS-CSF produces cell death and neuroinflammatory response when applied to cells. The addition of ALS-CSF to animals produces motoneuron deficits and neuroinflammation. CSF from age-matched controls does not have these effects.

Objective:
To remove the toxic proteins found in the CSF that are believed to be contributing to the death of motoneurons and the progression of ALS.

Methods:
EnClear developed a novel CSF access and recirculation system with a proprietary cartridge that can remove specific proteins. Our device can continually remove toxic proteins from the CSF.
Results: We have demonstrated how our system can remove proteins such as TDP-43, Dipeptide Repeats (DPRs) and tau from the CSF. We have examined the effect of such removal in both cell and animal models. We will be submitting an FDA application and will test our system in ALS patients in the first half of 2021.

Discussion:
CSF is known to go through changes as we age and in various central nervous system disease states such as ALS. The removal of the build-up of toxic components from CSF should help reduce the accumulated protein aggregates in the nervous system and slow or stop the progression of ALS.

References:
1. Mishra et al., Mishra et al. Acta Neuropathologica Communications (2020) 8:65
2. Mishra et al. Journal of Neuroinflammation (2017) 14:251
3. Gómez-Pinedo, et al., Neurología. (2018) 33(4):211—223
4. Bauer et al. Int. J. Mol. Sci. (2020) 21, 3564
5. Sumitha et al., Mol Neurobiol (2019) 56:1014–1034
6. Shobha et al., Neuroscience Letters (2010) 473: 56–61

TST-02: AAV9-Ighmbp2 gene therapy significantly improves motor performance in severe SMARD1-like mouse model, nmdem3

Sarah Holbrook[2], Dr. Amy Hicks[1], Dr. Paige Martin[1], Dr. Kathrin Meyer[3], Dr. Greg Cox[1]
[1]The Jackson Laboratory/ University Of Maine, Bar Harbor, United States, [2]The University of Maine, Orono, United States, [3]Nationwide Children's Hospital, Columbus, United States

Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Autosomal recessive mutations in IGHMBP2, a ubiquitously expressed DNA/RNA helicase, have been linked to childhood neuromuscular degenerative diseases (NMDs). C57BL/6J-Ighmbp2em3Cx is a SMARD1-like strain, or Spinal Muscular Atrophy with Respiratory Distress, created via CRISPR-Cas9 targeting of the IGHMBP2 gene and hereafter referred to as em3. SMARD1 is characterized by muscle weakness starting in the distal extremities and diaphragmatic paralysis leading to respiratory failure. Most patients are diagnosed in early infancy and die in early childhood. The em3 mouse has more severe muscle atrophy than the historical SMARD1-like model (nmd2J) in the hind limb, diaphragm, and intercostal muscles. The em3 mouse model also has an average lifespan of ~3 weeks compared to the 2J's ~3 month lifespan.
Gene therapy has shown promise in another NMD, Spinal Muscular Atrophy (SMA). In collaboration with the Meyer lab at Nationwide Children's Hospital in Columbus, OH, we are testing 2 different AAV9-Ighmbp2 vectors. Each has a different promoter with one having a Chicken β-Actin (CBA) Promoter [higher expression levels than endogenous levels] and the other having a truncated Methyl-CpG binding protein 2 (MECP2 aka P546) promoter [expression levels close to endogenous levels expressed by muscles and neurons]. We did p1 intracerebroventricular injections on em3/em3, em3/+, and +/+ pups to determine the efficacy of each treatment, respectively.
We were blinded as to which vector was which and therefore, in this poster, the viruses are labeled A, B, and C respectively [with one of the virus treatments being an empty vector]. We saw that Virus A and Virus C had significant improvements in lifespan, body weight, muscle weight, femoral motor axons counts, wire hang grip strength, and neuromuscular junction occupancy compared to mutant em3/em3 mice. Virus B was not statistically different from em3/em3. We also saw that Virus C did better with wire hang grip tests, NMJ occupancy, and muscle area than Virus A mice. However, both Virus A and Virus C did not completely rescue the treated mice with treated Virus A and Virus C em3/em3 mice still being smaller and having less axons in the femoral motor nerves than +/+ mice with Virus C. No toxic effects have been seen in treated +/+ mice. The endpoint for this current trial was 8 weeks with longitudinal measures such as wire hang and body weight being measured every week and tissues such as nerves, the medial gastrocnemius, etc being collected at 8 weeks to determine if treated em3/em3 mice would survive past the 3 week timepoint and, if so, how well impacted tissues were rescued. In conclusion, neither virus completely rescues the phenotype via ICV injection at p0 and Virus C shows the best improvement overall out of the vectors tested.

TST-04: Efficacy of Ciprofloxacin/Celecoxib combination in zebrafish models of amyotrophic lateral sclerosis

Hagit Goldshtein[1], Alexandre Muhire[1], Miss Virginie Légaré[2], Mrs Avital Pushett[3], Ron Rotkopf[4], Dr Jeremy Shefner[5], Randall Peterson[6], Prof Gary Armstrong[2], Dr Niva Russek- Blum[1]
[1]The Dead Sea Arava Science Center, Auspices of Ben Gurion University, , Israel, [2]Department of Neurology and Neurosurgery, Montreal Neurological Institute, Faculty of Medicine, McGill University, Montreal, Canada, [3]NeuroSense Therapeutics Ltd., Herzeliya, Israel, [4]Bioinformatics and Biological Computing Unit, Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel, [5]Barrow Neurological Institute, University of Arizona College of Medicine, Phoenix, United States, [6]College of Pharmacy, University of Utah, Salt Lake City, United States

Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
Due to the complex range of cellular events that lead to ALS (including aberrations in RNA regulation, defects in axonal transport and neuroinflammation), a combined treatment of multiple downstream pathways may be a beneficial therapeutic strategy. Ciprofloxacin is an approved synthetic antibiotic compound belonging to the fluoroquinolone family that was found to possess a substantial RNA interference (RNAi) enhancing activity. Celecoxib is an approved non-steroidal anti-inflammatory drug (NSAID) that specifically inhibits COX2, potentially interfering with glutamate-induced excitotoxicity, inflammation and oxidative stress (OS)-related toxicity. Combined activity of Ciprofloxacin and Celecoxib has been previously demonstrated and Celecoxib has been shown to cause the accumulation of Ciprofloxacin inside cells and in cerebrospinal fluid.

Objective:
To evaluate the efficacy of a fixed- dose combination of two approved drugs, Ciprofloxacin and Celecoxib, as a potential therapeutic treatment for ALS.

Methods:
Toxicity and efficacy of Ciprofloxacin and Celecoxib were tested, each alone and in distinct ratio combinations in a SOD1 G93R transgenic zebrafish model for ALS. Quantification of swimming measures following stimuli, measurements of axonal projections from the spinal cord, neuromuscular junction structure and morphometric analysis of microglia cells were performed in the combination- treated vs non- treated mutant larvae. Additionally, quantifications of touch-evoked locomotor escape response were conducted in treated vs non- treated zebrafish expressing the TARDBP G348C ALS variant.

Results:
When administered individually, Ciprofloxacin had a mild effect and Celecoxib had no therapeutic effect. However, combined Ciprofloxacin and Celecoxib (Cipro/Celecox) treatment caused a significant increase of ▯84% in the distance the SOD1 G93R transgenic larvae swam. Additionally, Cipro/Celecox elicited recovery of impaired motor neurons morphology and abnormal neuromuscular junction structure and preserved the ramified morphology of microglia cells in the SOD1 mutants. Furthermore, larvae expressing the TDP-43 mutation displayed evoked touch responses that were significantly longer in swim distance (110% increase) and significantly higher in maximal swim velocity (▯44% increase) when treated with Cipro/Celecox combination.

Discussion:
Cipro/Celecox combination improved locomotor and cellular deficits of ALS zebrafish models. These results identify this novel combination as effective, and may prove promising for the treatment of ALS (Goldshtein et al. 2020). NeuroSense Therapeutics Ltd., which holds the patent rights to Cipro/Celecox combination, developed a unique formulation, called PrimeC. Following these exciting pre-clinical results, PrimeC is now being clinically tested both in Israel and the US.

References:
Goldshtein, H., A. Muhire, V. Petel Légaré, A. Pushett, R. Rotkopf, J. M. Shefner, R. T. Peterson, G. A. B. Armstrong, and N. Russek-Blum. 2020. 'Efficacy of Ciprofloxacin/Celecoxib combination in zebrafish models of amyotrophic lateral sclerosis', Ann Clin Transl Neurol, 7: 1883-97.

Acknowledgements: This work was supported by NeuroSense Therapeutics (NST), the Israeli Ministry of Science and Technology (MOST) and ALS Canada.

TST-05: Novel small molecules against a target involved in lysosome biology rescues across multiple genetic mutations in ALS and show dose-dependent target engagement in-vivo.

Dr Irene Choi[1], Dr Mark Rosen[1], Grace Kim[1], Dr Swati Naphade[1], Dr Brian Kopec[1], Wendy Liang[1], Katherine Kurylo[1], Ekaterina Stomakhina[1], Lyn Batia[1], Dr Ningzhe Zhang[1], Dr Victor Hanson-Smith[1], Alice Zhang[1]
[1]*Verge Genomics, South San Francisco, United States*

Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
Verge Genomics has developed several novel potent small molecules that demonstrate efficacy across multiple genetic mutations in ALS. Evaluating gene expression between sporadic ALS patients and healthy individuals, we identified a gene network that is robustly down-regulated across multiple ALS patient cohorts. Using this network, we validated a target involved in lysosome biology that has since been found to be disrupted in multiple genetic mutations in ALS. Leveraging druggable mechanisms in this pathway, we have developed several small molecules that are orally available and show dose-dependent reductions in a target-engagement biomarker.

Objectives:
Novel chemical structures were synthesized, optimizing for potency against PIKfyve kinase as well as oral bioavailability for development of a small molecule therapeutic.

Methods:
Compounds were evaluated for potency in a cell-free biochemical assay measuring for the reduction of the enzyme substrate (phosphatidylinositol-3-phosphate) with increasing concentrations of the small molecule (conducted at Nanosyn). Compounds demonstrating kinase inhibition were then evaluated in the human embryonic kidney (HEK-293T) cells transfected to express TDP43 M337V, which shows increased cell death compared to healthy HEK cells. Reduction in cell death was measured in a dose-dependent manner. Further, compounds were tested in ALS-C9 patient-derived motor neurons from inducible pluripotent stem cells (iPSC MN), individually tracked for survival following nutrient removal for 10 days in culture. Oral availability was conducted in the mouse pharmacokinetic study comparing oral and intravenous administration of the compound. Further in-vivo target engagement was determined measuring changes in Il-12p40 concentrations following compound administration and lipopolysaccharide (LPS) challenge.

Results:
We developed a library of compounds that show robust PIKfyve potency (IC50 < 30nM) which significantly correlate with rescue in the HEK/TDP43 assay. These compounds also demonstrate significant rescue in C9 iPSC MN survival. Furthermore, in-vivo testing shows these compounds are orally bioavailable with long plasma half-lives and exhibit dose-dependent activity, demonstrating significant reductions in Il-12p40, a target engagement biomarker.

Discussion and Conclusions:
Verge has generated a library of compounds that are well positioned for potential development candidates to advance to the clinic. Evaluation of these compounds demonstrated that potent PIKfyve kinase inhibitors are able to show rescue in multiple mutations of ALS. Furthermore, Verge compounds are on track for development as an oral therapeutic with preclinical in-vivo studies showing oral bioavailability and on-target engagement activity in a dose-dependent manner. Current studies are underway to evaluate efficacy in a rodent model of ALS as well as general safety and tolerability to advance to IND-enabling studies.

TST-06: PARP inhibition reduces axonal degeneration in a C. elegans model of ALS

Mr Gilles Tossing[1], PhD Alex Parker[1]
[1]*Université De Montréal, Montreal, Canada*

Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Axonal degeneration is observed in early stages of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). This degeneration generally precedes apoptosis and is therefore may be a promising therapeutic target. An increasing number of genes have been identified to actively regulate axonal degeneration and regeneration, however, only a few potential therapeutic targets have been identified in the context of neurodegenerative diseases. Here we investigate DLK-1, a major axonal regeneration pathway and the contribution to axonal degeneration phenotypes in several C. elegans ALS models. From this pathway, we identified the PAR polymerases (PARP) PARP-1 and PARP-2 as the most consistent modifiers of axonal degeneration in our models of ALS.

TST-07: TBK1 Autophagy Pathway Disease Mechanisms in ALS

Erika Norabuena[1], Clinton Bourbonais[1], Dr. Kasper Roet[1], Dr. Kevin Eggan[2], Dr. Daniel Elbaum[1], Dr. Sandy Hinckley[1]
[1]QurAlis, Cambridge, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Harvard University, Cambridge, United States

Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are linked to deficits in the autophagy pathway. Recently, mutations that cause haploinsufficiency in TANK-Binding Kinase 1 gene (Tbk1), a gene critical for normal autophagy pathway function, have been found in ALS patients with both familial and sporadic forms of the disease, and in patients with FTD. Patients harboring TBK1 mutations suffer from haploinsufficiency leading to reduced TBK1 activity. Thus, rescuing TBK1 function in patients with any of these mutations could restore normal autophagy processes and potentially halt disease progression. We and others have found that both functional protein and RNA levels in TBK1 ALS patient cells are approximately 50% of WT. TBK1 patient cells also show a ~50% decrease in activation of TBK1. We have discovered that loss of TBK1 function impairs autophagy in human neurons. QurAlis has identified a drug target that negatively regulates the TBK1 pathway. Target knockdown restores pathway activity and protects against toxicity induced by proteasome inhibition in human neurons. Our therapeutic strategy is to inhibit this target either by using an antisense oligonucleotide to reduce expression or by using a small molecule inhibitor of this target in ALS patients, thereby restoring TBK1 pathway activity, to slow or halt disease progression. Given that TBK1 interacts with several other ALS-associated proteins in the autophagy pathway, the addressable patient population could be significant; patients with defects in these genes comprise approximately 10-15% of ALS patients worldwide. Overall, our goal is to discover and develop a novel therapeutic compound to address protein homeostasis dysfunction in ALS patients.

Acknowledgements:
Research support includes NINDS SBIR 1R43NS110339 – 01 and WA1131 ALS Association

TST-09: Identification and Validation of Small Molecules Preventing TDP-43 Aggregation as Therapeutics for Amyotrophic Lateral Sclerosis

Marc Shenouda[1,2], Ross S. Mancini[3], Kevin C. Hadley[4], Mark Reed[5], Christopher Barden[5], Avijit Chakrabartty[4,6,7], Donald F. Weaver[3,8,9], Janice Robertson[1]
[1]Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada, [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, [3]Department of Fundamental Neurobiology, Krembil Research Institute, University Health Network, Toronto, Canada, [4]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada, [5]Treventis Corporation, Toronto, Canada, [6]Department of Medical Biophysics, Princess Margaret Cancer Centre , University of Toronto, Toronto, Canada, [7]Department of Biochemistry , University of Toronto, Toronto, Canada, [8]Department of Chemistry, University of Toronto, Toronto, Canada, [9]Department of Medicine, University of Toronto, Toronto, Canada

Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
Nuclear depletion and neuronal cytoplasmic aggregation of the transactive response DNA-binding protein 43 (TDP-43) is the most characteristic pathology of Amyotrophic Lateral Sclerosis (ALS). The cytoplasmic aggregation of TDP-43 has been correlated with inducing neuronal loss. As such, preventing TDP-43 aggregation could have therapeutic potential for ALS and associated diseases. It has been shown in vitro that aggregation of TDP-43 can occur through structural transformations of an amyloid core region localized with the C-terminal low complexity domain.

Objective:
To identify and validate small molecules targeting the amyloidogenic core region of TDP-43 to prevent its aggregation.

Methods and Results:
The first stage of the discovery pipeline consisted of an in silico screen of over 50,000 compounds based on structural predictions of the amlyoidogenic core of TDP-43 using the Common Conformational Morphology (CCM) methodology (Treventis™ Corporation). The in silico screen identified >500 hit compounds. These compounds were then screened in an in vitro turbidity assay [2] to identify compounds that inhibit aggregation of TDP-35 and TDP-25, two pathological isoforms of TDP-43 that spontaneously aggregate in vitro and form cytoplasmic aggregates in cell culture. The top 20 compounds identified from the in vitro assays were screened in HEK293T cells transiently expressing either GFP-TDP-35 or GFP-TDP-25. Over two rounds of lead optimization, 278 analogues of the most potent compounds were synthesized and/or purchased, tested in vitro and the top leads in the cell culture model. To date, we have successfully identified and validated 8 potent compounds in cells, with the top compound reducing the number of cells with aggregated GFP-TDP-35 and GFP-TDP-25 by 50% ($p<0.0005$).

Discussion and Conclusion:
We have identified small molecule inhibitors of TDP-43 aggregation both in vitro and in cells. The top hit compounds identified from the cell-based assays are currently being screened in primary cortical and motor neurons expressing TDP-35 and TDP-25, and the top compounds will be tested in an in vivo model of TDP-43 aggregation with the goal of providing a drug(s) for clinical testing.

Acknowledgements:
This work was supported by the James Hunter and Family ALS Research Initiative, the Weston Brain Institute, ALS Society of Canada, Brain Canada and Fondation Vinçent-Bourque.

References:
[1] Jiang, L.L., et al., Structural transformation of the amyloidogenic core region of TDP-43 protein initiates its aggregation and cytoplasmic inclusion. J Biol Chem, 2013. 288(27): p. 19614-24.
[2] Sun, Y., et al., Physiologically Important Electrolytes as Regulators of TDP-43 Aggregation and Droplet-Phase Behavior. Biochemistry, 2019. 58(6): p. 590-607.

TST-10: Mitometin, a novel therapeutic drug class targeting mitochondrial dysfunction significantly prolong life in SOD1 rodent models of amyotrophic lateral sclerosis

Dr. Anne Skøttrup Mørkholt[1,2], MD Michael Sloth Trabjerg[2], Pam Huntjens[1], Kirsten Egelund Oklinski[1,2], Preben Bruun-Nyzell[1], Dr. John Dirk Nieland[1,2]
[1]2N Pharma, Aalborg, Denmark, [2]Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
A shift in the balance between the glucose and fatty acid metabolic pathways can lead to metabolic dysfunction, a contributing factor in the development of neurodegenerative diseases such as ALS. This way of looking at CNS diseases like ALS has been overlooked in the past. Taking this into account along with the urgent medical need, it is time to revise the general pathologic view of ALS. Therefore, 2N Pharma has on this basis developed a ground-breaking treatment strategy by targeting mitochondrial metabolic dysfunction, which we have shown to be an underlying disease driver of ALS.

Objectives:
To develop an effective therapy for patients with ALS by focusing on the extremely upregulated lipid metabolism often observed, by shifting systemic cellular metabolism into glucose metabolism, thus prolonging the life and quality of life for ALS patients.

Methods:
2N Pharma has developed a new reversible CPT1 inhibitor, Mitometin, that is being optimized for clinical use. 2N Pharma has obtained preclinical data on the effect of blocking CPT1 by using the SOD1 G93A animal model.

Results:
Pre-clinical data have shown that Mitometin slows down disease progression and has life prolonging properties in SOD1 G93A mice. Genetic downregulation of CPT1A activity in mice results in significant delay in disease symptoms and in humans a 10-fold reduction of disease incidence. Moreover, pharmacological (first generation CPT1 blockers) downregulation of CPT1 activity in mice results in amelioration of disease symptoms, inflammation, mitochondrial function and oxidative stress, whereas upregulation by initiating high-fat diet or corticosterone administration results in a more aggressive progression of disease. Lastly, by downregulating CPT1 the gut microbiota communities promote a shift towards a protective phenotype in SOD1 G93A mice.

Conclusions:
These findings reveal that mitochondrial metabolic dysfunction and specifically CPT1 plays a pivotal role in the disease progression in the SOD1 G93A mouse model. Treatment with the CPT1 blocker Mitometin demonstrates a reduction of disease progression, thereby underpinning CPT1 as a promising future therapeutic target in ALS.

TST-11: Neuroprotection by a CNS penetrant class I histone deacetylase inhibitor in culture and animal models of ALS

Mr. Mario Fernandez[1], Mr Frederic Fiore[2], Ms Maria del Carmen Pelaez Brene[3], Dr Nancy Larochelle[1], Ms Sandra Minotti[1], Dr Afrooz Dabbaghizadeh[1], Dr Josephine Nalbantoglu[1], Dr Chantelle Sephton[3], Dr Richard Robitaille[1], Dr Heather Durham[1]
[1]McGill University, Montreal, Canada, [2]Universite de Montreal, Montreal, Canada, [3]Universite Laval, Ville de Quebec, Canada

Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
Nucleosome remodeling is abrogated in ALS including disruption of nBAF chromatin remodeling complexes and post-translational modifications of histones (1). Histone acetylation and the chromatin landscape influence response to activity and expression of stress-response pathways including heat shock genes. Upregulation of heat shock proteins is a therapeutic strategy for disorders of proteostasis, including ALS; however, neurons can be resistant. We reported that inhibition of class I histone deacetylases (HDAC) enables the heat shock response in cultured spinal motor neurons in a stress-dependent manner, and improved the efficacy of HSP-inducing drugs (including arimoclomol) in murine spinal cord cultures subjected to thermal or proteotoxic stress (2).

Objectives:
In this study, we are testing a novel CNS-permeant HDAC class I inhibitor, RGFP963 (BioMarin), on functional biomarkers in culture models of fALS 1, 6 and 10 and in B6SJL-Tg(SOD1*G93A)1Gur/J (JAX) and Meox2Cre FUSR521G mice (3).

Methods and results:
ALS culture models (Durham/Nalbantoglu lab):
RGFP963 induced a dose-related decrease in HDAC activity and increase in histone acetylation, without changing histone levels. Plasmids encoding SOD1G93A, TDP43G348C or FUSR521H were expressed in motor neurons of dissociated murine spinal cord cultures by intranuclear microinjection. Treatment with RGFP963 (1µM) significantly preserved nuclear FUS and TDP43, along with nuclear expression of Brg1, the nBAF chromatin remodeling complex ATPase.

Preservation of neuromuscular function in SOD1G93A mice (Robitaille lab):
Mice were injected i.p. with 20mg/kg of RGFP963 or vehicle 5 days/week for 25 days, from P50-P75. Nerve-muscle (EDL) preparations were assessed physiologically and morphologically in comparison to nontransgenic B6SJL mice. At P75, RGFP963-treated SOD1G93A mice generated 50% more muscle (EDL) force with either nerve or direct muscle stimulation and had more completely innervated neuromuscular junctions and fewer completely denervated NMJs than vehicle-treated 75-day old SOD1G93A mice.

Restoration of cognitive performance in FUSR521G mice (Sephton lab):
Mice were treated with vehicle, 10mg/Kg RGFP963 by IP injection daily for 28 days beginning from postnatal day 60. At treatment onset, mice were significantly impaired in the novel object recognition and passive avoidance tests. RGFP963 restored novel object recognition, but not passive avoidance, to control levels passive avoidance (20mg/Kg is being evaluated).

Conclusion:
RGFP963 was neuroprotective in culture models of ALS through multiple mechanisms; in vivo, treatment significantly prevented loss of neuromuscular innervation and function in SOD1G93A mice and restored novel object recognition in FUSR521G mice. These data add to the evidence that class I HDAC inhibitors with good CNS bioavailability and safety profile have potential in ALS alone or in combination.

(1) Tibshirani et al., HMG 26):4142 2017PMID: 28973294
(2) Kuta et al., Cell Stress Chaperones 25:173 2020 Jan 3. PMCID: PMC6985055
(3) Sephton et al., PNAS 111:E4769 2014 PMID: 25324524

Supported by ALS Canada - Brain Canada; RGFP963 was provided by BioMarin

TST-12: NU-9 eliminates degeneration of upper motor neurons diseased by mSOD1 toxicity and TDP-43 pathology

Dr. Baris Genc[1], Dr. Mukesh Gautam[1], Ms. Oge Gozutok[1], Dr. Ina Dervishi[1], Mr. Santana Sanchez[1], Dr. Gashaw Goshu[1], Ms. Nuran Kocak[1], Mr. Edward Xie[1], Dr. Richard Silverman[1], Dr. Hande Ozdinler[1]
[1]Northwestern University, Chicago, United States

Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
There are no preclinical assays that investigate cellular responses of upper motor neurons (UMNs). Our goal is to incorporate improved UMN viability as selection criteria for preclinical studies, prior to moving into clinical trials.

Hypothesis:
Increasing success rate of future clinical trials requires information obtained directly from diseased neurons. We generated the UCHL1-eGFP reporter line, in which the UMN express eGFP allowing visualization and detailed cellular analysis of UMN both in vitro and in vivo. As we change our focus from mice to motor neurons, it is possible to learn directly from diseased neurons so that the compounds that improve their survival and health can be further considered for future clinical trials.

Methods:
The Silverman lab characterized a class of small chemical compounds for their ability to protect against mutant SOD1-induced cytotoxicity and reduced protein aggregation. NU-9, one of the compounds in this group, lacks toxicity and has the ability to cross the blood brain barrier with favorable pharmacokinetic values. We focused on NU-9's efficacy to improve UMN survival in vitro and in vivo in hSOD1G93A and prpTDP-43A315T ALS mouse models. NU-9 was administered daily by oral gavage from P60 to P120, and motor behavior was tested every 7 days. UMN in motor cortex and lower motor neurons (LMN) in lumbar spinal cord were quantified. Mixed cortical cultures were treated with NU-9 for 3 days, and axon length and complexity were measured.

Results:
NU-9 treatment improves the health of UMN both in vitro and in vivo. NU-9 crosses the blood brain barrier, has favorable pharmacokinetic properties, improves the ultrastructural integrity of mitochondria and the endoplasmic reticulum within diseased UMNs, helps retain cytoarchitectural integrity of UMNs, stabilizes their degenerating apical dendrites, reduces levels of misfolded SOD1 in UMNs, and improves their axon outgrowth better than the FDA-approved drugs, edaravone and riluzole.

Conclusions:
Identification of compounds that can improve the health of UMNs in vitro and in the cortex of animal models in vivo, represents a new paradigm for deciding which compounds to progress to clinical trials. Because information is gathered directly from diseased UMNs, and UMNs in mice are equivalent to those in humans at the cellular level, we propose that our approach will reveal compounds that improve the health of UMNs in patients, and thus the success rates of clinical trials. The ability of NU-9 to repair the ultrastructural defects of both mitochondria and ER of diseased UMNs, and to eliminate degeneration of UMNs that become diseased from two independent underlying causes in vivo, makes it the first compound to improve the health of UMNs, which could be a paradigm-changing advance in UMN disease drug discovery.

Acknowledgement:
NIH-NIA Grant R01 AG061708 (P.H.O, R.B.S), Ellen McConnell Blakeman ALS Fellowship (M.G.).

TST-13: Targeting mglu5 receptor to reduce the reactive phenotype of spinal cord astrocytes in the SOD1G93A mouse model of amyotrophic lateral sclerosis

Professor Marco Milanese[1], Dr. Carola Torazza[1], Dr. Francesca Provenzano[1,2], Dr. Silvia Ravera[3], Dr. Tiziana Bonifacino[1], Dr. Mandeep Kumar[1], Prof. Cesare Usai[4], Prof. Giambattista Bonanno[1,5]

[1]University Of Genoa - Dept. of Pharmacy, Genoa, Italy, [2][present affiliation] University of Tübingen - Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, Tübingen, Germany, [3]University Of Genoa - Dept. of Experimental Medicine, Genoa, Italy, [4]National Research Council, Institute of Biophysics, Genoa, Italy, [5]IRCCS Ospedale policlinico San Martino, Genoa, Italy

Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
Several causes have been suggested to contribute to ALS aetiology and glutamate (Glu)-mediated excitotoxicity plays a major role. Besides motor neuron (MN) death, both in-vitro and in-vivo studies demonstrated also the degeneration of non-neuronal cells, such as astrocytes and microglia. Thus, a precise functional modulation of each cell population it might be crucial for a targeted effective therapy. Group I metabotropic Glu receptors (mGluR1, mGluR5) likely play a role in ALS, since their expression and functions, especially in glial cells, are dramatically altered. We demonstrated that mGluR1 and mGluR5 sustain the excessive Glu release in the spinal cord of the SOD1G93A ALS mouse model and, most importantly, in-vivo knocking-down mGluR1/5 significantly prolongs survival and ameliorates the clinical progression.

Methods:
We here investigated in-vitro the effect of mGluR5 genetic downregulation, on the reactive phenotype of spinal cord astrocytes cultured from late symptomatic SOD1G93A mice, age-matched SOD1G93A_Grm5+/- (heterozygous mGluR5 animals) and WT mice. Histological, functional and biochemical experiments have been performed to characterise the phenotype of astrocytes and their cytotoxicity towards MNs.

Results:
The elevated cytosolic Ca2+ concentration was significantly reduced in SOD1G93A_Grm5+/- astrocytes, both in resting and stimulated condition with the Group I mGluRs agonist 3,5-DHPG [n=6; p value<0.05]. The over-expression of GFAP, vimentin and S100β as markers of activated astrocyte was significantly reduced in SOD1G93A_Grm5+/- astrocytes, compared to spinal cord SOD1G93A astrocytes [n=6; p value<0.05]. The pathological accumulation of cytosolic misfolded-SOD1 was also reduced in SOD1G93A_Grm5+/- astrocytes [n=6; p value<0.05]. Cultured SOD1G93A astrocytes showed bioenergetic alterations in terms of oxygen consumption and ATP synthesis, that were partially normalised in SOD1G93A_Grm5+/- astrocytes. The reactive phenotype of SOD1G93A astrocytes determines a massive increase in the release of IL1b, TNFa and IL6 respect to WT astrocytes. The mGluR5 downregulation strongly reduced the pathological synthesis and release of toxic cytokines in SOD1G93A_Grm5+/- astrocytes [n=6; p value<0.05]. Finally, to verify the impact of mGluR5 down-regulation on MNs viability we co-cultured SOD1G93A spinal MNs with spinal cord astrocytes from SOD1G93A, SOD1G93A_Grm5+/- or WT mice. Neuronal cell death was strongly reduced when MNs were seeded on SOD1G93A_Grm5+/- astrocytes compared to co-cultures with SOD1G93A astrocytes [n=8; p value<0.05]. Interestingly, both the silencing with antisense oligonucleotide (ASO) specific for mGluR5, or the pharmacological negative modulation of mGluR5 with CTEP, efficiently mimic the positive effects obtained with the genetic ablation of the receptor [n=3; p value<0.05].

Conclusion:
We conclude that a lower constitutive level of mGluR5 in SOD1G93A mice has positive effects on spinal cord astrocyte, supporting the role of mGlu5 receptor as a therapeutic target to obtain a shift from pathological toward a less noxious phenotype of reactive astrocytes.

Acknowledgement
Supported by the Italian Ministry of University and Research - MIUR (SIR project RBSI14B1Z1) to MM

TST-14: WVE-004, an investigational stereopure antisense oligonucleotide for the treatment of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)

Dr Yuanjing Liu[1], Amy Andreucci[1], Naoki Iwamoto[1], Yuan Yin[1], Hailin Yang[1], Fangjun Liu[1], Saurabh Patil[1], Susovan Mohapatra[1], Erin Purcell-Estabrook[1], Kristin Taborn[1], Elena Dale[1], Chandra Vargeese[1]
[1]*Wave Life Sciences , Cambridge, United States*

Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
A large hexanucleotide GGGGCC (G4C2) repeat expansion in the first intronic region of the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The G4C2 repeat expansion reduces the normal expression of the C9orf72 gene and causes the production of mutant repeat-containing transcripts that form pathogenic nuclear RNA foci and dipeptide repeat (DPR) proteins. Wave Life Sciences is developing an investigational stereopure antisense oligonucleotide, WVE-004, as a potential disease-modifying therapy for C9orf72-associated ALS and FTD.

Objectives:
To determine the effects of WVE-004 on C9orf72 G4C2 repeat-containing transcripts, DPR proteins, and total C9orf72 protein levels. To determine distribution of WVE-004 in central nervous system tissues.

Methods:
In vitro activity of WVE-004 was assessed in C9orf72 patient-derived induced pluripotent stem cell (iPSC) motor neurons under gymnotic (free uptake) conditions. In vivo activity and distribution studies were performed with WVE-004 in BAC transgenic mice containing the full human C9orf72 gene with a repeat expansion. Mice were given intracerebroventricular (ICV) injection of WVE-004 (50 µg) or PBS at day 0 and day 7. Transcript levels were analyzed using quantitative PCR (Taqman assay), poly-GP DPR was measured by Meso Scale Discovery (MSD) assay. Relative C9orf72 protein levels were measured by Wes capillary-based immunoassay and normalized to HPRT. Oligonucleotides in situ were visualized using ViewRNA® assay (ThermoFisher Scientific).

Results:
WVE-004 potently and selectively reduced repeat-containing transcripts in patient-derived iPSC motor neurons under gymnotic conditions (IC50 201.7 nM). ICV injection of WVE-004 into C9orf72 BAC transgenic mice significantly reduced repeat-containing transcripts by 60-80% ($p<0.0001$) in the spinal cord and by 40-50% ($p<0.0001$) in the cortex 6 months after dosing. DPRs were significantly reduced by 94.2% ($p=0.001$) and 87% ($p<0.0001$) in the spinal cord and cortex, respectively, at 6 months. Total C9orf72 protein levels were unaffected. In situ hybridization histochemistry demonstrated widespread and sustained distribution of WVE-004 in the nuclei of neurons in the brain including the spinal cord and cortex.

Conclusion:
In transgenic mice, WVE-004 produces substantial reductions in repeat-containing C9orf72 transcripts and DPR proteins that are sustained for at least 6 months, without disrupting total protein expression. These results support further development of WVE-004 and the potential for a potent, durable effect with preferential targeting of repeat-containing transcripts in patients with C9orf72-associated ALS and FTD. Wave Life Sciences expects to initiate clinical development of WVE-004 with the submission of a clinical trial application in the fourth quarter of 2020.

TST-15: Can localized stimulation of the motor cortex through TMS paradigms rescue the disease phenotype in a familial mouse model of ALS?

Ms Laura Reale[1], Ms Carlie Cullen[1], Ms Kaylene Young[1], Ms Tracey Dickson[1], Ms Catherine Blizzard[1]
[1]*Menzies Institute For Medical Research, 17 Liverpool Street, Australia*

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Alterations in excitability in the motor cortex are one of the earliest detectable clinical changes seen in ALS (1-2). Therefore, early intervention localised to the motor cortex may be an ideal strategy to mitigate progression of ALS. It is unknown if manipulating excitability is an effective therapeutic target, and due to the complex nature of excitability changes, whether increased or decreased excitation leads to neuronal demise. To address this important research gap, transcranial magnetic stimulation (TMS) can be employed. Repetitive TMS is a safe and non-invasive technique, allowing localized application of magnetic currents that can increase or decrease neuronal firing in the brain depending on the TMS paradigm (3). Therefore, TMS could provide a therapeutic intervention for ALS.

Objectives:
This research investigates if stimulating altered excitability directly to the motor cortex through excitatory and inhibitory TMS paradigms is sufficient to alter disease phenotype.

Methods:
This study utilised a familial mouse model of ALS expressing mutant TDP-43A315T. Low intensity TMS was applied to the motor cortex using a custom built circular rodent TMS coil. It was administered as an intermittent (inducing excitation) or continuous (inducing inhibition) theta burst (iTBS/cTBS) pattern or sham stimulation, 5 days a week across 40 days. We evaluated the effect of TMS on rotarod performance, hindlimb clinical score and survival.

Results:
Preliminary analysis revealed that iTBS (n=9) improves rotarod performance by week 2 ($p=0.038$) and week 3 ($p=0.040$) of TMS. Furthermore, iTBS improved the clinical score of TDP-43A315T mice relative to sham ($n=7$; $p<0.0001$) and cTBS ($n=9$; $p<0.01$) mice but did not alter survival. cTBS did not alter rotarod performance or clinical score, but decreased survival ($p<0.05$).

Discussion/Conclusions:
These findings suggest altering cortical excitability through different TMS paradigms can alleviate motor phenotypes in this model and alters life expectancy. Therefore, selectively and differentially enhancing excitability in a specific region may be a feasible therapeutic strategy to stop progression of ALS. These preliminary results provide new possibilities for future experiments where TMS will be employed to different regions of the corticomotor system and in sporadic models of ALS.

References:
1. Menon, P., Higashihara, M., van den Bos, M., Geevasinga, N., Kiernan, M.C. and Vucic, S., 2020. Cortical hyperexcitability evolves with disease progression in ALS. Annals of Clinical and Translational Neurology, 7(5), pp.733-741.

2. Vucic, S., Nicholson, G.A. and Kiernan, M.C., 2008. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. Brain, 131(6), pp.1540-1550.

3. Tang, A., Thickbroom, G. and Rodger, J., 2017. Repetitive transcranial magnetic stimulation of the brain: mechanisms from animal and experimental models. The Neuroscientist, 23(1), pp.82-94.

Acknowledgements:
This work was supported by the Motor Neurone Disease Research Australia [2017 Betty Laidlaw Prize] and the Tasmanian Masonic Research Foundation.

TST-16: Development of a novel splice-switching molecular therapy for amyotrophic lateral sclerosis

Dr Loren Flynn[1,2,3], Mr Nik Gavriel[1,2], Dr Samantha Barton[4], Dr Ianthe Pitout[1,2,5], Ms Doris Tomas[4], Mr Thomas Swanson[3], Prof Sue Fletcher[2,5], Dr Craig Metz[3], Prof Steve Wilton[1,2], A/Prof Bradley Turner[4], Prof P Anthony Akkari[1,2,3]

[1]Perron Institute for Neurological and Translational Science, Perth, Australia, [2]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, Australia, [3]Black Swan Pharmaceuticals, Durham, USA, [4]Florey Institute of Neuroscience and Mental Health, Melbourne, Australia, [5]PYC Therapeutics, Perth, Australia

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Strategies to suppress superoxide dismutase (SOD1) expression have shown promise in clinical trials for SOD1 mutation positive patients, whereby mutations in SOD1 result in misfolded protein that aggregates, leading to neuronal toxicity (1). An overwhelming body of evidence now suggests that the misfolding of wildtype SOD1 can also lead to neuronal toxicity in other forms of ALS. Others have demonstrated misfolded wildtype SOD1 in patients carrying mutations in C9ORF72 and FUS (2), and importantly, in sporadic ALS patients with no identifiable mutation (3). These data provide evidence for SOD1 toxicity as a common pathological mechanism for a much broader ALS patient population, suggesting that therapies designed to suppress SOD1 expression will be broadly therapeutic for ALS.

Objective:
The aim of this study was to determine whether an antisense SOD1 suppression therapy could ameliorate ALS cellular mechanisms and, therefore, be therapeutic in neuronal cells from SOD1-linked and sporadic ALS patients.

Methods:
We have developed splice-switching morpholino oligomers (PMOs) to induce targeted SOD1 suppression. Lead PMOs were transfected into neuronal cells from both SOD1 mutation positive and sporadic ALS patients. SOD1 suppression was assessed by PCR and western blot, and the resultant effect on protein aggregation and cellular pathways was investigated.

Results:
Following PMO transfection in multiple patient cell strains we observe up to 93% reduction in SOD1 protein levels (p=0.001), leading to an improvement in cell viability and cellular response to oxidative stress. Furthermore, SOD1 suppression PMOs appear to be effective at preventing SOD1 aggregation in sporadic ALS cells exposed to environmental stressors.
Direct comparison of exon skipping PMOs with alternative SOD1-suppressing AO strategies in vitro, demonstrates that exon skipping PMOs are more effective at suppressing SOD1. Importantly, our data suggests that exon skipping PMOs have a superior safety profile when compared to phosphorothioate AOs that induce RNase H; given that PMOs do not induce off-target protein binding or cellular toxicity.

Discussion:
SOD1 suppression in vitro in neuronal cells from SOD1-linked and sporadic ALS patients demonstrates an improvement in cellular response to oxidative stress and prevents SOD1 aggregates from forming, suggesting that SOD1 suppression will provide therapeutic benefit in broad ALS application. Furthermore, the in vitro safety and efficacy data distinguish our PMOs from current international efforts and support further development of splice-switching PMOs towards clinical trials.

References:
1. Miller, T.M., et al., Lancet Neurol, 2013. 12(5): p. 435-42.
2. Forsberg, K., et al., J Neurol Neurosurg Psychiatry, 2019. 90(8)
3. Forsberg, K., et al., PLoS One, 2010. 5(7)

Acknowledgements:
This study was supported by funding from MND Research Australia and FightMND.

TST-17: Effects of antiretroviral therapy on endogenous retrovirus activity and associated TDP-43 proteinopathy in a motor neuron disease mouse model

Mrs Megan Dubowsky[1], Associate Professor Jill Carr[1], Dr Adam Walker[2], Professor Julian Gold[3], Dr Mary-Louise Rogers[1]
[1]Flinders Health and Medical Research Institute, Flinders University, Bedford Park, Australia, [2]Queensland Brain Institute, Queensland University, St Lucia, Australia, [3]The Albion Centre and Faculty of Medicine and Health, University of Sydney, Surry Hills, Australia

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
The cause of Motor Neuron Disease (MND) remains unknown and treatment options are limited. It has been proposed that re-activation of endogenous retroviruses (ERVs), specifically human endogenous retrovirus type K (HERV-K), may be a causative factor. It has been suggested that HERV-K causes progression of MND through interaction with TAR DNA binding protein 43 (TDP-43) and immune regulators (1). It was hypothesised that a treatment that reduces the levels of HERV-K, may slow the progression of MND. A phase II clinical trial of an antiretroviral therapy, Triumeq, to target endogenous retrovirus activity showed slower average decline in patients on the Triumeq treatment compared to pre-treatment levels. Serum levels of HERV-K were also significantly reduced over the treatment course (2). However, the mechanism of benefit from Triumeq and its influence on TDP-43, HERV-K and inflammation is still unknown.

Objectives:
To evaluate endogenous retrovirus activity, TDP-43 proteinopathy and immune response after administration of Triumeq in a TDP-43 mouse model of MND compared to untreated TDP-43 mice.

Methods:
Triumeq (7mg/day; equivalent to human dose of 1 tablet/day) was trialled on hTDP-43ΔNLS mice (3) (N=23) and motor strength was assessed using the wirehang test prior to treatment administration and 15 and 30 days post-treatment onset. TDP-43 pathology was analysed using immunofluorescence. HERV-K levels and inflammatory markers have been measured using PCR.

Results and Discussion:
Motor analysis of the treated hTDP-43ΔNLS mice identified a significantly higher ($p=0.01$) latency to fall during the wirehang test at 15 days post treatment onset for the Triumeq treated hTDP-43ΔNLS TDP-43 mice (N=4) compared to the untreated hTDP-43ΔNLS mice (N=2). Immunohistochemistry identified differences in treated and untreated hTDP-43ΔNLS mice based on TDP-43 mislocalisation. Further research will determine any further motor, immunohistochemical and inflammatory differences between Triumeq treated and untreated hTDP-43ΔNLS mice. Also, further analysis of the TDP-43-HERV-K association and the involvement of inflammatory pathways will be investigated through analysis of induced pluripotent stem cells from MND patients. This research has significant implications for identifying a potential cause of MND and a potential treatment that may slow the progression of the disease and increase quality of life.

References:
1 Douville & Nath, Handbook of Clinical Neurology 2015 Elsevier.
2 Gold et al., Amyotrophic lateral sclerosis and frontotemporal degeneration 2019;63:1-10.
3 Walker et al., Acta Neuropathologica 2015; 130: 643-660.

Acknowledgements:
Funding for this was work from a 2019-2021 MND PhD Scholarship Top-up Grant and 2020 MNDRIA Innovator Grant

TST-18: HDAC6 as a potential therapeutic target for ALS

Mrs Fariha Kabir[1], Dr Rachel Atkinson[1], Dr David Gell[1], Associate Professor Tony Cook[1], Associate Professor Nuri Guven[1], Dr Jessica Collins[1], Dr Bill Bennett[1], Professor Anna King[1]
[1]*University Of Tasmania, Hobart, Australia*

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Defects in axonal transport are considered as one of the key pathological mechanism for developing both sporadic and familial Amyotrophic Lateral Sclerosis (ALS) and may be related to deacetylation of microtubules. Microtubule acetylation is regulated by the histone deacetylase 6 (HDAC6) enzyme and there is evidence that deletion of HDAC6 in animal models of ALS results in increased acetylation of α-tubulin, one of the subunits of microtubules, which slows motor neuron degeneration [1]. Thus, inhibiting HDAC6 may be a potential therapeutic for ALS. Among the HDAC inhibitors that are being developed for neurodegenerative diseases, ACY 738 has shown promising results in an Alzheimer's disease model and fused in sarcoma (FUS) transgenic mouse models through specific inhibition of HDAC6 [2, 3].

Aims:
Our goal is to test ACY738 in mouse models of ALS. In this part of the project, we are testing the effect of ACY738 on microtubule acetylation and acetylation of histones, with or without the current ALS treatment riluzole, both in vivo and in vitro.

Methods:
For in vitro experiments, neurons are cultured from embryonic day E15.5 C57/Bl6 mice and treated with a range of concentrations of riluzole and ACY738. For in vivo experiments, C57/Bl6 mice are treated with 100mg/Kg/day ACY738 (or vehicle) provided in food with or without 13mg/Kg/day riluzole provided in drinking water for 1 and 4 months.

Results:
Our preliminary results in vivo indicate that at 1 month ACY738 causes 1.5 fold increase in tubulin acetylation in the brain which is not affected by riluzole (N= 6). In preliminary cell culture studies, a combination of ACY 738 (1µM) and riluzole (5µM) significantly ($p<0.05$; 3 technical repeats from 1 culture) increased the level of acetylated tubulin compared to the vehicle. We will now further investigate the effect of 1 and 4 month treatment with ACY 738 (+/- riluzole) to acetylate microtubules in different CNS tissue including brain, spinal cord, optic nerve and sciatic nerve. Also, we will investigate the IC50 for microtubule acetylation by ACY 738 (+/- riluzole) in primary mouse neurons and this will lead to a study determining the effects of ACY 738 (+/- riluzole) in the ALS TDP-43Q331k mouse model.

Conclusion:
This study opens avenues for to develop effective treatment options for neurodegenerative diseases by determining the effectiveness of current HDAC6 inhibiting drugs in ALS models.

References:
1. Taes, I., et al., Human molecular genetics, 2013. 22(9): p. 1783-1790.
2. Majid, T., et al., Alzheimer's & Dementia: Translational Research & Clinical Interventions, 2015. 1(3): p. 170-181.
3. Shen, S. and A.P. Kozikowski, Expert Opinion on Therapeutic Patents, 2020. 30(2): p. 121-136.

TST-19: Probiotic Lacticaseibacillus rhamnosus HA-114 rescues ALS phenotypes via mitochondrial beta-oxidation

Miss Audrey Labarre[1,2], Ericka Guitard[1,2], Gilles Tossing[1,2], Eric Bareke[1], Marjorie Labrecque[1,3], Martine Tétreault[1,2], Alex Parker[1,2]
[1]CRCHUM, Université De Montréal, Montréal, Canada, [2]Département de neurosciences, Université de Montréal, Montréal, Canada, [3]Département de biochimie, Université de Montréal, Montréal, Canada

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
The human microbiota is believed to influence health. Microbiome dysbiosis may be linked to neurological conditions like Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). Over the last year, new evidence showed perturbations in microbiota and in lipid composition in ALS. However, there is a considerable lack of understanding of the effect of these perturbations in ALS. We partnered with Lallemand Health Solutions to develop an assay in C. elegans to screen probiotic strains for their effects on fat accumulation. We successfully established a novel assay that recapitulated findings from mouse models but could be done at a fraction of the cost and time required for mammalian models. We were curious if these probiotics had additional effects, so we tested them in a variety of assays, including lifespan and stress resistance, as well as in our worm ALS models. We were surprised to discover that one strain, Lacticaseibacillus rhamnosus HA-114, suppressed motility defects and motor neuron degeneration in our C. elegans models of ALS.

Objectives:
To determine how L. rhamnosus HA-114 contributes to rescue ALS disease phenotypes.

Methods:
We used a combination of genetics and gene expression profiling to identify genes and pathways that are influenced by microbiota and are responsible for neuroprotection in our worms and mice models.

Results:
We report here the ability of a probiotic bacterial strain in reversing neurodegeneration phenotypes. We show that L. rhamnosus HA-114 is neuroprotective in C. elegans models of ALS. Our results show that neuroprotection from L. rhamnosus HA-114 is unique from other L. rhamnosus strains, and resides in its fatty acid content. Neuroprotection by L. rhamnosus HA-114 requires acdh-1/ACADSB, kat-1/ACAT1 and elo-6/ELOVL3/6, which are key fatty acid metabolism and mitochondrial β-oxidation genes. Moreover, L. rhamnosus HA-114 delayed disease onset and suppressed motor neuron degeneration in an aggressive mouse model of ALS. Our data suggest that disrupted lipid metabolism contributes to neurodegeneration and that dietary intervention with L. rhamnosus HA-114 restores lipid homeostasis and energy balance through mitochondrial β-oxidation.

Discussion and conclusions:
We have identified key genes responsible for neuroprotection in our C. elegans models. These findings may confirm a link between microbiota and ALS and can lead to future therapies, through modulation of the intestinal environment. L. rhamnosus HA-114 is suitable for human consumption opening the possibility of modifying disease progression by dietary intervention.

Acknowledgments:
ALS Canada/Brain Canada, FRQS, LHS, NSERC

TST-20: QRA-244 a Potent, Selective KCNQ2/3 Opener and a Potential Therapy for Motor System Hyperexcitability induced Disease Progression in ALS patients

Dr Dan Elbaum[1], Dr. Sandy Hinckley[1], Dr Emanuele Sher[3], Dr Birgit Priest[2], Dr Mark Chappell[2], Helen Szekeres[3], Maria Whatton[3], Dr Andrew Williams[3], Dr Yu-Hua Hui[2], Dr Matthew Abernathy[2], Dr Keith Phillips[2], Louisa Appel[3], Dr Keith Wafford[3], Dr. Kasper Roet[1]
[1]Quralis, Cambridge, United States, [2]Eli Lilly and Company, Indianapolis, United States, [3]Eli Lilly and Company, Basingstoke, United Kingdom

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Recent studies have demonstrated that approximately half of ALS patients show hyperexcitability in the motor cortex and spinal motor axons, a phenotype that is linked to poor survival. ALS patients with motor system hyperexcitability can be targeted using neurophysiological biomarkers. In patient iPSC derived motor neurons this hyperexcitability leads to neurodegeneration and was traced to reduced Kv7.2/7.3 activity. This motor neuron degeneration was rescued by the Kv7.2/7.3 agonist Retigabine which also reversed hyperexcitability in a clinical trial of ALS patients. The trial demonstrated a statistically significant beneficial effect on several markers of excitability including short interval cortical inhibition (SICI) and strength duration time constant (SDTC), two biomarkers linked to patient survival. Despite these beneficial effects, retigabine was associated with significant adverse events consistent with its prior clinical use in epileptic patients which strongly limits its use as a therapeutic. We have been working to discover, characterize, and develop a novel KCNQ2/3 activator with an improved channel specificity, which is expected to translate into a better clinical safety profile with comparable or better efficacy. Here we show that QRA-244 activates KCNQ2/3 channels selectively in the KCNQ family, increases rheobase and decreases SDTC in rats. In side by side experiments with Retigabine we demonstrate a significantly improved safety profile in rat models of dizziness (rotarod) and fatigue (REM/NREM sleep). Unlike Retigabine, QRA-244 has no effect on human bladder strips at clinically relevant concentrations. Overall, QurAlis is developing QRA-244, a more potent and selective Kv7.2/7.3 activator aimed at normalizing excitability of the ALS motor system, with a significant reduction in off-target driven adverse events. We believe that this compound offers a promising therapeutic approach to counteract disease progression induced by hyperexcitability in ALS patients.

TST-21: Repeated intrathecal delivery of a monoclonal full-length antibody against TDP-43 reduces TDP-43 mislocalization and NF-kB activation in neurons.

Dr Silvia Pozzi[1], Dr Philippe Codron[2], Mrs. Geneviève Soucy[1], Ms. Laurence Renaud[1], Dr. Pierre Junior Cordeau[1], Dr. Kallol Dutta[1], Mrs. Christine Bareil[1], Dr. Jean-Pierre Julien[1,3]
[1]Cervo Brain Research Centre, Québec, Canada, [2]UMR CNRS 6015, INSERM U1083, University of Angers, Angers, France, [3]Department of Psychiatry and Neuroscience, University of Laval, Québec, Canada

Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

TDP-43 proteinopathy, an event characterized by a consistent cytoplasmic mislocalization and aggregation of the protein TDP-43, is a pathological hallmark of Amyotrophic Lateral Sclerosis (ALS) and FrontoTemporal Lobar Degeneration (FTLD). We previously demonstrated that the viral-mediated delivery of a single chain antibody against the RRM1 domain of TDP-43 was able to rescue motor and cognitive impairments in TDP-43 mutant mouse models by reducing the amount of cytoplasmic accumulated protein and its interaction with p65, the active subunit of NF-kB (1). Here we investigated the target specificity and therapeutic efficacy of the monoclonal full-length antibody (named E6), previously used to derived single chain antibodies, in TDP-43 related conditions. We observed that the antibody recognizes specifically the cytoplasmic fraction of TDP-43 in cells, animal models and human tissues. We demonstrated that in neuronal cells the antibody can reduce cytoplasmic TDP-43 levels by activating the TRIM-21/proteasome degradative pathway. We delivered the antibody by repeated intrathecal injections for five weeks in nine-months old TDP-43 A315T mice and demonstrated a wide diffusion in the spinal cord and a specific uptake by large neurons and microglial cells. In tissues of treated mice, we measured the levels of cytoplasmic TDP-43 and nuclear p65 observing a significant reduction of both events in conditions where E6-antibody was delivered.

We therefore demonstrated for the first time the feasibility and efficacy of a therapeutic approach based on the repeated intrathecal delivery of a full-length monoclonal antibody against TDP-43 in an ALS/FTLD mouse model.

References:
1. Pozzi S et al. Virus-mediated delivery of antibody targeting TAR DNA-binding protein-43 mitigates associated neuropathology. J. Clin. Invest. 2019;129(4):1581–1595.