The Relationship between CSF Biomarkers and Efficacy of Treatment with NurOwn (MSC-NTF cells)

ALS ONE, Industry Presentations

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October 7, 2022

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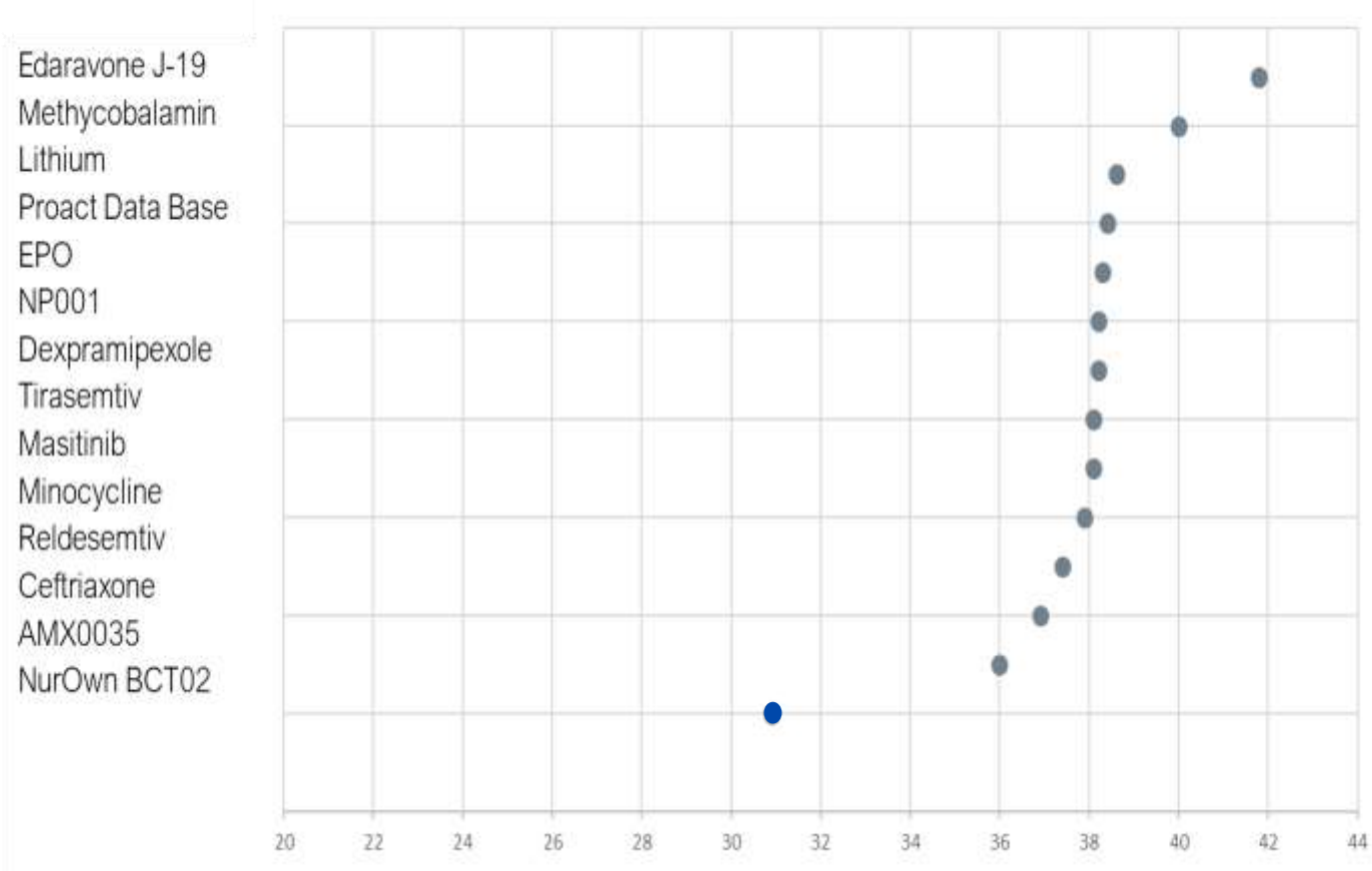
Participants with baseline ALSFRS-R ≤ 25 are impacted by the floor of the scale*

ALSFRS-R Subscale	% participants with value of 0 at baseline
Bulbar	7%
Fine Motor	42%
Gross Motor	37%
Respiratory	1%

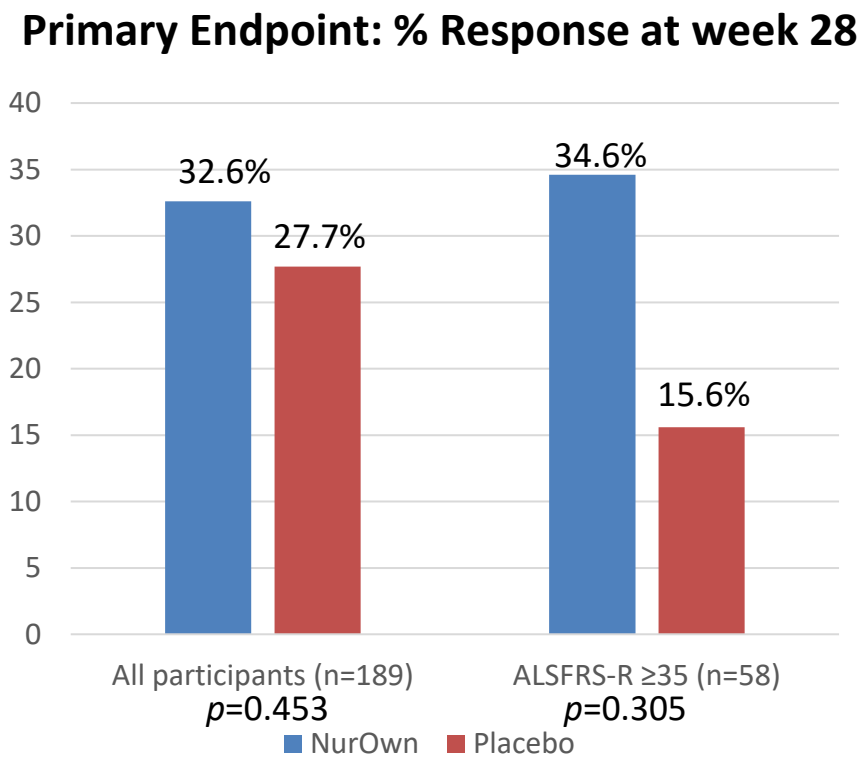
NurOwn Phase 3 featured participants with advanced ALS disease resulting in a floor effect

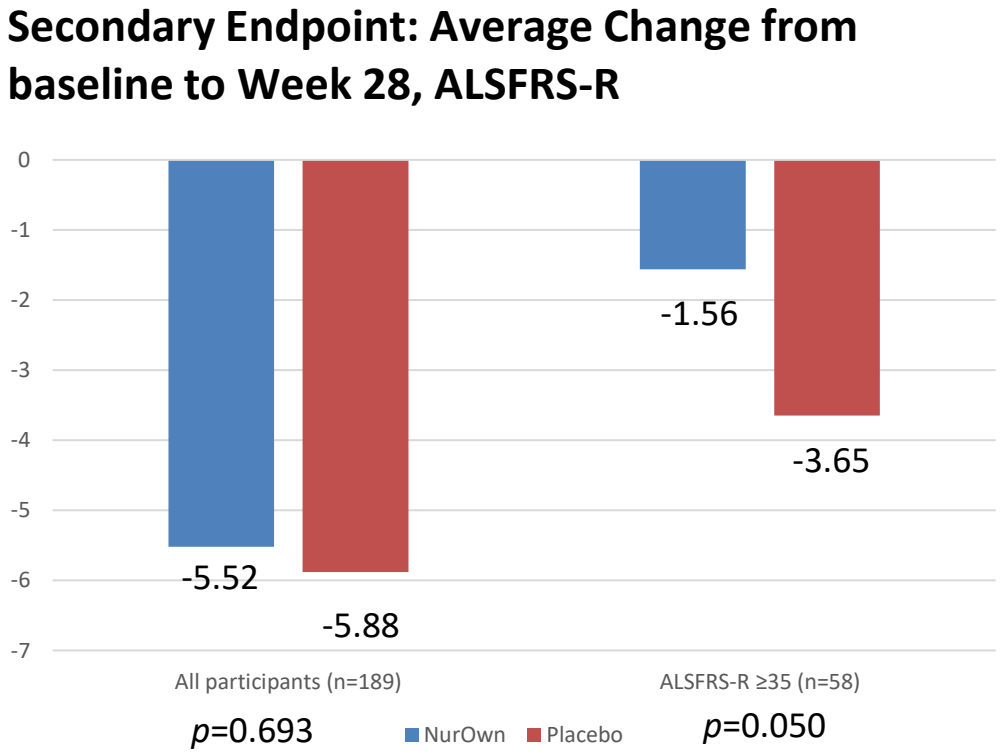


* *Number of participants with baseline ALSFRS-R ≤25 = 44 of 189 (23.3%)*

NurOwn treatment suggests effect in participants with less advanced disease

Evidence from trial aligns with historical data when the ALSFRS-R floor effect is accounted for





Cudkowicz, M, Lindborg S, Goyal N, et al. Musc Nerve, Jan 2022
Supplemental File & Erratum published Aug 2022

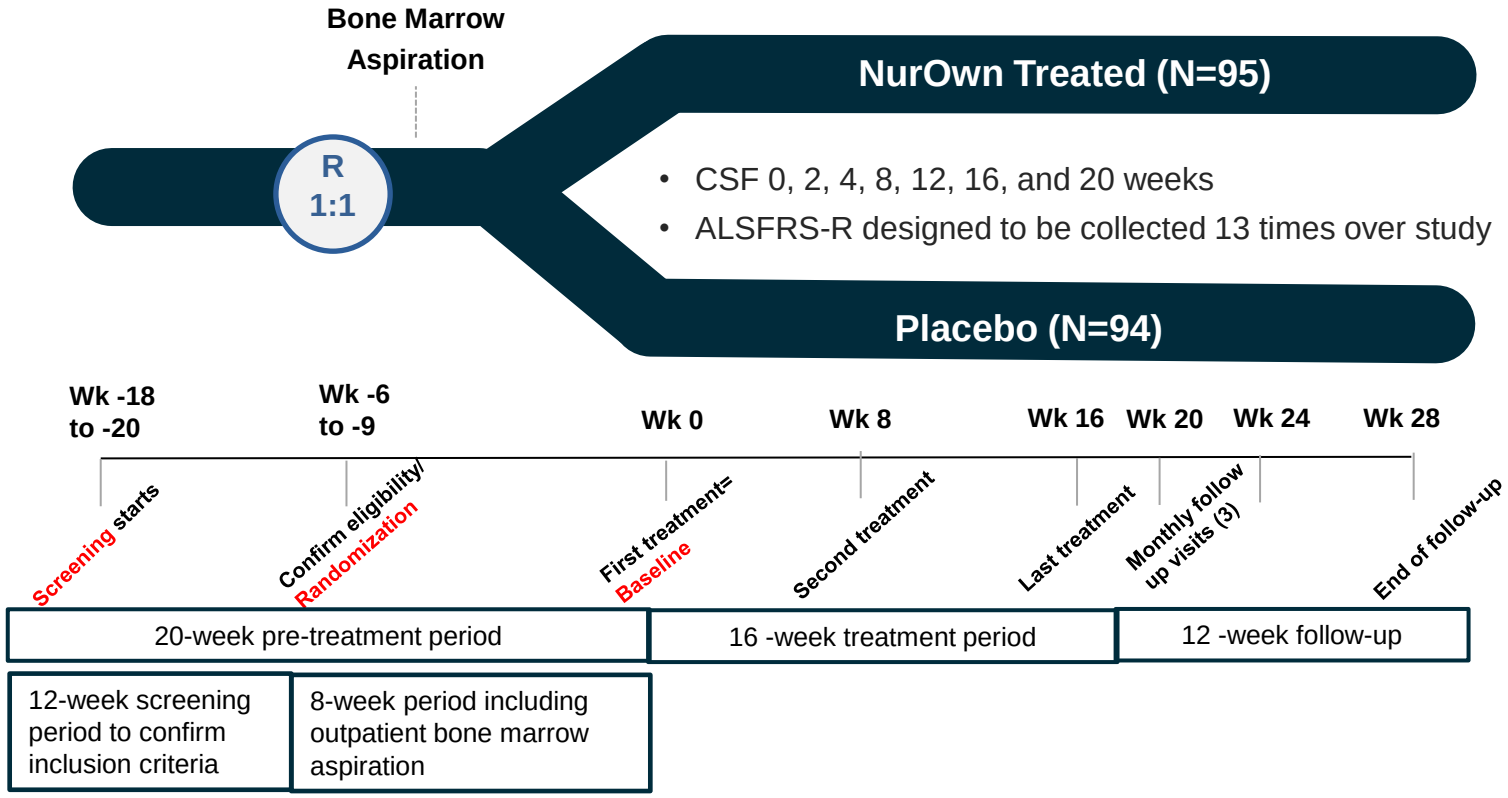
OBJECTIVE

Evaluating the effect of NurOwn and placebo on CSF biomarkers across pathways of neuroinflammation, neurodegeneration and neuroprotection from a Phase 3 RCT

- using statistical methods that focus on univariate and multivariate data
- to understand the role that baseline ALSFRS-R values plays with biomarker trajectories
- to understand the predictive power of biomarkers on clinical outcomes

Phase 3 Trial Study Design: Designed to exclude slow progressors

Double-blind, Placebo-controlled, Randomized Trial



Designed to exclude slow progressors.

Inclusion criteria specified:

- ALSFRS-R ≥ 25 at Screening Visit (Not at Baseline).
- Decline in ALSFRS-R total score of 3 or more points in the 12 weeks before randomization.

Cudkowicz, M, Lindborg S, Goyal N, et al. Musc Nerve, 2022

Biomarker Analysis Methods

- **7 CSF samples over 20 weeks - collected prior to and after each treatment**
  - Validated assays at Thermo Fisher, Quanterix, Olink, Ray Biotech and Norgen
  - Biomarker Statistical Analysis Plan submitted to FDA before trial unblinding
  - Due to skewness in the distribution, biomarker data are log transformed for statistical analysis

Three Main Analyses

1. **Principal Component Analysis -** used biomarker data from the treatment period, and pre-specified biological pathways to identify groups of relevant biomarkers.
2. **Individual Biomarker Trajectories -** plotted using an MMRM model
3. **Prediction of NurOwn Response** - mixed stepwise regression model selected significant biomarkers predicting response to NurOwn.

Clinical Outcomes Considered as Measure of Efficacy

Two different clinical measures based on ALSFRS-R specified in the biomarker SAP:

Clinical outcome based on change between pre-treatment and post-treatment: Clinical Response (primary endpoint)[a]

See Cudkowicz, et al. Musc Nerve, Jan 2022 for results

Clinical outcome based on a post treatment change (rate of decline): piecewise slope[b]

[a] ≥1.25 points/month improvement in post-treatment vs. pre-treatment slope in ALSFRS-R score through week 28

[b] Powerful model which jointly borrows information available from the pre-treatment period to better characterize the slope post-treatment

Pre-specified biomarkers passed validation and were analyzed by pathway

16 Neuroinflammatory/Anti-inflammatory markers:

Inflammation: CHI3L1/YKL-40, Chitotriosidase-1, ICAM-1, IP-10, MCP-1, OPG, S100B, SDF-1a, TREM-2, GFAP
Anti-inflammation: Fetuin-A, IL-37, LAP/TGFβ1, MSR1, miR 146a, miR 146b

8 Neurodegeneration markers:

Caspase-3, DR6, miR 142-5p, NfL, pNfH, Tau, TWEAK, UCH-L1

9 Neuroprotection markers:

BDNF, Clusterin/ApoJ, Galectin-1, G-CSF, GDF-15, HGF, LIF, NMNAT1, VEGF-A

- Only biomarkers that passed validation at the time of analyses were included. Additional samples were sent for analysis for each biomarkers that didn't pass validation and were included in analyses once available.
- Biomarkers that have >20% missing data are not included in the multivariate analyses (PCA, stepwise model selection)*

* Includes 5 biomarkers: Neuroinflammatory (OPG, miR 146a, miR 146b), Neurodegenerative (miR142-5p) and Neuroprotective (LIF)

ALS Phase 3: Biomarker Evaluation

NurOwn decreases Neuroinflammation and Neurodegeneration, and increases Neuroprotection over 20 weeks (BCT-002)

Patterns observed with individual biomarkers confirmed in analysis across biomarkers leveraging Principal Component Analysis

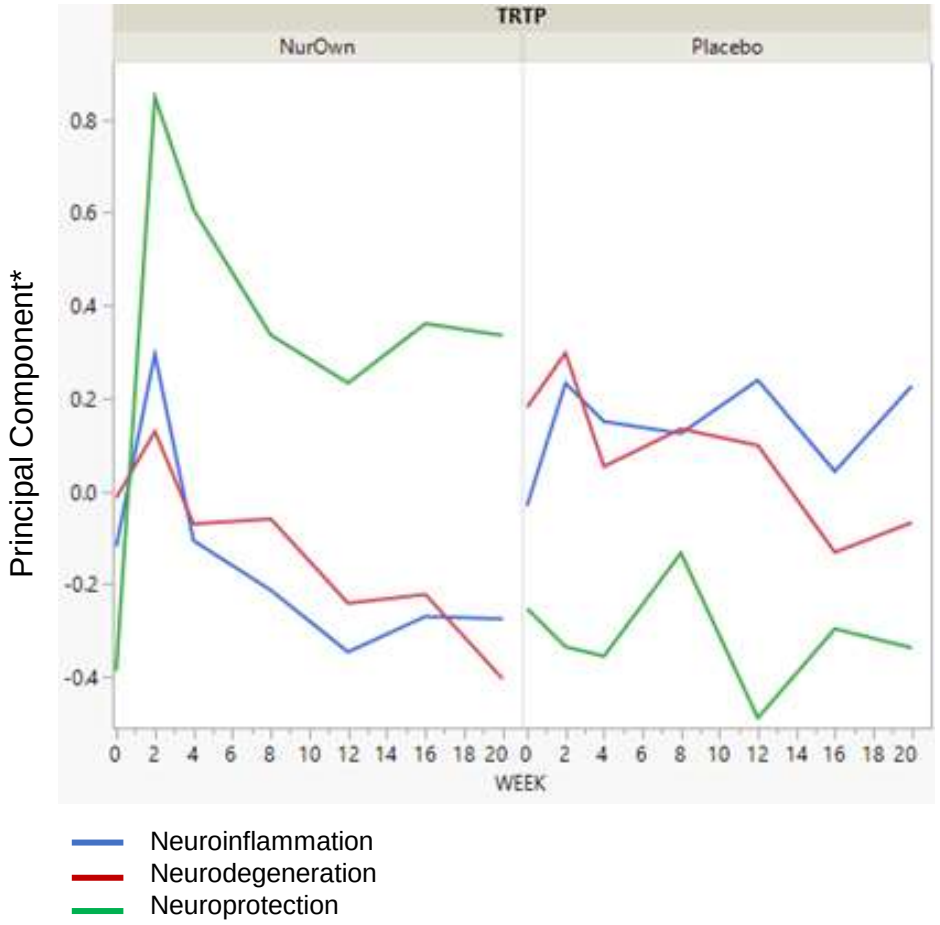
Neuroinflammation decreased with NurOwn
Placebo Neuroinflammation levels remain high

Neurodegeneration decreases with NurOwn
Placebo Neurodegeneration levels decrease across the study, remain elevated relative to NurOwn.

Neuroprotection increased with NurOwn treatment, maintained over 20 weeks
Placebo Neuroprotective levels remain low

Biomarkers driving Principal Component Analysis:

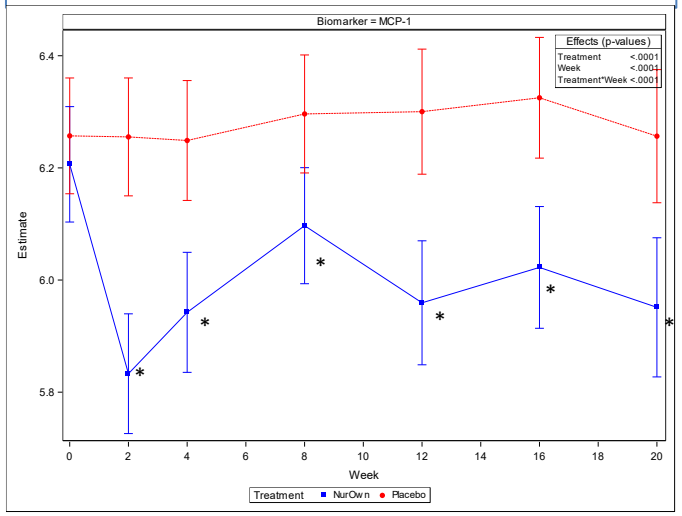
- Neuroinflammation: CHI3L1, Chit-1; IP-10, MCP-1, Trem2
  - 63% of the variation in the data is described by two principal components (36% PC1, 27% PC2)
- Neurodegeneration: NfL, UCH-L1, pNfH
  - 75% of the variation in the data is described by one principal component
- Neuroprotection : Follistatin, VEGF, BDNF; Clusterin
  - 67% of the variation in the data is described by two principal components (45% PC1, 22% PC2)



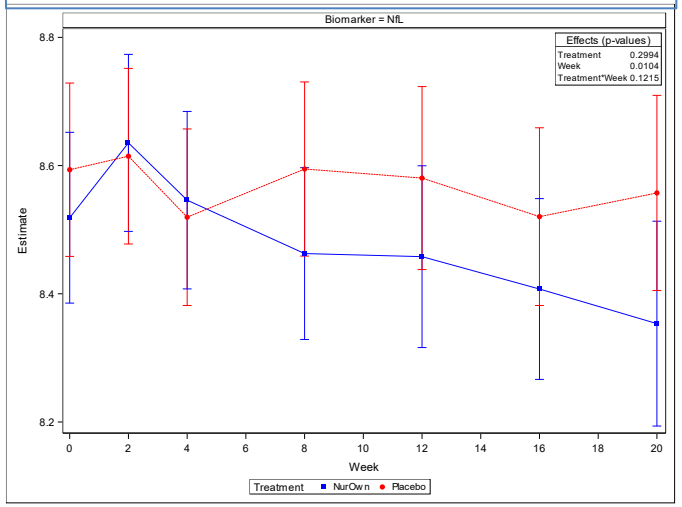
Graph produced the first Principal component from each pathway

NurOwn Improves Biomarkers Across Multiple Pathways Over Time, LS Mean (95%CI)

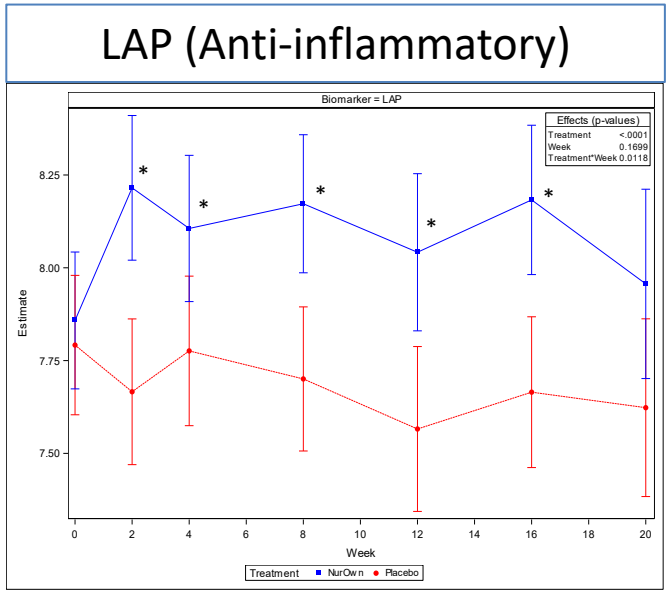
MCP-1 (Pro-Inflammatory)



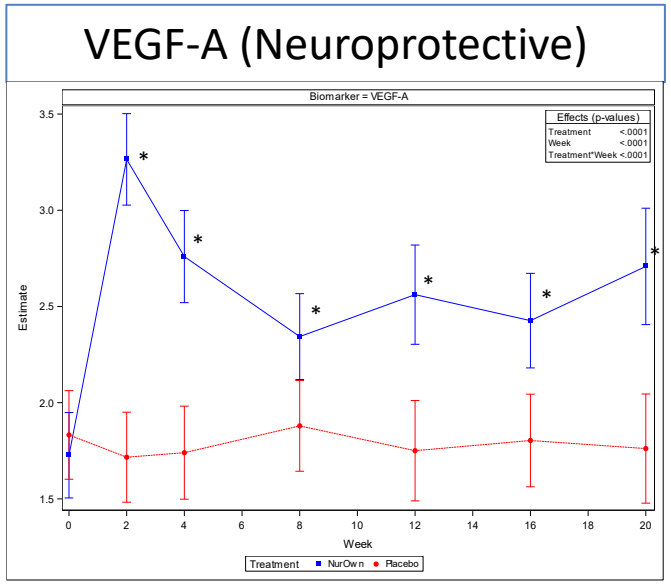
NfL (Neurodegenerative)



LAP (Anti-inflammatory)



VEGF-A (Neuroprotective)



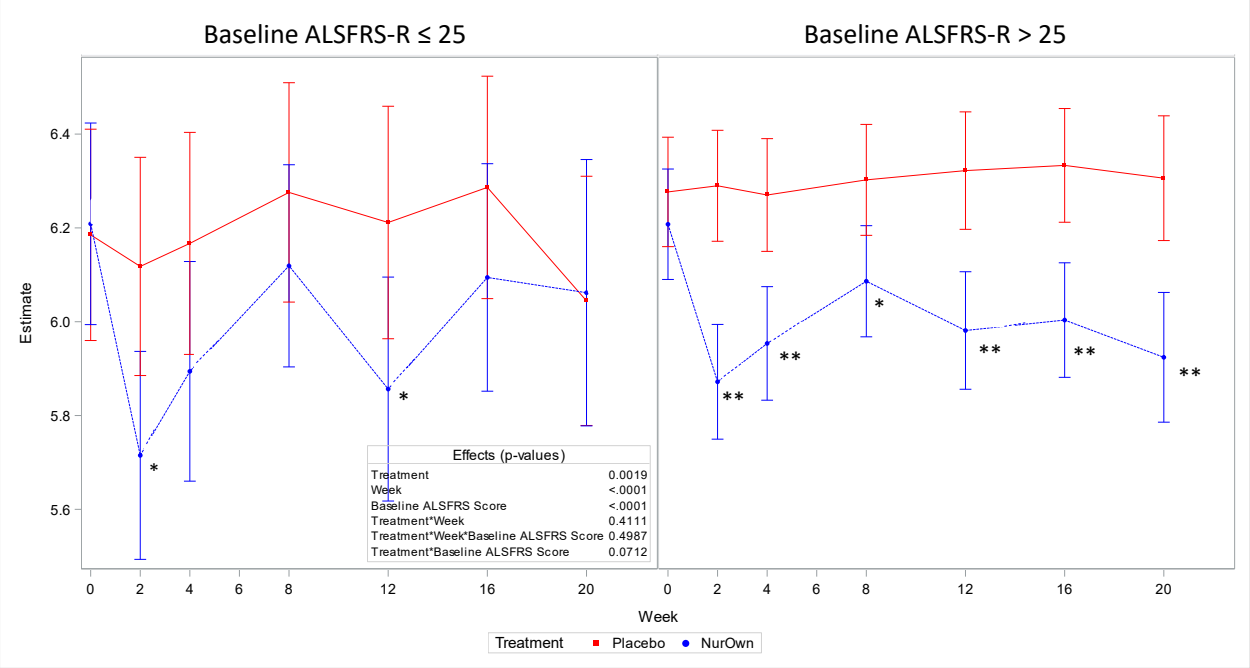
NurOwn

Placebo

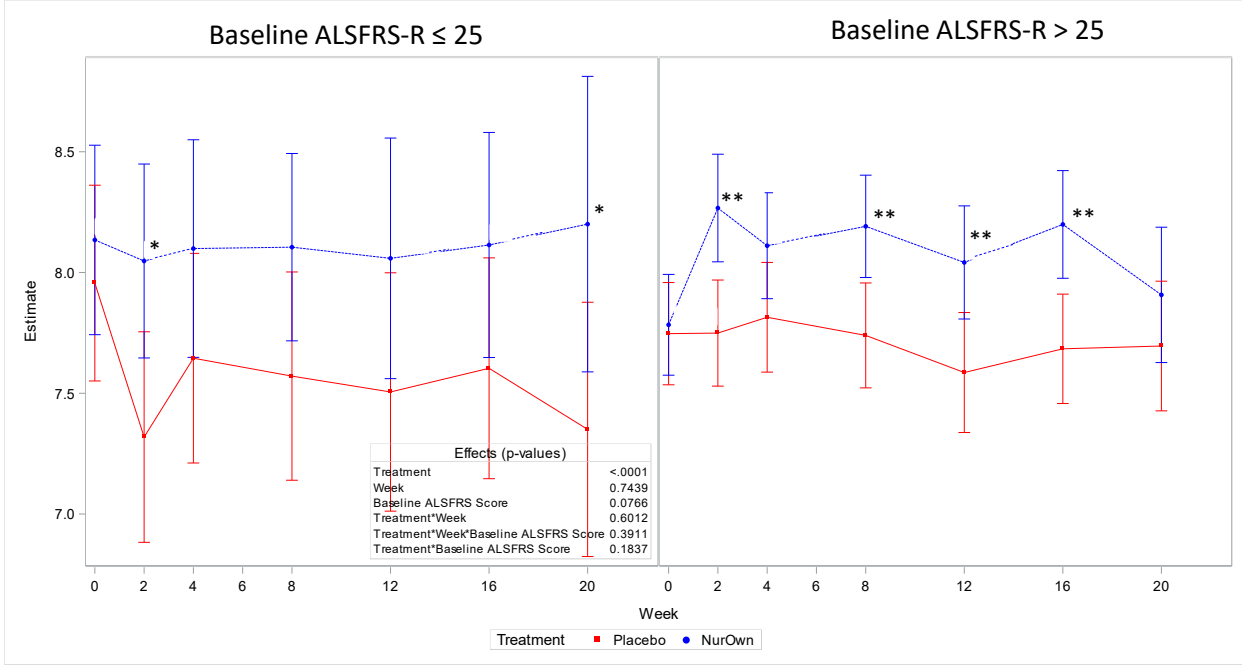
*p < 0.05

Biomarkers are not impacted by the floor effect of the ALSFRS-R

MCP-1



LAP



NurOwn

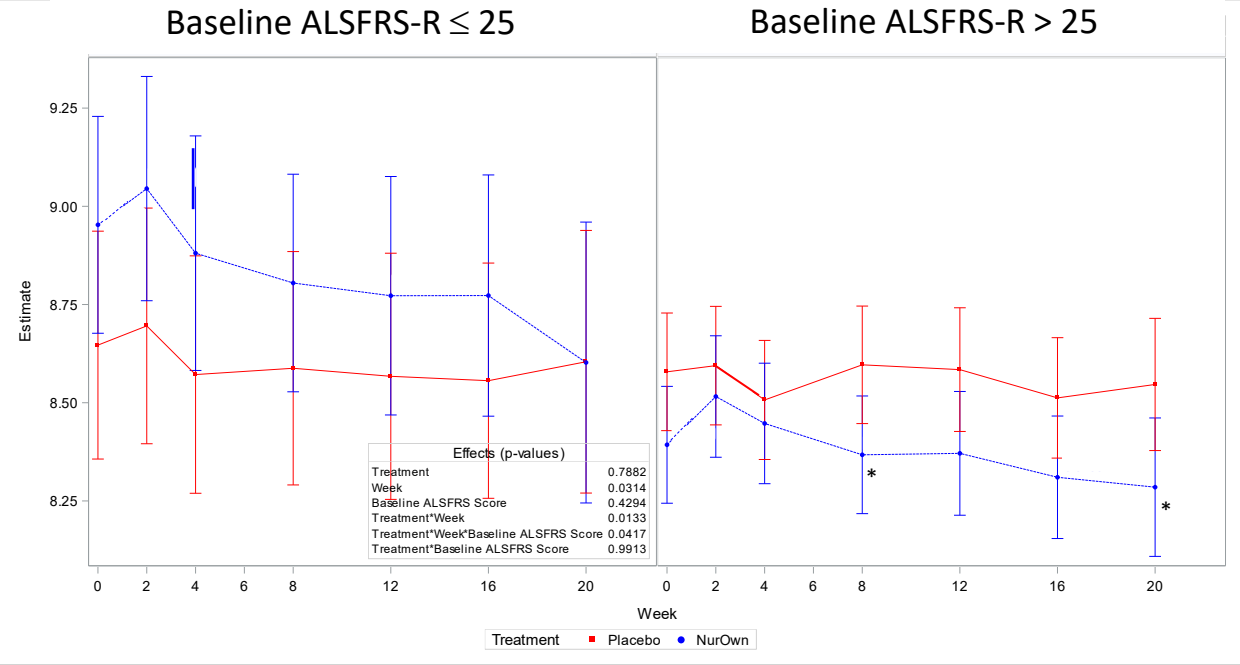
Placebo

* p-value < 0.05
** p-value < 0.01

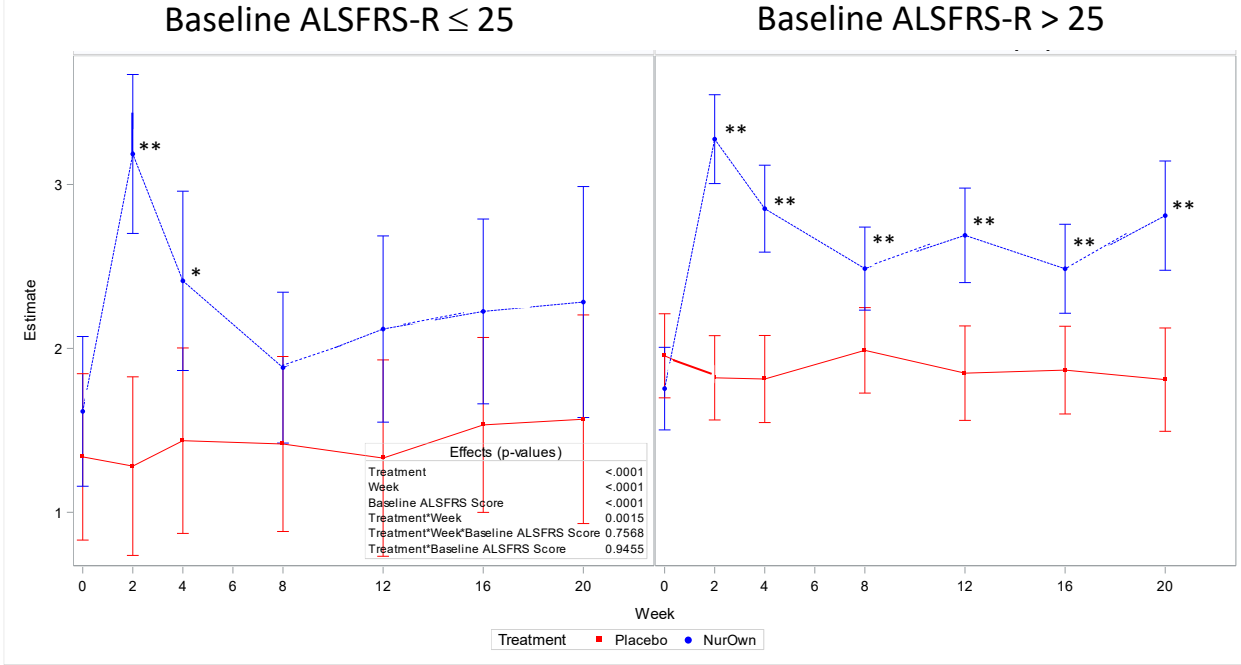
- Pro- and Anti-Inflammation Biomarkers: A large difference is observed between NurOwn and Placebo at the first post-treatment visit, indicating a treatment effect in reducing inflammation quickly following treatment with NurOwn
- Large p-values from the interaction terms with Baseline ALSFRS-R score indicate no significant difference in the ≤25 subgroup and >25 subgroup

Biomarkers are not impacted by the floor effect of the ALSFRS-R

NfL



VEGF-A



NurOwn

Placebo

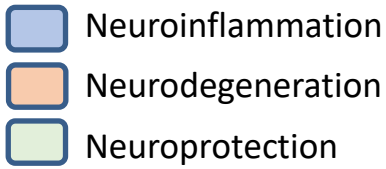
* p-value < 0.05
** p-value < 0.01

- Neurodegeneration: NfL values decreased for NurOwn participant while the placebo group showed a flat trend
- Neuroprotection: VEGF values increase rapidly in both subgroups relative to placebo, remain elevated.

The Relationship Between Biomarkers and Clinical Outcomes reveal biomarkers across 3 pathways are important to predicting the clinical response observed

Clinical outcome based on a post treatment change (rate of decline): piecewise slope

Stepwise Regression Model for NurOwn Group
Baseline LAP
Baseline NfL
mean Change Galectin-1



- Statistical model identifies 3 biomarker that are predictive of clinical outcomes
- NfL selected as a biomarker associated with both clinical outcome measures
- Similar to the biomarkers selected related to the primary endpoint, these biomarkers span all three key pathways
- good model fit

Summary

Changes in biomarkers following NurOwn treatment are statistically linked to clinical outcomes from the trial

- Treatment with NurOwn elevates markers of neuroprotection, and lowers markers of neuroinflammation and neurodegeneration over time compared to placebo
- The same biomarker responses, including patterns by treatment, are observed across biomarker pathways in participants with less advanced ALS, and advanced ALS
- Statistical modeling identifies biomarkers that have the potential to predict a clinical response with NurOwn, with two different clinical endpoints
  - Markers of neuroinflammation, neurodegeneration and neuroprotection were all selected in the final model.
  - The Relationship Between Biomarkers and Clinical Outcomes reveal biomarkers across 3 pathways are important to predicting the clinical response observed.
- Novel therapies that simultaneously target multiple pathways may offer great potential

Manufacturing Sites

City of Hope Center for Biomedicine & Genetics

Dana-Farber Harvard Cancer Center

Cell Manipulation Core

Treatment Centers

University of Massachusetts Medical School

Massachusetts General Hospital

Mayo Clinic

UC Irvine

Cedars-Sinai

Sutter Health
Sutter Pacific Medical Foundation

Funding

CIRM
California's Stem Cell Agency

ALS Association

I AM ALS