Neurizon Therapeutics Limited
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Neurizon's NUZ-001 Reduces Aggregation of Key ALS Disease Target TDP-43 in Preclinical Study

Highlights:

- **Positive preclinical results in human *in vitro* iPSC Motor Neuron models of Amyotrophic Lateral Sclerosis (ALS)**
- **Neurizon's lead drug NUZ-001 and its major active metabolite significantly and dose-dependently prevented the aggregation of TAR DNA-binding protein 43 (TDP-43) by ~50% and ~55% respectively, in M337V Motor Neurons in response to a stressor**
- **TDP-43 aggregration is a key hallmark pathological feature of ALS**
- **Treatment with NUZ-001 and its major metabolite significantly improved electrophysiological dysfunction of TDP-43 mutated M337V Motor Neurons**
- **Provides valuable insights into the mechanism of action of NUZ-001 in ALS and strengthens promising efficacy results from the Phase 1 MEND study completed earlier this year in patients with ALS**

15 November 2024 – Melbourne, Australia: Neurizon Therapeutics Limited (ASX: NUZ & NUZOA) ("Neurizon" or "the Company"), a clinical-stage biotech company advancing treatments for neurodegenerative diseases, is pleased to announce positive results from a preclinical study of its lead candidate, NUZ-001. These innovative studies reveals NUZ-001 unique mechanism of action in preventing the aggregation of TAR DNA-binding protein 43 (TDP-43), a key pathological feature of ALS, and the ability of NUZ-001 to significantly improve the electrophysiological dysfunction of TDP-43 M337V mutated motor neurons, showcasing the potential for NUZ-001 to be a transformative treatment for ALS. Importantly, these findings reinforce the promising efficacy results seen in Neurizon's Phase 1 MEND study and bolster confidence in NUZ-001 therapeutic capabilities for patients with ALS.

Two separate preclinical studies (see full study descriptions below) were conducted in collaboration with Ncardia, a leading human induced pluripotent stem cell (iPSC) technology company. The first study evaluated the ability of NUZ-001 and its major active metabolite (NUZ-001 Sulfone) to reduce TDP-43 aggregation in M337V Motor Neurons co-cultured with astrocytes in response to a stressor. TAR DNA-binding protein 43 (TDP-43) is a known driver of ALS pathology. The results show NUZ-001 and NUZ-001 Sulfone significantly and dose-dependently reduced TDP-43 aggregation in M337V Motor Neurons treated simultaneously with aggregation stressor MG-132 by 50% ($p=<0.005$; 0.625 µM) and 65% ($p=<0.0005$; 1.25 µM), respectively.

The second study evaluated the ability of NUZ-001 and NUZ-001 Sulfone to restore the normal electrophysiological function of TDP-43 mutated M337V Motor Neurons. The TDP-43 M337V mutation is associated with the development of ALS and has been shown to impair neuronal electrical activity at multiple levels. NUZ-001 and NUZ-001 Sulfone rescued the electrical activity of TDP-43 M337V Motor Neurons, by enhancing increasing bursting (NUZ-001 0.625 µM $p=<0.05$) and network burst activity and reducing inter-burst intervals and network burst activity decreasing inter-burst interval (NUZ-001 0.625 µM $p=<0.005$; NUZ-001 2.5 µM $p=<0.0005$ and NUZ-001 Sulfone 2.5 µM $p=<0.00005$) to wild type Motor Neuron activity levels.

Dr. Michael Thurn, Managing Director and Chief Executive Officer of Neurizon, commented: "The positive results from these preclinical studies are a significant milestone, providing validation of our hypothesis that NUZ-001 and its major metabolite prevent the damaging accumulation of TDP-43 in diseased neuronal cells. This finding also highlights the power of NUZ-001 to improve neuronal electrophysiology, an essential step towards providing patients with ALS with a meaningful treatment option."

"This advancement brings us closer to delivering a much-needed therapeutic option for patients with ALS," Dr. Thurn continued. "NUZ-001's positive results present a compelling case for continued development and create exciting opportunities for partnerships as we advance our clinical studies. We are committed to making a significant difference in the lives of patients with ALS and are eager to move forward with our next clinical trial of NUZ-001 in early 2025."

About the Human *In Vitro* Models and Study Results

HTRF TDP-43 Aggregation Assay

Detection of TDP-43 protein aggregates in motor neurons is considered a pathological hallmark of ALS and it is a crucial target to develop therapies targeting pathways that can alter this phenotype and ultimately the disease progression. TDP-43 aggregation assay detects endogenous levels of aggregated TDP-43 protein in cells by using Homogeneous Time Resolved Fluorescence (HTRF) technology.

Methods

Co-cultures of TDP-43 M337V Motor Neurons with astrocytes were treated with aggregation stressor (MG-132) and compounds for 7 days (from day 14 to day 21) at every medium change. At day 21, lysates were prepared from cultures and TDP-43 aggregation kit supplier's (Revity) recommendations were followed to perform the assay. Briefly, the samples were submitted to a disaggregation procedure and incubated overnight at room temperature with HTRF pre-mixed antibodies (donor and acceptor). Samples were read in an HTRF compatible plate reader and ratio acceptor/donor was calculated for the signal 665 nm/620 nm of each sample followed by calculation of aggregation ratio (ratio disaggregated sample/ratio control sample).

Results

A seven day treatment regime with NUZ-001 and NUZ-001 Sulfone significantly reduces TDP-43 aggregation in M337V Motor Neurons co-cultured with astrocytes treated simultaneously with aggregation stressor MG-132. A ~50% reduction of TDP-43 aggregation for NUZ-001 at a concentration of 0.625 µM (p=<0.005) and a ~25% reduction for NUZ-001 at 1.25 µM was observed while NUZ-001 Sulfone reduced TDP-43 aggregation by ~55% at a concentration of 1.25 µM (p=<0.0005) and 25% at 2.5 µM.

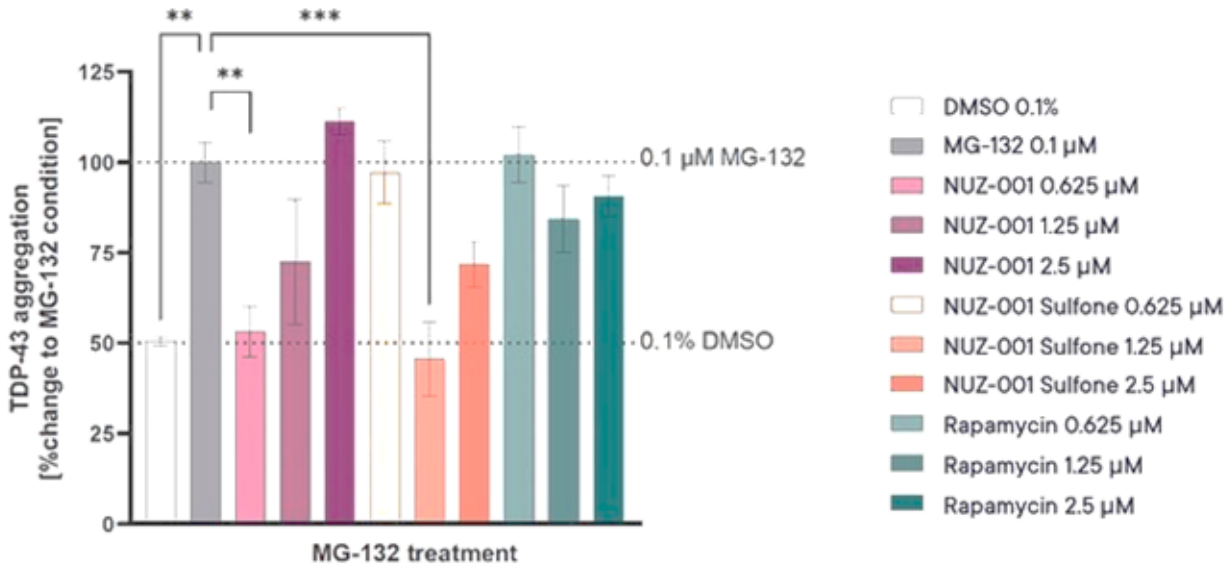


Figure 1: HTRF TDP-43 M337V Aggregation Assay
(**p=<0.005; ***p=<0.0005)

Multi-electrode Array (MEA) Study

MEA is a powerful tool for evaluating and monitoring the activity of electrically active cells, such as neurons. MEA recordings are non-invasive to the cells which allows the electrical activity and formation of neuronal networks to be monitored over time. This feature is particularly valuable in studying neuronal maturation, the development of cultures, and disease-associated phenotypes. The TDP-43 M337V mutation, associated with ALS, has been shown to impair neuronal electrical activity at multiple levels. These impairments include altered firing activity (measured by the weighted mean firing rate, wMFR), bursting activity (repeated neuronal firing), larger inter-burst intervals (IBI),

and organization of bursts in network bursts, a hallmark of neuronal maturation and pacemaker activity (organized rhythmic and synchronous firing activity).

Methods

MEA recordings were performed using pre-coated 48-well Cytoview MEA plates (Axion Biosystems), with the protocol for measuring electrical activity of iCell Motor Neurons provided by the Cell Supplier. Both iCell WT Motor Neurons and iCell TDP-43 M337V Motor Neurons were plated in co-culture with iCell Astrocytes. Motor neuron maintenance media was added and compounds were added to the cultures from day 14 to day 21, with MEA recordings taken three times a week.

Results

The results showed NUZ-001 and NUZ-001 Sulfone significantly improved the ALS TDP-43 M337V associated phenotype/MEA activity by increasing the number of bursts (NUZ-001 0.625 µM p=<0.05) and network burst activity and decreasing inter-burst interval (NUZ-001 0.625 µM p=<0.005; NUZ-001 2.5 µM p=<0.00005 and NUZ-001 Sulfone 2.5 µM p=<0.00005) at day 18 (corresponding to 4 days of treatment with compound).

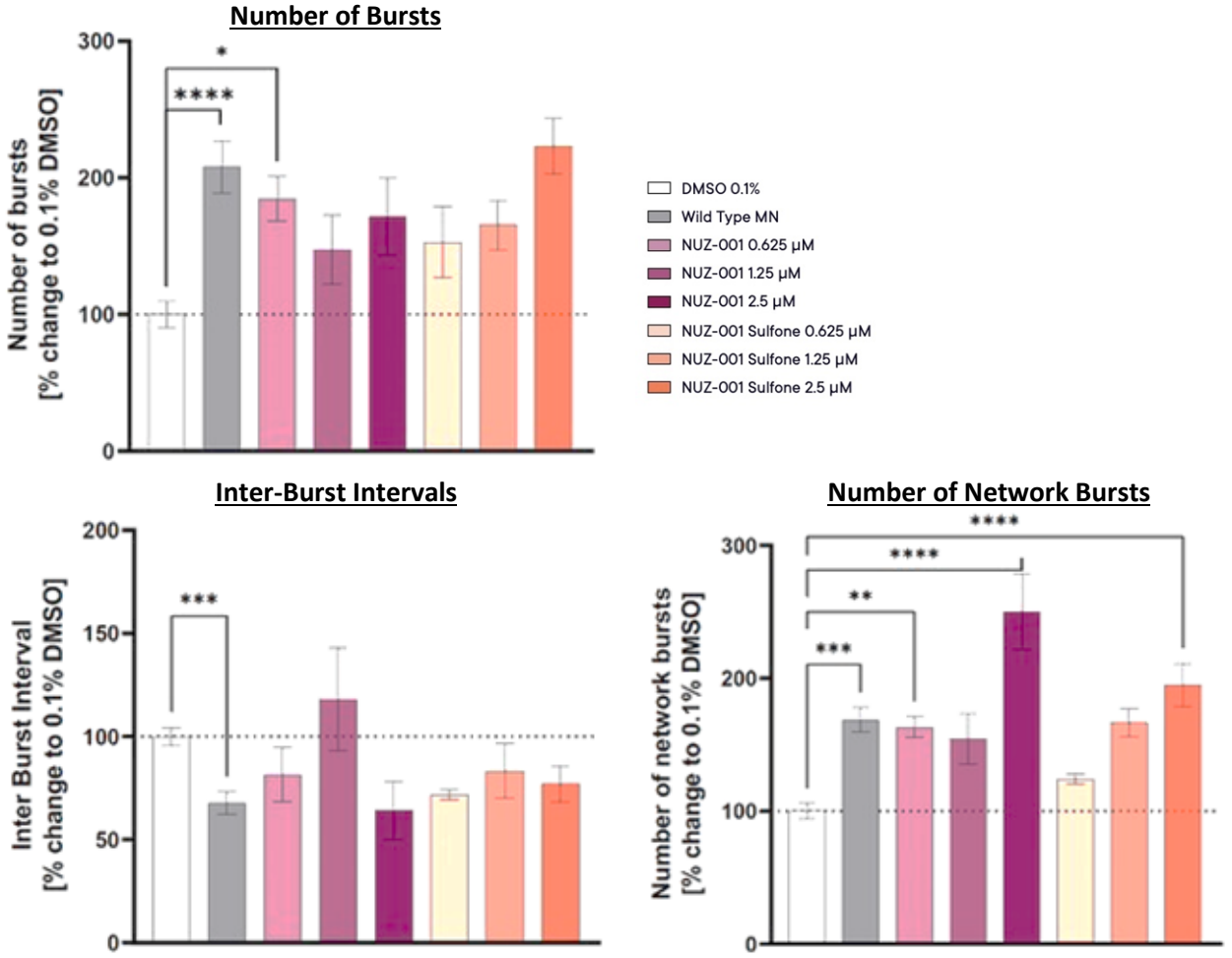


Figure 2: MEA Activity Day 18
(*p=<0.05; **p=<0.005***p=<0.0005; ****p=<0.00005)

Impact on ALS Therapy and the Market

The ability of NUZ-001 to target TDP-43 aggregation and repair motor neuron function positions it as a promising lead candidate for the treatment of ALS, where effective treatments remain scarce. With its novel mechanism, NUZ-001 has the potential to address ALS pathology at its core, offering hope to patients and caregivers affected by this debilitating disease. The ALS treatment market, projected to grow significantly in the coming years, presents a critical opportunity for NUZ-001 to fill a longstanding therapeutic gap.

About TDP-43

TDP-43 protein aggregation is common in several neurodegenerative diseases, including ALS, frontotemporal dementia (FTD), Alzheimer's disease (AD), and limbic predominant age-related TDP-43 encephalopathy (LATE). In ALS, cytoplasmic accumulation of TDP-43 disrupts cellular processes, leading to motor neuron dysfunction and degeneration. By targeting TDP-43 pathology, NUZ-001 offers a new approach to mitigating ALS progression and highlights the potential for expanded applications in other neurodegenerative diseases.

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This announcement has been authorized for release by the Board of Neurizon Therapeutics Limited.

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About Neurizon Therapeutics Limited

Neurizon Therapeutics Limited (ASX: NUZ) is a clinical-stage biotechnology company dedicated to advancing treatments for neurodegenerative diseases. Neurizon is developing its lead drug candidate, NUZ-001, for the treatment of ALS, which is the most common form of motor neurone disease. Neurizon's strategy is to accelerate access to effective ALS treatments for patients while exploring NUZ-001's potential for broader neurodegenerative applications. Through international collaborations and rigorous clinical programs, Neurizon is dedicated to creating new horizons for patients and families impacted by complex neural disorders.

About Ncardia

Ncardia is a human iPSC technology company that operates worldwide with facilities, offices, and staff throughout Europe and North America. Ncardia is built on the belief that stem cell technology will help bring better therapies to patients faster. The company's goal is to enable biopharmaceutical companies in drug discovery to accelerate their development processes through the integration of human iPSC technologies.

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