Metabolic Targeting: A Promising Therapeutic Approach for ALS

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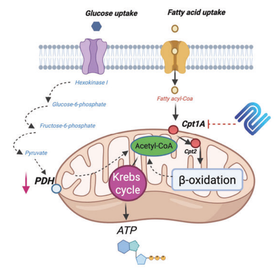
INTRODUCTION

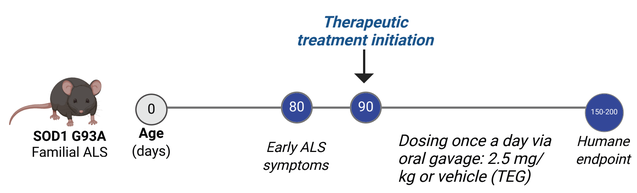
Amyotrophic Lateral Sclerosis (ALS) presents a major challenge in neurodegenerative disease research due to the limited therapeutic options currently available. 2N Pharma is at the forefront of exploring a novel neurometabolic approach to ALS treatment, with a focus on mitochondrial dysfunction and lipid metabolism. Our latest compound, 2N050, has demonstrated promising results in preclinical studies, particularly using the SOD1 mouse model, a widely recognized model for ALS research.The SOD1 mouse model, which carries mutations in the superoxide dismutase 1 (SOD1) gene, mimics several key features of ALS pathology, including motor neuron degeneration, oxidative stress, and protein misfolding. 2N050 targets carnitine palmitoyltransferase 1 (CPT1), the rate limiting enzyme for lipid metabolism, with the aim of restoring mitochondrial function and slowing disease progression. By focusing on CPT1 inhibition, we aim to correct the metabolic disturbances observed in ALS.

MODE-OF-ACTION



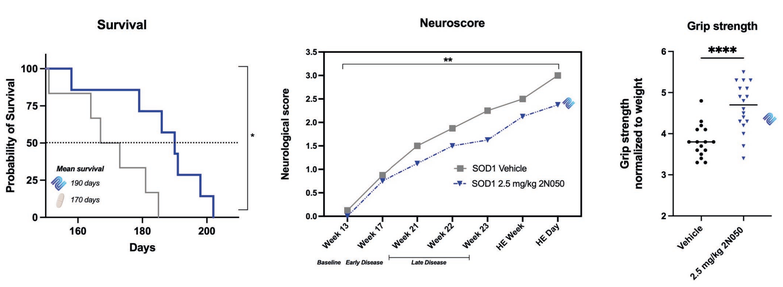
METHODS

Male and female SOD1 G93A were randomized by weight to either treatment or vehicle group. WT mice were included as controls. When animals had lost 5% of their weight they were supplemented with protein. Once animals displayed motor symptoms, they started receiving treatment once a day via oral gavage. Once animals reached a clinical neuroscore of 3 or lost more than 20% of their baseline weight they were euthanized. Brain, spinal cord, tibialis anterior muscle, liver, and serum was collected for downstream analysis which included proteomics, transcriptomics, metabolomics, and immunohistochemistry.

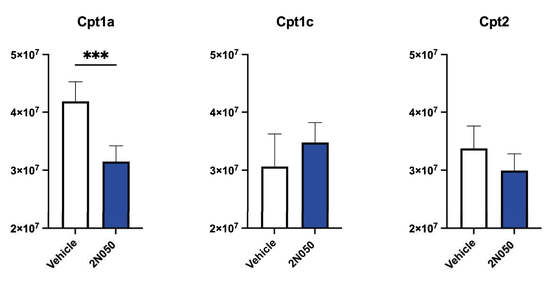


SCAN ME

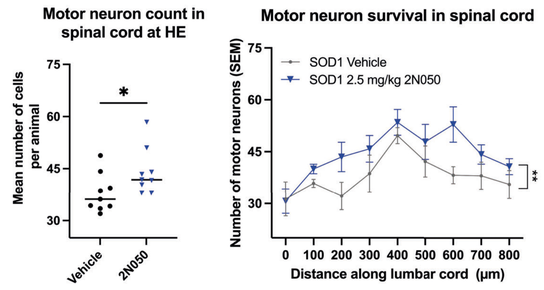
RESULTS



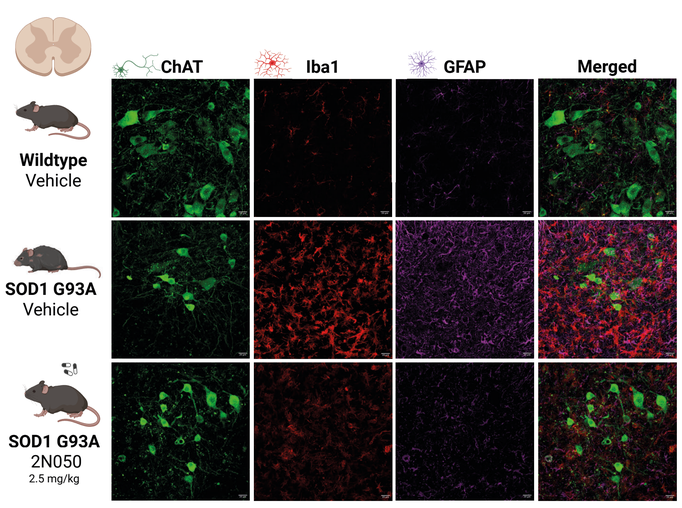
The treated mice exhibited a notable **improvement in survival**, with an average extension of life span by 20 days. Animals treated with 2N050 demonstrated a consistently **lower neuroscore** compared to those receiving the vehicle. In the early stages of the disease, **grip strength was significantly improved**.



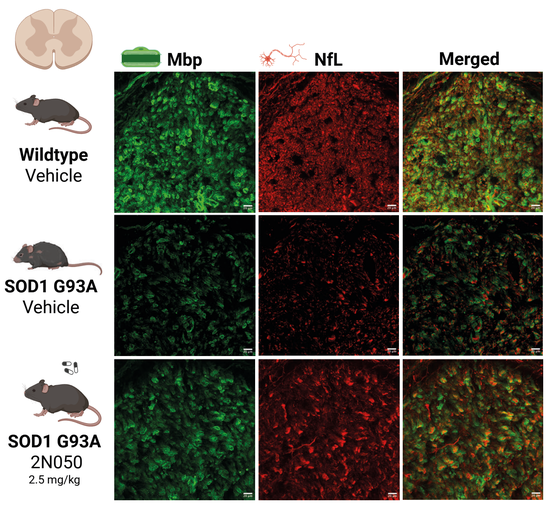
In the motor cortex of animals treated with 2N050, there is a marked decrease in the expression of the *Cpt1a* protein compared to those receiving a vehicle treatment. The expression levels of *Cpt1c* and *Cpt2* remain stable, underscoring the **compound's selective targeting of Cpt1a.**



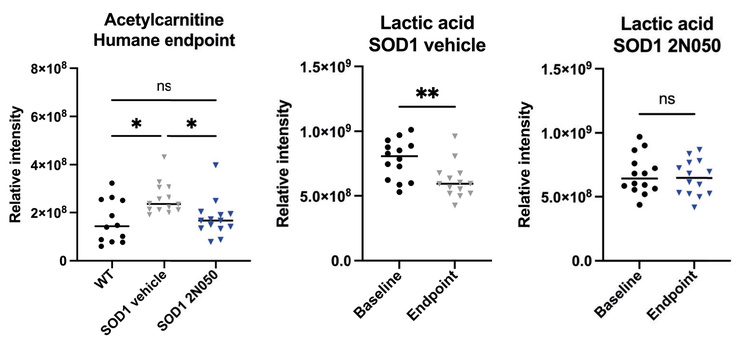
Animals treated with 2N050 exhibited significantly **higher motor neuron survival** in the corticospinal tracts in the spinal cord compared to those treated with a vehicle. Quantification of motor neuron numbers across the entire lumbar spinal cord displays higher number of motor neurons throughout the entire lumbar cord.



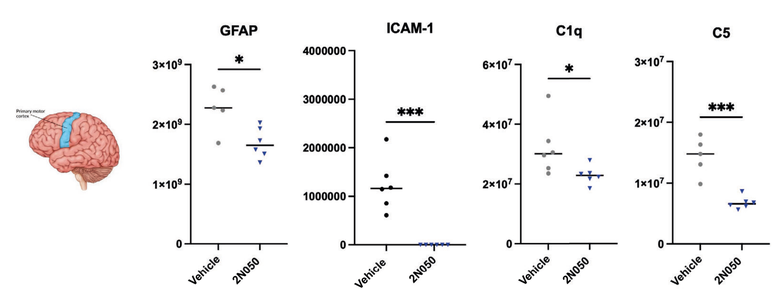
Investigating spinal cord molecular changes reveals that increased motor neuron survival is indicated by stronger *ChAT* signals. In SOD1 mice treated with 2N050, there is a significant reduction in microglial (*Iba1*) and astrocyte activation (*Gfap*), highlighting the drug's neuroprotective and anti-inflammatory effects. Similarly, astrocyte activation is also diminished.These results collectively demonstrate the **neuroprotective** and **anti-inflammatory** properties of our drug.



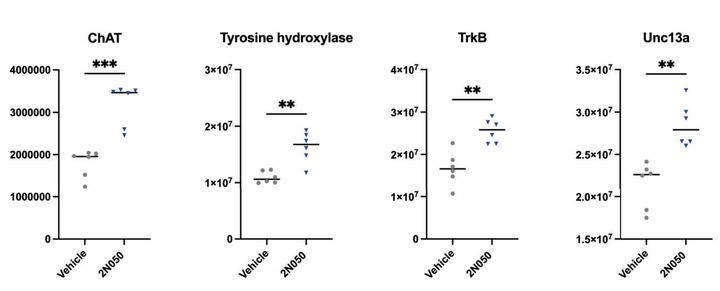
Demyelination of motor neurons significantly contributes to the decline in motor function and the progressive weakness observed in ALS. Our findings demonstrate **increased myelination** (*Mbp*) of motor neurons in the treated mice. The preservation of *NfL* levels in the spinal cord underscores the neuroprotective effects of 2N050, extending beyond the protection of motor neurons to encompass the overall **integrity of the neuronal population**.



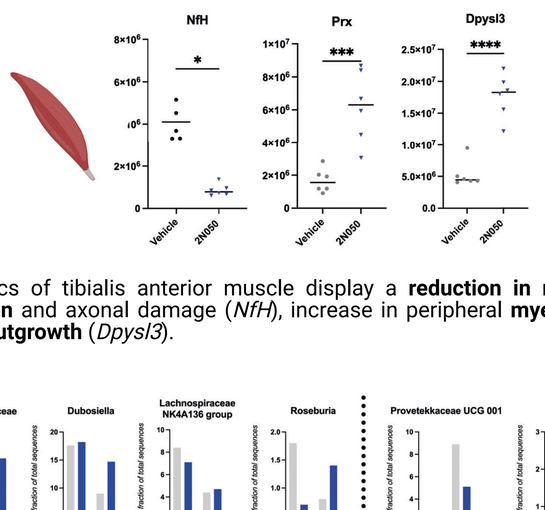
Serum metabolomics reveal a reduction in serum *acetylcarnitine* levels observed in the 2N050 treatment group suggesting **enhanced mitochondrial efficiency** and a decrease in systemic metabolic stress. Stabilized *lactic acid* levels suggest a mitigation of hypoxia-like conditions or excessive cellular energy demands, indicating **improved cellular energy balance**.



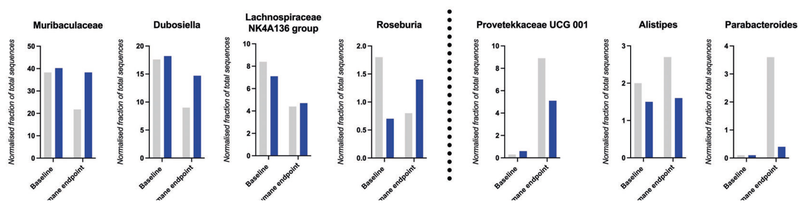
Treatment with 2N050 **reduces neuroinflammation** in the motor cortex by downregulating astrocyte activation (*GFAP*), leukocyte adhesion and blood-brain barrier infiltration *(ICAM-1*), and complement cascade components (*C1q and C5*). 2N050 is therefore able to collectively target key neuroinflammatory pathways implicated in motor neuron degeneration in ALS. This synergistic anti-inflammatory effect of 2N050 is crucial for **disease modifying effect** in ALS.



Treatment with 2N050 displays **neuroprotective effects** in motor cortex by increasing the expression of ChAT, tyrosine hydroxylase, TrKB, and Unc13A. *ChAT* synthesizes acetylcholine for motor neuron communication, while *tyrosine hydroxylase* produces catecholamines crucial for neuronal signaling. *TrkB* supports neuronal survival and synaptic plasticity, and *Unc13A* enhances synaptic transmission.



Proteomics of tibialis anterior muscle display a **reduction in motor neuron degeneration** and axonal damage (*NfH*), increase in peripheral **myelination** (*Prx*) and **neurite outgrowth** (*Dpysl3*).



Microbiome analysis display that 2N050 preserves or increases gut bacteria that are **anti-inflammatory and health promoting**, and represses the growth of proinflammatory and disease inducing bacteria.

ACKNOWLEDGEMENT

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CONCLUSION

Addressing metabolic imbalance in cellular energy production, an alteration that 2N Pharma proposes occurs prior to neuronal degeneration and inflammatory injury, represents a novel and promising strategy for disease-modifying therapy in ALS.