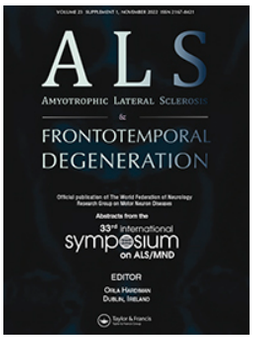

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Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

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ALS

Amyotrophic Lateral Sclerosis

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Frontotemporal Degeneration

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Abstracts from the

33rd international symposium on ALS/MND

EDITOR

Orla Hardiman
Dublin, Ireland

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33nd (VIRTUAL) INTERNATIONAL SYMPOSIUM ON ALS/MND

Welcome

Welcome, and thank you for participating in this, the **33rd International Symposium on ALS/MND.**

We had hoped to be writing this introduction with COVID-19 well and truly behind us and a sense of normality returned to all our lives. Sadly, this is not the case: with political and climate-related events, an ever-increasing cost of living and the continued shadow of COVID-19, we are certainly not out of the woods yet. Despite this, we sincerely hope you have managed to find some light in these uncertain times, and that you and your family, friends and colleagues have been safe and well.

The above issues highlight the interconnectivity of our world. Collaboration and understanding through the sharing of information (while filtering out disinformation) is key to moving us forward to find answers - just as our community of researchers, healthcare professionals, people living with and effected by ALS/MND, industry and government are trying to do every day in the search for answers.

We share with you the belief that the Symposium is an essential part of the ALS/MND calendar. The International Symposium plays a fundamental role not only in facilitating the exchange of exciting new knowledge and information, but also serving as an interdisciplinary melting pot, stimulating new ideas and fostering new collaborations.

Following the success of last year's virtual Symposium and the ongoing challenges caused by the global pandemic, the MND Association s 33rd International Symposium on ALS/MND will once again be held online.

As many of you know, we were due to be hosted in San Diego by the ALS Association (ALSA) and we consulted in detail with ALSA and the International Alliance of ALS/MND Associations prior to making the decision to hold another virtual event. Whilst the COVID-19 omicron wave has now receded in many countries, it has also demonstrated the frightening speed at which new variants can spread across the world. Given the prospect of new COVID spikes this winter, and taking into account the risks linked to significant contractual obligations, we did not have the logistical or financial certainty we needed to go ahead with planning an on-site event of the scale and international reach of the Symposium. We understand the disappointment felt by this decision and really do thank each and every one of you for your understanding.

We have again made every effort to meet the challenges of the online format in catering for the interests of a diverse range of delegates, located across multiple time zones, and we have attempted to include the traditional mix of basic and clinical science, therapy development and clinical management that is synonymous with the event. We anticipate that the virtual format will again facilitate access to people living with or affected by ALS/MND who wish to attend the meeting, including participants selected by the independent Patient Fellows Program. We (along with other organisations across the globe) will once again work to disseminate news and content from the Symposium in lay terms through our communication channels.

We extend our thanks to the Symposium Programme Committee, chaired by Professor Ammar Al-Chalabi, for its tireless work and valuable advice, and to our plenary speakers, who have graciously agreed to deliver their presentations online. This year, we have once again been able to review and select submitted poster presentations to be upgraded to oral presentations from the outstanding pool of poster abstracts submitted, to add to the program.

The Poster Sessions, undoubtedly a highlight of the annual meeting, have also moved into the virtual world. It is a challenge to generate the 'buzz' of the face-to-face event, but we hope to encourage a similar feel between poster presenters and delegates through 'live' online interaction, including Q&A sessions, networking, gamification and three-minute 'lightening' video presentations of each poster.

We hope you will join us online in our collective effort to understand, treat and – ultimately – defeat ALS/MND.

We wish you a successful and enjoyable Symposium.

Research Development Team
Motor Neurone Disease Association of England, Wales and Northern Ireland

Thank you to Cytokinetics for supporting the
33rd International Symposium on ALS/MND

AMYOTROPHIC LATERAL SCLEROSIS

&

FRONTOTEMPORAL DEGENERATION

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SESSION 1 OPENING SESSION

C01 The contribution of aging to neurodegenerative disease

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Alzheimer's disease (AD) is a highly prevalent neurodegenerative disease that exclusively affects elderly people. Here, we used the direct conversion of primarily sporadic AD patient fibroblasts into induced neurons (iNs) to generate an age-equivalent neuronal model. Patient-derived iNs exhibit strong AD-specific transcriptome neuronal signatures characterized by down-regulation of mature functional and morphological properties and up-regulation of immature neuronal and neural stem cell-associated pathways. Mapping AD and control iNs to longitudinal transcriptome data from maturing human neurons demonstrated that AD iNs are fully converted into iNs, but reflect a de-differentiated neuronal identity. Epigenetic landscape profiling revealed an aberrant cellular program underlying their immature neuronal state, which shares similarities with malignant transformation and age-dependent epigenetic erosion. To probe for the involvement of aging, we generated iPSC neurons from the small cohort, which, indeed, showed non-significant disease-related transcriptome signatures. This is consistent with epigenetic aging clock and brain oncogenesis mapping, which indicated that, unlike iPSC neurons, iNs more closely reflect adult and old brain stages, rendering them a valuable tool for studying adult-specific, age-related neurodegeneration. In this model, AD-related neuronal changes appear less as a mere accumulation of damaging events, but rather as an age-dependent cellular program that impairs neuronal identity.

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SESSION 2 CLINICAL TRIALS

C02 Results from a randomized, double-blind, placebo-controlled trial of RNS60 in people with ALS

E. Pupillo[1], E. Bianchi[1], C. Bendotti[1], V. Bonetto[1], S. Paganoni[2,3], L. Mazzini[4], E. Beghi[1] and RNS60 study group o

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Background: The only available drugs for the treatment of ALS are riluzole and edaravone. We evaluated the efficacy of RNS60, a novel anti-inflammatory and cytoprotective drug that has shown positive results in animal models of neuroinflammation and neurodegeneration (1–3), in people with ALS.
Primary objective: to measure the effect of RNS60 on candidate markers of inflammation and neurodegeneration in peripheral blood: MCP-1, PPIA, Tyrosine Nitrated-Actin (Actin-NT), 3-nitrotyrosine (3-NT), IL-17, NfL and Tregs (measured via FOXP3 and CD25 mRNA). Secondary objectives: to evaluate the effect of RNS60 on Functional Rating Scale-Revised (ALSFRS-R); respiratory function (forced vital capacity (FVC)% predicted of normal value, Forced Expiratory Volume 1 (FEV1)); quality of life (ALS Assessment Questionnaire-40 (ALSAQ-40)); self-sufficiency (defined as having a score of 3 or 4 on all three selected ALSFRS-R questions (swallowing, cutting food and handling utensils, and walking)); survival; tolerability and safety(adverse events (AEs)).
Methods: Phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial. Participants diagnosed with Definite, Probable, or Probable lab-supported ALS, with satisfactory respiratory function (FVC $\geq$80% of predicted normal value) and self-sufficiency were randomly assigned to receive either RNS60 or placebo. The study drug was administered intravenously (375ml) once a week and by nebulization (4ml/day) on non-infusion days for 24 weeks. After the treatment period, participants were followed for an additional 24-week off-treatment period.
Results: 147 participants, 99 women, and 48 men, aged 30–77 years were recruited. Spinal onset ALS was documented in 85.7% of cases. 37 participants (25.2%) did not complete the 48-weeks follow-up (13 died), 70.3% of whom occurred after treatment discontinuation. Candidate biomarkers did not differ significantly between the two groups. In the intention-to-treat analysis, the mean rate of decline in FVC and the eating and drinking domains of the ALSAQ-40 scale was significantly slower in the RNS60 arm compared to placebo over a 24-week treatment period (FVC: −0.46 per week for RNS60, −0.87 per week for placebo, $p = 0.0101$; ALSAQ-40: 0.19 per week for RNS60, 0.38 per week for placebo, $p = 0.0319$). A significant difference in the rate of decrease of FEV1 between the two treatment groups was observed (−0.45 per week for RNS60, −0.52 per week for placebo, $p = 0.0146$) during the on-treatment period. AE was similar in the two arms.
Discussion: RNS60 showed no effects on candidate biomarkers in people with ALS, but positive effects on measures of respiratory and bulbar functions. These findings support the need for further clinical trials of RNS60 in ALS, including earlier treatment, longer treatment periods, and additional measures of respiratory and bulbar function.

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C03 Modifying immune response and outcomes in ALS (MIROCALS): design and results of a phase 2b, double-blind randomized placebo-controlled trial of low dose interleukin-2 (ld IL2) in ALS

G. Bensimon[1,2] and P.N. Leigh[3]; on behalf of the MIROCALS Study Group*

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There is strong evidence that microglial activation and 'inflammation' contributes to neuronal damage in amyotrophic lateral sclerosis (ALS; motor neuron disease, MND) and in many other neurodegenerative disorders. Peripheral regulatory T cells (Tregs) appear to modify inflammatory responses and higher numbers of circulating Treg numbers are associated with longer survival in ALS. Treg numbers and function are critically dependent on interleukin-2 (IL2). In a previous phase Ib dose finding RCT (1) we showed that low dose (ld) IL2 boosted Treg numbers and suppressive function while decreasing plasma CCL2, a marker of inflammation. We, therefore, designed a randomized placebo-controlled trial primarily to evaluate the efficacy and safety of ld IL2 and to test the hypothesis that enhancement of Tregs modifies disease progression in ALS. Secondary objectives were to validate a new phase-II study design to facilitate early detection of drug response using biomarkers of neuronal damage and neuroinflammation.
MIROCALS is a double-blind randomized placebo-controlled trial of low-dose IL2, delivered by sub-cutaneous injections (2MU) in 5-day cycles every 28 days, as in IMODALS. Volunteers with ALS (disease duration $\leq$24 months, VC $\geq$70%, not previously treated with riluzole, age 18–76, El Escorial Definite, Probable, Laboratory-Supported Probable, or

Possible) were recruited to a 3-month 'run-in' while starting riluzole. Volunteers who tolerated riluzole were randomized to ld IL2 or a matching placebo. At selection and randomization, all volunteers were required to have blood and CSF sampling. A third CSF sample was collected at week 17 post-randomization. Blood sampling at other time points was taken for biomarker assays and for safety monitoring. 220 volunteers were randomized over 28 months in the UK ($n = 83$) and France ($n = 137$) in 17 ALS Centers. Treatment was continued for 18 months (548 days) but blinded follow-up and ascertainment of survival were maintained for all randomized volunteers until 21 months (640 days) after randomization.
The Primary efficacy outcome was time to death (survival) at 21 months (640 days) post-randomization in the ITT population. Pre-specified survival analyses for treatment effect include stratified Log-rank and Cox proportional hazard models. Secondary efficacy outcome measures were: ALSFRS-R slope; SVC slope. Core biomarkers were CSF pNFH; CSF CCL2; and Tregs (number and percent of CD4 cells). EQol-5D was used to assess the quality of life. Exploratory analyses included additional blood and CSF CNS biomarkers; chemokine/cytokines; transcriptomics, deep immunophenotyping and functionality of Tregs and other PBMCs, whole genome sequencing, DNA methylation, and changes in MRI parameters in a sub-group of volunteers. In this presentation, we will summarize MIROCALS study design and population demographics, and present the primary and major secondary efficacy outcomes and safety analyses.

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*The MIROCALS Study Group
https://symposium.mndassociation.org/wp-content/uploads/2022/10/The-MIROCALS-Study-Group.pdf

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C04 Evidence for a survival benefit in ALS with CNM-Au8 treatment: interim results from the RESCUE-ALS trial long-term open label extension

S. Vucic[1], P. Menon[1], W. Huynh[2], C. Mahoney[2], K. Ho[3], A. Rynders[3], J. Evan[3], J. Evan[3], R. Glanzman[3], M. Hotchkin[3] and M. Kiernan[2]

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Background and methods: RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 in early sporadic ALS, with an ongoing open-label extension (OLE) to evaluate the long-term safety and efficacy of CNM-Au8. CNM-Au8 is a suspension of catalytically active clean-surfaced gold nanocrystals shown to enhance neuronal metabolic energy, reduce oxidative stress, and improve protein homeostasis. Study participants were randomized 1:1 to receive 30mg CNM-Au8 or placebo daily for 36 weeks during the double-blind portion of the study, followed by an OLE with CNM-Au8 (30mg/day). The trial enrolled 45 participants [$n = 23$ active (CNM-Au8), $n = 22$ matched placebo].
Results: Long-term observed survival was compared by treatment group (originally randomized active versus placebo) from randomization through the latest OLE observation with a data-cut of 5-July-2022. This analysis compared early treatment (original active randomization) versus either no-treatment or a 9-month delayed treatment start for ex-placebo participants who transitioned into the OLE. Current vital status was obtained for 43 of the 45 study participants. Data were right censored at the last study contact for participants lost to follow-up (active, $n = 1$; placebo, $n = 1$), or as of the data cutoff. There were 5 deaths in the originally randomized active group and 14 deaths in the originally randomized placebo group. Median survival from randomization for the originally randomized active group was undefined due to insufficient mortality events, and median survival for the originally randomized placebo group was 99.7 weeks (23.1 months). Unadjusted Kaplan-Meier survival analyses demonstrated a significant mortality benefit with participants initially randomized to CNM-Au8 treatment versus those initially randomized to placebo, resulting in a 70% decreased risk of death, log-rank hazard ratio =0.301 (95% CI: 0.122–0.742, $p = 0.0143$). Sensitivity analyses substituting death in place of lost to follow-up censoring resulted in concordant findings (Hazard Ratio: 0.338, 95% CI: 0.143–0.797, $p = 0.0182$).
Discussion: CNM-Au8 treatment was well-tolerated and there were no significant safety findings reported during the OLE. These results demonstrate improved survival with early CNM-Au8 treatment.

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C05 Evaluating efficacy and safety of tofersen in adults with SOD1-ALS: Results from the Phase 3 VALOR trial and open-label extension

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Background: Tofersen is an investigational drug for ALS associated with mutations in the *SOD1* gene.
Objectives: The Phase 3 trial (VALOR) and its open-label extension (OLE) evaluated efficacy and safety of tofersen.
Methods: Participants were randomized in VALOR to intrathecal tofersen 100 mg or placebo for approximately 6 months, after which, participants had the opportunity to enroll in the OLE. Data from VALOR and its OLE were integrated to evaluate the effects of early (in VALOR) vs. delayed (in the OLE) initiation of tofersen on target engagement (total CSF SOD1), axonal injury and neurodegeneration (plasma neurofilament light chain; NfL), clinical function (ALSFRS-R), respiratory function (SVC), strength (HHD megascore), quality-of-life (QoL, [ALSAQ-5, EQ-5D-5L]), and survival. An interim data cut of the OLE was performed in January-2022, when all participants had the opportunity for at least 12 months of follow-up from VALOR baseline.
Results: One hundred and eight participants were randomized (tofersen [$n = 72$], placebo [$n = 36$]) in VALOR, and 95 (88%) subsequently enrolled in the OLE. As of the January-2022 data cut, 67 (62%) participants remained ongoing. Tofersen led to robust reductions in total CSF SOD1 protein and plasma NfL which were sustained over time. Over 52 weeks, the early-start tofersen group experienced less worsening than the delayed-start group on measures including ALSFRS-R (difference: 3.5; $p = 0.03$), SVC (difference: 9.2, $p = 0.02$), HHD megascore (difference: 0.28, $p = 0.02$), and QoL measures. Median time to death or permanent ventilation (PV) could not be estimated due to the limited number of events. However, data suggest a reduced risk of death or PV with earlier initiation of tofersen. Most adverse events were mild/moderate. Serious neurologic events, including myelitis, aseptic meningitis, and papilledema, were observed in 6.7% of tofersen-treated participants. Additional analyses on body weight, and subgroups analyses of time to death, permanent ventilation, or withdrawal due to disease progression will be presented.
Discussion: Longer-term integrated data from VALOR and its OLE suggest meaningful biological and clinical disease-modifying effects of tofersen.

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TMM: advisory board and clinical research support for Biogen; licensing agreement with, and consulting for, Ionis Pharmaceuticals; consultant for Cytokinetics; licensing agreements with C2N; advisory boards for Disarm Therapeutics and UCB Pharma. MEC: advisory board for Biogen; compensation from QurAlis, RRD, Sunovian, Cytokinetics, Takeda, Regeneron, Transposon, and Locust Walk; member of Praxis Board of Directors. AG: consultant for genetic testing for Biogen; consultant on ALS trial design for Alexion, AL-S Pharma, Calico, Cytokinetics, and Sanofi; and CMO at QurAlis. PJS: advisory board for Biogen, Aclipse Therapeutics, Quell Therapeutics, BenevolentAI, and QurAlis and research support from Quell Therapeutics, Aclipse Therapeutics, Pfizer, and SwanBio. Support for clinical trials participation received from Biogen, Alexion, the EU Horizon programme and UK NIHR. GS: compensation from Mitsubishi Tanabe Pharma, Takeda, and Sumitomo Dainippon Pharma. TC, IN, DG, PS, MM, LF, TAF, and SF: employees of and hold stock/ options in Biogen.

SESSION 3 METABOLISM

C06 Investigating the role of hypermetabolism in ALS

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The maintenance of optimal energy balance and body composition is critically dependent on maintaining energy intake with energy expenditure. An imbalance between dietary intake and nutrient absorption with resting and activity-associated energy expenditure contributes to weight gain or loss. During disease, impairments in energy balance leading to weight loss are often associated with worse outcomes.

Evidence of metabolic dysfunction in ALS first emerged in the 1980s, and subsequent findings have confirmed that impairments in whole-body physiology and energy balance are common presentations in the disease. Of the metabolic changes that occur in ALS, hypermetabolism (defined by an increase in resting energy expenditure relative to predicted resting energy expenditure) has been observed in multiple patient cohorts. While reports of hypermetabolism in ALS have increased, measures that define the prevalence and impact of hypermetabolism in ALS are likely to have been confounded by the varying strategies used to identify individuals who are hypermetabolic across study cohorts. Second to this, whether hypermetabolism is a phenomenon that exists in a persistent state from one individual to the next remains to be answered. As such, our understanding of the clinical significance of hypermetabolism in ALS is still emerging; the current consensus is that hypermetabolic patients are likely to present with a more aggressive disease that is associated with faster progression and increased risk for earlier death. Thus, the targeting of hypermetabolism and/or metabolic perturbations is a potential therapeutic strategy for ALS.

Here, I will provide an overview of recent work in deciphering hypermetabolism in ALS, how the use of prediction equations might influence research outcomes, and our efforts to track the presentation and persistence of hypermetabolism throughout the disease, and across the spectrum of MNDs. Finally, I will touch on our current approach to targeting hypermetabolism in patients with ALS.

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C07 Dysregulated energy metabolism related to worse prognosis of ALS

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Background: Dysregulated energy metabolism has been reported in ALS patients and models. Resting hypermetabolism has been identified as a prevalent phenomenon in approximately 40%–60% of ALS patients, although the end-stage patients always had significantly reduced food intake and muscle content. Estimation of exercising energy metabolism, measured by oxygen uptake during peak exercise (VO2 peak) in ALS is needed. Associations between energy metabolism and clinical features of ALS are controversial.

Objectives: To evaluate the characteristics of resting and exercising energy metabolism in ALS patients and to explore their clinical value for severity assessment and prognosis prediction of ALS.

Methods: After strict control, 156 eligible subjects (78 ALS patients and 78 controls) underwent clinical tests, neuropsychological assessment using the Edinburgh cognitive and behavioral ALS screen, and comprehensive metabolic assessments including body composition, resting and exercising energy metabolism. Patients were followed up every 6 months for disease evaluation.

Results: Of 268 eligible participants, there were 118 ALS patients and 150 age- and sex-matched healthy controls. Increased resting energy metabolism and decreased exercising energy metabolism ($P < 0.001$) were observed in ALS patients compared with controls. Resting energy metabolism decreased with disease progression as graded by King's College staging system ($P = 0.001$). Exercising energy metabolism was correlated with impairment severity, cognitive level, and progression rate ($P < 0.001$). An increase in resting energy metabolism indicated a worse prognosis (HR =1.03; $P = 0.003$), while an increase in exercising energy metabolism indicated improved survival (HR =0.82; $P = 0.002$).

Discussion: In this study, a systematical metabolic profile with increased resting energy metabolism and decreased exercising energy metabolism indicating a worse prognosis was identified in ALS. They were also significantly associated with disease severity and disease progression, supportive of values of disease evaluation. Such alterations in energy metabolism could provide insights into the metabolic modification of ALS, with potential use as an informative tool for prognosis in the clinical setting and an effective outcome measure for objective evaluation in clinical trials of ALS.

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C08 A 18F-FDG-PET study exploring the metabolic signature of pure bulbar and pure spinal ALS

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Background and objectives: MRI studies reported that ALS patients with bulbar and spinal onset showed focal cortical changes in the corresponding regions of the motor homunculus (1). Since to date functional studies focused on this issue showed inconsistent findings (2), we aimed at evaluating the capability of brain 18F-FDG-PET to disclose the cerebral metabolic features characterizing patients with pure bulbar or spinal motor impairment.
Methods: We classified as pure bulbar (PB) ALS patients with bulbar onset who showed a normal score in the spinal items of the ALSFRS-R at the time of PET at diagnosis. We considered as pure spinal (PS) ALS patients with spinal onset, displaying a normal score in the bulbar items of the ALSFRS-R at the time of PET at diagnosis. Healthy Controls (HC) were also enrolled. We compared PB and PS, and each patient group with HC through the two-sample t-test model of SPM12. Metabolic clusters showing a statistically significant difference between PB and PS were tested to evaluate their accuracy in discriminating the two groups. First, we performed a Leave- One-Out Cross-Validation (LOOCV) over the entire dataset. Four classifiers were considered for comparison: Support Vector Machines (SVM), K-Nearest Neighbors, Linear Classifier, and Decision Tree. Then, we used a separate test set, composed of 10% of patients, with the remaining 90% composing the training set.
Results: We included 63 PB, 271 PS, and 40 HC. PB showed a relative hypometabolism compared to PS in the bilateral precentral gyrus in correspondence with the regions of the motor cortex involved in the control of bulbar function. SVM showed the best performance, resulting in the lowest error rate in both LOOCV (4.19%) and the test set (9.09 ± 2.02%).
Discussion: We found clusters of relative hypometabolism in the bilateral motor cortex in PB compared to PS, closely overlapping with the somatotopic representation of bulbar functions in the motor homunculus. The metabolism of such regions showed a very high capability to discriminate between PB and PS. Our data provide in vivo support for the concept of the focality of ALS onset and strengthen the idea that 18F-FDG-PET can play a role as a biomarker for precision medicine-oriented clinical trials.

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SESSION 4 CELL BASED MODELS

C09 iPSC-derived models for compound screening and the identification of new therapeutic strategies

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The combination of human-induced pluripotent stem cell (iPSC) derived neuronal models and gene editing techniques such as CRISPR have created a powerful opportunity to understand the disease, to identify drug targets, and to screen for potential therapeutics.

Whole genome transcription analysis and functional studies of ALS patient-derived human stem cell models which exhibit loss of function of TDP-43, identified STATHMIN-2 as a key driver of TDP-43 driven neuropathology (1). The predictive value of the stem cell models was confirmed by recent animal models which show that loss of Stathmin-2 leads to motor neuropathy, a hallmark of ALS (2). Further confirmation comes from the identification of a novel STATHMIN-2 variant as a risk factor for the development of ALS and as a modifier of the clinical phenotype (3). QRL-201, an investigational therapy is being developed by QurAlis to increase the levels of STATHMIN-2 in motor neurons, for the treatment of ALS.

Traditional patch clamp techniques in combination with an unbiased high throughput screen using human ALS motor neurons and an annotated compound library have identified and solidified Kv7.2/7.3 as a drug target to counteract hyperexcitability-induced neurodegeneration (4,5). This was confirmed by the results of a Phase 2a clinical trial (6). QurAlis is developing QRL-101 which acts on Kv7.2/7.3 and is intended to treat motor neuron hyperexcitability-induced disease progression in ALS patients, which is estimated to be up to 50% of all ALS cases.

New developments in single cell high content high throughput imaging and cloud computing have opened up new multiparametric analysis, machine learning and artificial intelligence (AI) methods. A cloud-based image processing and analysis platform were developed that captures the intricate activity profile of ALS patient-derived motor neurons (7). This platform identified compounds that revert the multiparametric disease profile of 153 parameters to that found in healthy cells.

Taken together, patient-derived stem cell models are a powerful technology that is helping us to understand ALS and to identify new therapeutic precision medicine strategies which will hopefully lead to effective treatments for ALS patients.

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C10 Sporadic ALS motor neurons recapitulate neuronal vulnerability

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Background: Patient induced pluripotent stem cell (iPSC)-derived motor neurons provide a powerful and clinically relevant model of ALS, however published iPSC studies model familial ALS (FALS) which is not representative of sporadic disease. There have been no large scale phenotyping studies of motor neurons generated from sporadic ALS (SALS) patients to date.

Objectives: To conduct large-scale deep phenotyping of motor neurons from SALS patients at the cellular, molecular and transcriptomic levels.

Methods: Skin biopsies, blood samples, and clinical profiles were collected from over 200 study participants. Whole genome sequencing was performed on all participants. Fibroblasts were re-programmed into iPSCs using non-integrating episomal vectors and detailed quality control and characterization of re-programmed cell lines were conducted to confirm pluripotency, trilineage potential, and genomic integrity. A total of 150 validated iPSC lines were generated, including 100 SALS, 15 FALS, and 30 control lines. iPSCs were differentiated into spinal motor neurons and virally transduced with a Hb9-GFP reporter. ALS phenotypes, including motor neuron survival, axonal length, TDP-43 mislocalisation, proteostasis, and mitochondrial content were measured using a live cell imaging and automated analysis pipeline, in addition to calcium imaging and RNAseq analysis.

Results: We generated highly pure and mature (>95% Hb9+ChAT+) spinal motor neurons from 100 SALS patient iPSC lines. Live cell imaging of motor neurons from 30 to 60 DIV revealed an axonal loss in FALS lines (SOD1, TDP-43, C9ORF72, VCP, and UBQLN2). Remarkably, we detected significant axonal degeneration and reduction in survival ($P < 0.05$) in 30% of SALS lines, consistent with spontaneous neurodegeneration in the absence of FALS gene mutations. This phenotype in SALS motor neurons correlated with TDP-43 pathology, impaired proteostasis, and mitochondrial abnormalities.

Discussion: These results establish a spontaneous neurodegenerative phenotype in motor neurons generated from a subset of SALS patients, implicating cell autonomous degeneration in the absence of upper motor neurons, interneurons, glia, and support cells. Importantly, the genome of SALS appears sufficient to drive ALS phenotypes in this system.

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C11 Reduced C9ORF72 expression exacerbates poly-GR toxicity in patient iPSC-derived motor neurons

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Background: C9ORF72 intronic repeat expansion GGGGCC is the most common known single genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This repeat expansion is believed to result in both haploinsufficiency and toxic gain-of-function through the production of toxic arginine-enriched dipeptide-repeat proteins (DRPs), namely polyGR and polyPR (1). Small-molecule inhibition of type I protein arginine methyltransferases (PRMTs) has been shown to protect against toxicity resulting from polyGR and polyPR challenge in NSC-34 cells and primary mouse-derived spinal neurons (2), but the effect in human motor neurons (MNs) has not yet been explored.
Objectives: Here, we sought to generate a panel of C9ORF72 homozygous and hemizygous knock-out iPSCs to examine the contribution of C9 haploinsufficiency toward disease pathogenesis. We differentiated these iPSCs into spinal motor neurons and examined the role of C9ORF72 in sensitivity to growth factor (GF)-deprivation, glutamate, AMPA, and poly-GR(15) challenge.
Methods: We used a CRISPR/Cas9 dual-guide approach to create knockout C9ORF72 lines and hemizygous C9 lines. We confirmed editing in iPSCs via Sanger sequencing using Synthego's ICE analysis, tested gene expression via qPCR, and measured protein levels via Western blot analysis. The generated C9-KO iPSCs were differentiated into spinal motor neurons using the protocol by Du et al. (3). We tested cell viability using CellTiter-Blue following GF-deprivation, glutamate, AMPA, and poly-GR(15) challenge. Poly-GR(15) was also applied in the presence of type I PRMT inhibitors to test the effect of inhibition of asymmetric arginine dimethylation inhibition in mediating neuronal toxicity.
Results: Genotyping analysis confirmed the successful generation of homozygous and hemizygous knockout iPSC clones. Follow-up by qRT-PCR and Western blotting confirmed C9ORF72 abundance was nearly undetectable in the homozygous clones and roughly 50% of wild-type in hemizygous clones. Poly-GR(15) had the greatest effect on MN survival, irrespective of genotype, compared to GF-deprivation, glutamate, and AMPA exposure. C9ORF72 hemizygous and homozygous KOs showed higher sensitivity to poly-GR(15) compared to wild-type MNs. Interestingly, patient-derived C9ORF72-expanded MNs showed a poly-GR(15) toxicity endpoint similar to homozygous KO cells. Type I PRMT inhibition shows a moderate rescue from 0.1 to 3.7uM against 1uM poly-GR(15) challenge, although the treatment duration for this test is currently being optimized.
Discussion and conclusions: We generated a panel of isogenic iPSCs to examine the role of C9ORF72 haploinsufficiency. In MNs we showed that reduced levels of C9ORF72 exacerbate poly-GR toxicity in a dose-dependent manner. Type I PRMT inhibition was tolerated during a 48hr treatment window and was able to partially rescue poly-GR(15) toxicity. Further work to better understand the rescue mechanism is currently underway.

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SESSION 5 DISEASE PRESENTATION AND RISK

C12 Identifying risk factors using mendelian randomization

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Mendelian randomization (MR) is an approach that can overcome some of the limitations of observational epidemiological studies. A contemporary review of MR approaches to understanding factors that lead to disease onset and disease progression will be provided, with attention given to how pharmacotherapeutic targets can be investigated in this way. The remaining challenges – including data challenges – will be presented, with reference to ALS.

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C13 Traumatic brain injury as a risk factor for neurodegenerative disease

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The World Health Organization estimates that 600 in every 100 000 people suffer from concussion/mild traumatic brain injury each year, which is likely an underestimate. Concussions are most commonly reported in contact sports, but are also frequent in motor vehicle accidents, falls, and trauma. Once trivialized, we are now aware that concussions can lead to serious somatic, affective, and cognitive sequelae. The long-term health-related effects of multiple concussions have become a growing concern as repeat concussions have been associated with mid to late-life depression and mild cognitive impairment in retired football players, with an earlier onset of Alzheimer's disease (AD) observed in some reported cases. More recently concussions and multiple concussions have been identified as modifiable risk factors for dementia. Multiple concussions are not only apparent in sports but also common in intimate partner violence, seniors' falls, motor vehicle collisions, and military and prison populations. Since concussions are very common there is a growing concern that multiple concussions can lead to neurodegeneration including accelerated atrophy and reduced white matter integrity. Moreover, multiple concussions are the most important risk factor for developing chronic traumatic encephalopathy" (CTE), a slowly progressive neurodegenerative disease manifesting years after concussive and subconcussive events that includes deleterious psychiatric, cognitive, and motor symptoms. Multiple concussions have also been associated with other neurodegenerative diseases including AD, Frontotemporal Dementia, Parkinson's disease, and Amyotrophic Lateral Sclerosis. There is, however, ongoing controversy as most of the evidence comes from small case series, and not all studies have identified head injury as a risk factor for any neurodegenerative diseases. Co-pathology of CTE with the other proteinopathies is increasingly being recognized with closer attention to tau pathology in post-mortem studies in patients with repeated concussions. Identifying reliable biomarkers for detecting CTE in vivo will further understand the relationship between concussion and neurodegenerative diseases. Given the high frequency of concussions, and growing evidence for severe cumulative effects, multiple concussions must now be considered a significant health problem.

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C14 Mild motor impairment and prodromal disease markers

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Amyotrophic lateral sclerosis (ALS) is traditionally regarded as a clinical syndrome. Emerging science, and insights from other neurodegenerative diseases, however, indicate that ALS should instead be viewed as a biological entity with both pre-symptomatic and clinically manifest stages of disease (1). This framework is supported in part by the observed increase in neurofilament light chain (NfL) concentration, an ALS biomarker, prior to phenoconversion in gene mutation carriers (2). The prognostic value of NfL in predicting imminent phenoconversion has even enabled the design of the first-ever pre-symptomatic ALS clinical trial (3).

While biomarker evidence of disease may be apparent prior to the emergence of clinically manifest ALS, the pre-symptomatic stage of the disease has traditionally been regarded as clinically silent. We have, however, found evidence to the contrary, demonstrating that prodromal clinical markers may also precede phenoconversion. Indeed, since motor neuron degeneration is incremental and cumulative over time, it stands to reason that the clinical (motor) syndrome might be preceded by a prodromal state characterized by minor motor abnormalities that are initially of insufficient severity or extent to permit a diagnosis of ALS. While this prodromal period of mild motor impairment (MMI) is usually missed, the study of pre-symptomatic gene mutation carriers offers a unique opportunity to observe what is typically unseen.

Based on our experience from the Pre-fALS study—through which we have documented MMI in multiple genotypes (SOD1, FUS, TARDBP, and C9orf72) so far—we conclude that MMI is an observable state that precedes clinically manifest disease in the most common genetic forms of ALS, and perhaps in all genetic ALS (4). We also hypothesize that this might be true of non-genetic ALS. Parenthetically, a prodromal period of mild cognitive impairment (MCI) and mild behavioral impairment (MBI) may also precede the emergence of clinically manifest disease in carriers of some genetic mutations. And the observed increase in NfL concentration may precede or follow the emergence of MMI, in part related to the tempo of underlying disease progression and the relative dominance of lower vs. upper motor neuron pathology.

As a diagnostic label, MMI provides the language to describe the indeterminate (and sometimes intermediate) transition between the unaffected state and clinically manifest ALS. Recognizing MMI as a distinct clinical entity should generate

fresh urgency for developing biomarkers that capture the earliest events in the degenerative cascade, with the potential to reduce the diagnostic delay and permit earlier therapeutic intervention.

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SESSION 6 GENETICS AND GENOMICS

C15 Decoding gene regulatory networks in ALS

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Gene regulatory networks describe the interaction of multiple different regulators that govern gene expression, which subsequently influences protein translation and ultimately the function of the cell. In order to identify and validate therapeutic agents to slow down, pause or even potentially reverse disease progression in amyotrophic lateral sclerosis (ALS), it is necessary to establish and understand the pathogenic mechanisms involved. Over the years, following the identification of mutated genes and dysregulated proteins and pathways, many therapies have been trialed targeting these pathogenic mechanisms including excitotoxicity, oxidative stress, neuroinflammation, and protein aggregation. However, only riluzole, targeting excitotoxicity, is widely available, whilst edaravone, targeting oxidative stress, is licensed in fewer countries.

Transcriptomics, or gene expression profiling, offers a snap shot of genes being expressed and this has been used to investigate disease mechanisms, monitor disease progression, and identify potential prognostic and diagnostic biomarkers in ALS. Major technological advances have also occurred, from quantifying the whole transcriptome on a microarray through to RNA sequencing on a single cell. As such, this work is significantly increasing our understanding of the disease and the gene regulatory networks that are being disrupted in ALS.

In 2017, I commented that gene expression profiling could become an integral part of clinical trials. This would allow us to understand the mechanism of action of a treatment, to begin to explain why there were variations in patient responses, and to identify predictive biomarkers at diagnosis that establish if a patient would respond to a drug.

The IMODALS clinical trial (https://clinicaltrials.gov/ct2/show/NCT02059759) was a phase II study to assess the safety and activity and low dose interleukin-2 (ld-IL-2) in a small cohort of ALS patients (1). The randomized, placebo-controlled, double-blind trial tested two doses of IL-2, and blood samples were taken at five time-points during the trial for transcriptomic analysis. Our collaborators in the trial highlighted that there was a variable response amongst patients to the treatment, as measured by increased numbers of regulatory T cells (Tregs), and we demonstrated that this was due to differential expression of genes in the T-cell gene regulatory network (2). In addition, two potential biomarkers were identified in the baseline sample that predicted the patient's response to treatment.

The MIROCALS clinical trial (https://clinicaltrials.gov/ct2/show/NCT03039673) is a larger phase II study to assess ld-IL-2 safety and clinical efficacy on survival in newly diagnosed ALS. Five blood samples were taken during the trial and analysis of this data from over 1000 samples will be presented, to illustrate the impact of ld-IL-2 on gene regulatory networks in ALS patients.

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C16 Unsupervised hierarchical clustering of postmortem motor cortex expression data identifies distinct molecular phenotypes which replicate the major mechanisms of ALS pathogenesis

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Background: Amyotrophic lateral sclerosis (ALS) displays considerable clinical and genetic heterogeneity, with a multitude of cellular processes known to contribute to ALS pathogenesis. Unsupervised clustering approaches have been previously employed to stratify people with ALS into molecularly homogeneous subgroups, however, they have either adopted a case-control framework or used samples from different tissues. Here, we perform hierarchical clustering of postmortem motor cortex samples obtained from the UK Project MinE cohort to characterize specific molecular phenotypes.

Methods: Bulk RNA-seq data from 112 UK ALS postmortem motor cortex samples were filtered and normalized before hierarchical clustering was performed using the 5000 most variably expressed genes. The resulting informative gene and

sample assignments were then subject to several downstream analyses to establish the molecular and phenotypic architecture of each cluster. Other omics data (DNA-seq and methylation) were utilized to assess if other phenotypic measures such as mitochondrial DNA copy number, telomere length, and biological/transcriptional age acceleration differed between clusters. Gene networks for each cluster were also constructed. Validation of these clusters was performed by applying linear discriminant analysis of informative genes to the TargetALS RNA-seq cohort (168 cases).
Results: Three molecularly and phenotypically distinct clusters were identified in our cohort. The largest cluster, characterized by 131 informative genes, defined 54% of cases and was strongly enriched for synaptic, interneuron, and neuropeptide signaling processes. The second cluster, comprised of 293 genes and defining 25% of cases, was linked to apoptosis and excitotoxicity. The final cluster, which contained 371 informative genes and defined 21% of cases, represented a pure neuroinflammatory molecular phenotype. This was evidenced by enrichment for the adaptive immune system and complement cascade pathways, a high microglial and astrocyte composition, and a major histocompatibility complex class II specific sub-network. Known ALS risk genes were identified among the informative genes of each cluster, suggesting potential for genetic profiling of the molecular phenotypes. Subgroup clinical phenotype analysis found distinct outcomes associated with the clusters in terms of progression, survival, and age of death, compatible with the transcriptional age of the cluster members. Cell-type composition analysis associated neurons, endothelial cells, and microglia with clusters 1, 2, and 3 respectively. These clusters are highly conserved, with correct cluster assignment probability ranging from 87.7 to 93.7 percent in the TargetALS dataset.
Conclusions: We identify three major molecular phenotypes, driven by different cell types, which reflect three known key drivers of ALS pathogenesis; neuronal signaling dysfunction, oxidative stress, and inflammation. These results show potential for enhancement of an effective targeted and personalized approach to the study of ALS biology and the development of treatments.

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C17 Interactions between genetic modifiers of survival in ALS: a population-based study

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Background: ALS is considered a multifactorial disorder, caused by an interaction between genetics and the environment. While relatively is known about the environmental contribution to ALS, at least 30 genes are currently considered to be related to the disease, the most common being C9orf72, SOD1, TARDBP, and FUS. Besides disease-causing genes, several other genes have been reported as modifiers of ALS phenotype, including survival. However, very little information is available about the interactions between these genes, in particular, if their co-presence has an additive effect on ALS course.
Aim: The aim of this study was to assess the role of various genetic variants that have been proposed to be implicated in the prognosis of ALS in order to determine if they have and additive effect.
Methods: Patients population included 1319 ALS cases diagnosed between 2007 and 2016 identified through the Piemonte and Valle d'Aosta Register for ALS (PARALS), and who underwent whole-genome sequencing on an Illumina HiSeqX10 sequencer. Patients with SOD1, TARDBP, and FUS mutations ($n = 74$) were excluded from this study. We considered the following genes: C9orf72 repeat expansion, UNC13A (rs12608932), CAMTA1 (rs2412208), ATXN2 (intermediate polyQ repeats $\geq$31), SLC11A2 (rs407135) and ZNF512B (rs2275294). First, the effect on survival of each gene expansion/polymorphism was evaluated separately. Second, all genes which reached at least a $p < 0.2$ in univariate analysis were assessed in Cox multivariable analysis. Third, the interaction of detrimental alleles was assessed in gene couples.
Results: A total of 1245 cases were included in the study. C9orf72 ($p = 0.015$), UNC13A ($p < 0.001$), and ATXN2 ($p < 0.001$) reached a significance in univariate analysis. CAMTA1 reached a $p = 0.17$, while SLC11A2 ($p = 0.66$) and ZNF512B ($p = 0.32$) were not significant. In Cox multivariable analysis which included the most relevant prognostic factors (age at onset, site of onset, diagnostic delay, gender, King's stage, ALSFRS-R decline, FVC%) C9orf72 (HR 1.39, 95% c.i. 1.11–1.75, $p = 0.005$), ATXN2 (HR 2.13, 95% c.i. 1.52–2.96, $p < 0.001$), and UNC13A (HR 1.31, 95% c.i. 1.09–1.57, $p = 0.004$) were confirmed to be independently related to survival, and also CAMTA1 resulted to be significantly related to survival (HR 1.14, 95% c.i. 1.01–1.29, $p = 0.045$). When assessing the interaction between pairs of genes we found that the copresence of ATXN2 intermediate repeat expansion and UNC13A or CAMTA1 deleterious alleles (both $p < 0.001$), the copresence of UNC13A and CAMTA1 deleterious alleles ($p < 0.0001$), and the association of C9orf72 expansion and UNC13A ($p < 0.0001$) had a strong negative effect on ALS survival.
Conclusions: In this population-based study, we confirmed that C9orf72 repeat expansion, UNC13A, CAMTA1, and ATXN2 intermediate repeats are robust modifiers of ALS survival and we showed that the combination of these genes had an additive detrimental effect. Our findings should be considered when designing clinical trials and have an impact on personalized medicine in ALS.

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SESSION 7 RNA AND PROTEIN PROCESSING

C18 Protein chaperones as a therapeutic target for ALS

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Protein misfolding and aggregation are associated with multiple forms of ALS/FTD. A logical therapeutic strategy is to boost the chaperoning capacity of neural cells by inducing heat shock proteins (HSPs) to manage the load of aberrant proteins; however, motor neurons have a high threshold for inducing expression of HSPs in response to stress and respond poorly to HSP-coinducers such as arimoclomol. This talk will review the steps in activating heat shock protein expression, and discuss how aging, epigenetics, and disease mechanisms further compromise the ability of neural cells to maintain proteostasis. Such mechanisms are multifactorial and could include: the level of the transcription factor HSF1; the complex regulation of HSF1 activation; mRNA production, transport and translation, and coordinated activity of chaperoning and proteolytic pathways in the face of a metastable proteome. Variants of RNA Binding Proteins (RBPs) linked to familial ALS are known to affect gene expression through multiple mechanisms including RNA splicing, mRNA transport, and translation. Mechanisms of nucleosome remodeling are abrogated in ALS; i.e. histone acetylation and expression of nBAF chromatin remodeling complexes that act cooperatively with transcription factors. including HSF1 to enable binding to heat shock elements (HSE) (1). We hypothesized that treatment with histone deacetylase (HDAC) inhibitors might not only relieve epigenetic abnormalities but enable HSP-inducers. Indeed, treatment with Class I HDAC inhibitors lowered the threshold for expression of stress-inducible HSPA1A (Hsp70) in cultured motor neurons and co-treatment improved the efficacy of multiple types of HSP-inducing drugs. However, this effect was stress-dependent and HSPA1A was detected in only a subset of neurons expressing SOD1G93A or TDP43G348C, but not in neurons expressing FUSR521G/H (2). Yet the treatments were neuroprotective through other, still to be defined, mechanisms. Neither drug increased HSP expression in the cortex, spinal cord or muscle of SOD1G93A or FUSR521G transgenic mice, but as in culture, both drugs showed efficacy, reversing cognitive impairment and improving neuromuscular function in FUSR521G mice. Current studies are examining the underlying mechanisms and how FUS, TDP43 and SOD1 variants, or dysregulated wild-type counterparts, alter the life cycle of constitutive and inducible HSPs to compromise their function. Conclusions: Therapeutic upregulation of HSPs remains elusive because of the biology of their regulation in nervous tissue, toxicity or suppression by disease mechanisms. ALS is not a homogeneous disease and subtypes could respond differently to treatments. The therapies we are testing have other targets and might be particularly useful in combination and in ALS associated with RNA metabolism and FTD mechanisms. Currently supported by ALS Canada-Brain Canada.

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C19 MicroRNA dysregulation in motor neuron disease

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microRNAs (miRNAs) are endogenous noncoding RNAs that play critical roles in maintaining brain integrity. miRNA dysregulation has been studied in ALS over the last 13 years, using multiple approaches. Specific miRNAs are involved in the regulation of pathways that are essential for neuronal survival or function and changes to the expression of specific miRNAs or miRNA ability to recognize their messenger RNA target sequences are associated with deleterious consequences. Furthermore, miRNAs are susceptible to fail when protein factors that are critical for miRNA biogenesis, such as the DICER complex, malfunction. In addition, cell-free miRNAs contribute to a new generation of biomarkers for neurodegeneration. Therefore, specific miRNAs and the miRNA biogenesis machinery contribute to mechanistic understanding and are intriguing targets for therapeutic interventions.

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C20 Diminished miRNA activity is associated with aberrant cytoplasmic intron retention in ALS pathogenesis

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Intron retention (IR) is now recognized as a dominant splicing event during motor neuron (MN) development. Traditionally, IR was regarded as a repressive mechanism; with the inclusion of an intron resulting in nuclear detention of the transcript and therefore preventing its translation. However, recent studies have identified the presence of stable intron-retaining transcripts (IRTs) within the cytoplasm,

though the role and regulation of these remains particularly understudied.

Using a directed differentiation paradigm on patient-derived human iPSC, we show that IR is a physiological process that is spatiotemporally regulated during MN lineage restriction. Importantly, we show that IRTs in the cytoplasm are detected in as many as 13% ($n = 2297$) of all genes expressed throughout this differentiation. We identify a major class of cytoplasmic IRTs, which are not associated with reduced expression of their own genes, but instead show a high capacity for RNA binding protein (RBPs) and miRNA occupancy. Interestingly, modifying the expression of these candidate RBPs and miRNA has no impact on the expression of the IRTs. The combination of these findings raises an exciting possibility that the IRTs are not regulated by these RBPs and miRNAs but are themselves regulators. Finally, we show that ALS-causing VCP mutations lead to a selective increase in cytoplasmic abundance of this particular class of IRTs, which in turn temporally coincides with an increase in the expression level of predicted miRNA target genes. Altogether, our work identifies a previously unrecognized class of cytoplasmic intronic sequences with a potential regulatory function beyond their own gene expression.

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SESSION 8 NEUROINFLAMMATION

C21 Modelling neuroinflammation in ALS

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Although major clinical symptoms in ALS arise from neurodegeneration and the death of motor neurons, it is now well established that non-neuronal cells such as microglia play an important role in disease pathogenesis. Indeed, there is increasing evidence suggesting that chronic deregulation of immunity may represent one of the key elements in the pathobiology of several neurodegenerative disorders including ALS. Inflammation in ALS is characterized by a marked and chronic activation of resident glial cells including astrocytes and microglia, changes in T cell activation patterns and infiltration as well as systemic immune system activation. Importantly, data from several clinical and experimental studies suggest that in addition to sporadic disease, multiple genetic mutations linked to ALS (including TARDBP SOD1, C9Orf72, OPTN, TBK1, etc.) have been associated with either enhanced and/or dysregulated neuroinflammatory signaling. Furthermore, the observed dysregulation and/or disease-associated dysfunction of immunity in ALS exhibit features of otherwise distinct immune dysfunctions such as excessive inflammation, autoimmunity, and inefficient immune responses. One of the important questions here is how the disease affects immune cells in the brain and/or spinal cord? In the central nervous system, the principal resident immune cells are microglia. A current view is that over the course of the disease, microglia change their phenotypes from initially beneficial into highly toxic and aberrant cells resistant to any conventional immunemodulatory therapeutic interventions. Recent evidence from several research groups including ours suggests that ALS microglial cells, and potentially other immune cell types gradually lose their protective functional phenotype and thus contribute to disease progression. This apparent change in functional phenotypes and development of the distinct and disease-associated molecular profiles may, at least in part explain, why various anti-inflammatory or immunosuppressive therapies in ALS have so far failed. Therefore, increasing our knowledge about the complex molecular mechanisms involved in ALS pathogenesis and neuroinflammation will be crucial for the development of novel and more efficient therapeutic strategies.

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C22 Suppressing neuroinflammation: regulatory T lymphocyte immunomodulatory therapy for ALS

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Neuroinflammation plays a prominent role in promoting the disease progression of ALS, mediated in part by the interaction of injured motoneurons with surrounding glia and dysregulated central and peripheral immunomodulatory cells. Prominent among such immunomodulatory cells are the CD4+ CD25highFOXP3+ T-lymphocytes that mediate neuroprotection by suppressing proinflammatory responses. However, Treg suppressive functions are impaired in ALS, but are restored and even enhanced following expansion ex vivo. Autologous infusions of these expanded T regs together with subcutaneous IL-2 injections formed the basis of two ALS clinical trials.

In a Phase 1 pilot study of 3 ALS patients, infusions were safe and well-tolerated and slowed progression rates during early and later stages of the disease. Treg numbers and suppressive function increased after each infusion and correlated with slowing of disease progression. However, the duration of the clinical benefit was limited, possibly related to the serum biomarkers of oxidative stress, 4-hydroxynonenal, and oxidized LDL These lipid peroxide biomarkers were increased prior to Treg infusions, fell with Treg infusions and slowing of disease progression, rose again as disease progression accelerated in the absence of infused Tregs, then fell again when Tregs were reinfused. Thus, the levels of 4-HNE and ox-LDL were effectively responsive to Treg infusions and mirrored the stabilization or deterioration of the subject's clinical status.

A Phase 2A study of autologous infusion of expanded Tregs in combination with subcutaneous IL-2 injections was undertaken at Houston Methodist and Massachusetts General Hospitals. The study was planned for 12 ALS pts enrolled in a 24-week randomized control trial (RCT) followed by a 24- week open label extension (OLE). In the RCT Treg/IL-2 treatments were safe and well-tolerated; with increased Treg suppressive function in the active group. Evaluation of relative progression rates in the RCT was precluded by the COVID pandemic which decreased the number of participants. However, 8 ALS patients did complete the 24-week OLE; Treg/IL-2 treatments were safe and well-tolerated, and Treg suppressive function and numbers were increased. Six patients showed slow to no progression in the OLE (mean change of −2.7 points on ALSFRS-R) Two patients progressed rapidly; they were unresponsive to Treg infusions and had elevated markers of peripheral inflammation (IL-17C and IL-17F) as well as elevated markers of oxidative stress (OLR1 and oxidized-LDL). The 6 participants in the slow progressing group had normal levels.

Whether Treg/IL-2 treatments can slow disease progression in ALS requires a large double-blind randomized controlled study. Nevertheless, our open-label studies, albeit in a limited population, suggest that Treg therapy is safe and well tolerated, and a promising approach to slowing ALS progression; lipid peroxide biomarkers may not only reflect a heightened pro-inflammatory milieu but may also be useful in monitoring clinical responsiveness to therapy.

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C23 Molecular signatures of neuroinflammation in patient tissue across sporadic, SOD1 and C9orf72-ALS cohorts

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Background: Amyotrophic lateral sclerosis (ALS) exists on a pathogenetic disease spectrum with frontotemporal dementia (FTD), with patients sometimes experiencing symptoms of both conditions (ALS-FTSD) (1). Clinical heterogeneity across this spectrum is a complicating factor in clinical trials, potentially obscuring outcome measurements in studies without appropriate stratification.
Objectives: For mutations associated with ALS-FTSD, the factors influencing where an individual may lie on this spectrum require further characterization. As such, our study aimed to investigate molecular signatures underlying heterogeneity across three genetic cohorts with ALS.
Methods: Here we used NanoString molecular barcoding (2) with a panel of 770 neuroinflammatory genes to interrogate this dysregulation at the level of gene expression in postmortem motor cortex tissue from 18 sporadic, 10 C9orf72, and 5 SOD1 ALS cases and 10 controls. Validation of select hits was performed using BaseScope(TM) in situ hybridization and immunohistochemistry. Heatmap clustering and gene ontology analyses were performed to investigate pathways related to signature-defining gene modules.
Results: We identified both shared and unique dysregulated neuroinflammatory genes across our 3 cohorts. Our analyses also revealed distinct neuroinflammatory subgroups, delineated by the direction of expression of different neuroinflammatory gene modules. These subgroups did not segregate with a genetic mutation, but rather with clinical and/or pathological findings.
Discussion: These data imply that distinct neuroinflammatory signatures can be detected within well-curated and deeply clinically phenotyped cohorts and highlight the need for molecular stratification in clinical trials involving inflammation-modulating treatments. The establishment of such signatures will allow for the more meaningful measurement of cXlinical outcomes, holding promise for future targeted therapies.

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SESSION 9 TRIAL DESIGN AND CLINICAL ENDPOINTS

C24 Improving clinical endpoints in therapeutic trials for ALS

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A key challenge in clinical trials for ALS is the lack of sensitive endpoints to determine the effectiveness of experimental compounds. This is for an important part driven by the extensive clinical heterogeneity between patients, complicating any measure that summarizes ALS into a single overall severity score. As such, a change in overall severity score may not accurately reflect the true value of an experimental compound on either a group-level or for individual patients. The alternative is to measure overall survival, but this requires relatively long and large clinical trials, which may not always be feasible, especially in the early phases of clinical development. In this talk, we will look at the toolbox available for ALS trialists and discuss the main considerations of the current clinical endpoints. The talk will continue with an exploration of how limitations may be overcome and improved for future studies.

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C25 Improving ALS clinical trial design

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ALS is a rare, fatal neurodegenerative disease characterized by loss of upper and lower motor neurons (1,2). People with ALS experience progressive loss of muscle mass, strength, and function in limb, bulbar, and respiratory muscles, usually leading to death within 3–5 years due to respiratory failure (3,4). In part due to the heterogeneity of the disease, clinical research in ALS has proven challenging. There are opportunities to improve and optimize the therapeutic trial design. Considerations for future ALS trial designs include duration of the study, choice of clinical outcome measures, and utility of biological markers of disease progression as co-variants to account for disease heterogeneity and as biomarkers of treatment response. Of particular interest, neurofilament has potential utility both as a covariant to account for baseline disease heterogeneity and as a biomarker of treatment response.
The experience with tofersen in SOD1-ALS highlights key learnings from clinical research and provides a foundation for discussion of improving ALS trial design and endpoints. Tofersen and BIIB078, investigated in C9orf72-ALS, provide important learnings related to the potential for neurofilament to inform clinical research efforts. This presentation will discuss these learnings, using tofersen and BIIB078 examples, and recommendations for future trial designs.

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C26 Registry of validated endpoints in ALS (REVEALS)- results from a multi-centre prospective study of respiratory measures and their clinical meaningfulness

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Background: Respiratory decline is a primary concern in ALS and respiratory failure is the principal cause of death. Regular assessment of respiratory function is recommended (1) but little is known about the impact of respiratory decline on respiratory tract infections (RTIs), respiratory adjunct use, clearing secretions and fatigue, breathlessness or QOL. REVEALS was a prospective study that had three aims. Firstly, to investigate the rates of decline in FVC, SVC, PCF and SNIP; and to measure their inter-correlations and correlations with the ALSFRS-R. Secondly, to prospectively measure RTIs. Thirdly, to quantify the clinical meaningfulness of respiratory decline.
Methods: This was a prospective observational study across 6 European sites. Participants with baseline Kings Stage 1 or 2 ALS were assessed every 3 months. Respiratory measures included SNIP, FVC, SVC and PCF, ALSFRS-R, and respiratory symptoms. RTIs and their associated clinical presentation were prospectively ascertained by text every two weeks. Bayesian multiple outcomes random effects models were constructed to quantify rates of decline.
Results: In total, 974 assessments were performed on 280 participants (mean age 63.3 ± 11.6: male 66.8%) with baseline Kings Stage 2 (59.6%) or 3 (40.4%). A median of 3 assessments was completed over 195 days (IQR 50.5, 382). At baseline, median time since diagnosis was 7 months, onset was

bulbar in 19%; spinal in 81%. In bulbar patients FVC declined at a rate of −0.02 L/day, SVC: −0.02 L/day, SNIP: −1.01 cmH2O/day and PCF: −4.06 L/min/day. In spinal patents, FVC (−0.02 L/day) and SVC (−0.02 L/day) declined at a similar rate to bulbar patients, but SNIP (−0.58cmH2O/day) and PCF (−2.5 L/min) declined more slowly. Respiratory measures did not correlate with ALSFRS-R respiratory sub-scores. SVC and FVC were highly correlated. 151 RTIs were reported by 31.4% of participants. The decline in respiratory function was not associated with an increased probability of an RTI. The decline in respiratory measures (particularly decline in PCF) was associated with a greater probability of reporting fatigue with clearing secretions. The use of respiratory adjuncts was low (Breath Stacking 15.4%, Cough Assist 2.9%, NIV 33.9%, Free Aspire 3.9%).

Discussion: Respiratory measures declined over time, but differentially according to the site of onset. SNIP and PCF showed the greatest ability to differentiate between bulbar- and spinal-onset ALS. RTIs were not associated with a decline in respiratory measures. Declining respiratory measures are clinically meaningful with consequences for the patient including increased difficulty, and fatigue with clearing secretions.

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SESSION 10 CLOSING SESSION

C27 BrainGate: clinical trials in intracortical brain-computer interfaces toward the restoration of communication and mobility

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Intracortically-based Brain-Computer Interfaces (iBCIs) are poised to revolutionize our ability to restore lost neurologic functions. By recording high-resolution neural activity from the brain, the intention to move one's hand can be detected and decoded in real-time, potentially providing people with motor neuron disease (ALS), stroke, or spinal cord injury with restored or maintained ability to control communication devices, assistive technologies, and their own limbs. A multi-site pilot clinical trial of the investigational BrainGate system has been demonstrating the feasibility of people with tetraplegia controlling a computer cursor, a tablet computer, and other devices quickly and intuitively. This presentation will review recent progress in iBCIs and the challenges and opportunities for restorative neurotechnologies in research and clinical practice.

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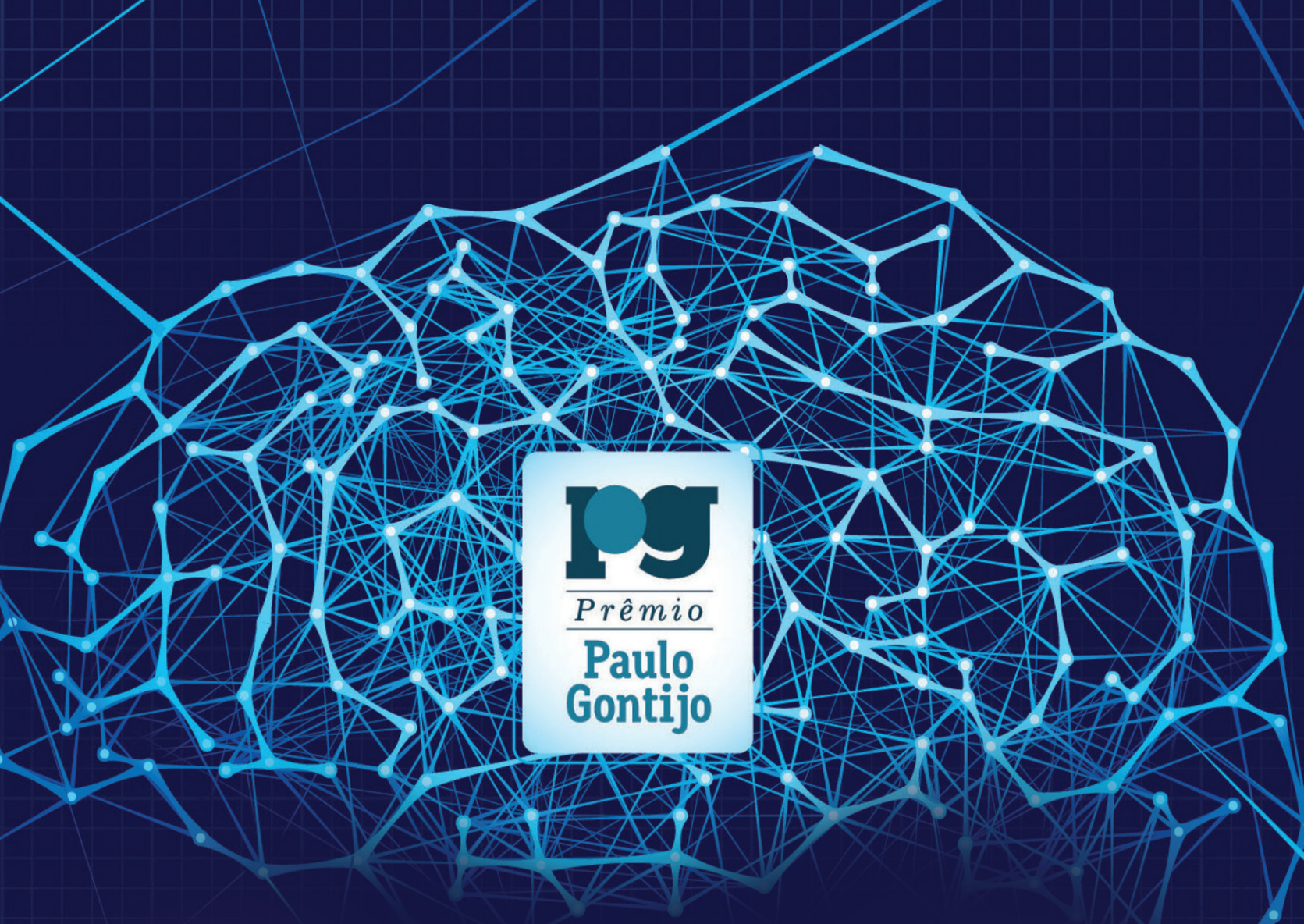

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