CSF biomarkers identified as predictive of clinical outcomes in ALS participants following NurOwn treatment in a phase 3 clinical trial: Reductions in NfL associated with less ALSFRS-R decline

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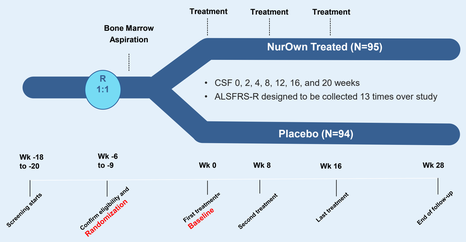
Background

Brainstorm Cell Therapeutics completed a randomized, placebo-controlled Phase 3 trial in amyotrophic lateral sclerosis (ALS) participants (n=189) evaluating efficacy and safety of 3 intrathecal doses of NurOwn (Cudkowicz et al. 2022). CSF biomarker samples were collected longitudinally in all trial participants.



7 CSF samples were collected by lumbar puncture for all trial participants

- Sample collected prior to each treatment.
- The last sample was collected at 20 weeks

Objectives

- Verify baseline NfL as a prognostic factor of clinical outcome
- Explore which biomarkers were significantly impacted by NurOwn
- Investigate the relationship of clinical outcomes and CSF biomarkers
- Explore the relationship between change in NfL and change in ALSFRS-R

Methods

Biomarker Statistical Analysis Plan signed off, submitted to FDA before unblinding the trial.

Two populations involved in the analysis of Clinical data, ALSFRS-R

- mITT population (189 participants): 95 NurOwn and 94 Placebo
- Population representing participants with no item level floor effect at baseline (106 participants): 49 NurOwn and 57 Placebo

All analyses associating NfL and ALSFRS-R data used the No item level Floor effect population

Correlation analyses performed between baseline NfL & change from baseline in ALSFRS-R (week 28), using placebo mITT data

MMRM analyses for change from baseline in biomarker values were performed for mITT population with baseline disease covariates pre-specified in primary study SAP for efficacy analyses and baseline NfL

A pre-specified forward stepwise regression model used to select biomarkers predictive of the primary endpoint (all biomarkers).

- Biomarkers with p-values required to be 0.25 to enter and p<0.05 to be retained
- Regression model adjusted for biomarkers with significant impact on clinical outcomes fit with baseline covariates per pre-specified efficacy model for mITT population

Causal inference used to isolate NurOwn effect on NfL and ALSFRS-R

- Population representing participants with no item level floor effect at baseline; participants with observed values at the last time of CSF sampling and ALSFRS-R measured (n=23)
- Build disease natural progression model using placebo data for NfL & ALSFRS-R
- Obtain the predicted NfL and ALSFRS-R values for NurOwn participants, based on the natural disease progression model, representing predicted values had participants not been treated with NurOwn
- Use observed NfL and ALSFRS-R values from NurOwn treated participants, conduct correlation analyses of change in ALSFRS-R at week 28 (last measurement) and change in NfL at week 20 (last CSF sampling time) adjusting for the model predicted placebo effect.

Pre-specified biomarkers passed validation and were analyzed

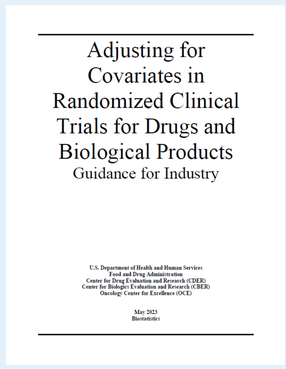
- 16 Neuroinflammatory/Anti-inflammatory markers:
  - Inflammation:
    Pro-Inflammatory: CHI3L1/YKL-40, Chitotriosidase-1, ICAM-1, IP-10, MCP-1, OPG, S100B, SDF-1a, TREM-2, GFAP
    Anti-inflammatory: Fetuin-A, IL-37, LAP/TGFβ1, MSR1, miR 146a-5p, miR 146b-5p
- 8 Neurodegeneration markers:
  Caspase-3, 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

CSF immediately centrifuged at 1,750g for 10 min., stored at -80°C.
Samples analyzed by Thermo Fisher, Quanterix, Olink, and Norgen using validated assays

What is a floor effect?

- Per FDA: "[a] floor effect can occur at the item level or at the scale score level. The floor effect occurs when the scale of measurement is not able to capture progression at the bottom of the scale."[1]
  - An **item level floor effect** is observed when large concentration of participants endorses the lowest response category within an item.
  - A **scale score level floor effect** is observed when a large concentration of participants' scores fall at or near the lower limit of the scale score of the instrument.

ALS Heterogeneity, Adjusting for Covariates


Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products
Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
Oncology Center for Excellence (OCE)

May 2023
Biostatistics

- Guidance focused on prognostic baseline covariates. Sponsors should **prospectively specify** covariate-adjusted analysis.
- Covariate adjustment is acceptable even if baseline covariates are strongly associated with each other.
- Given FDA guidance, biomarker MMRM analyses adjusted for covariates will use from the **Phase 3 Primary, pre-specified efficacy MMRM model**:
  - time since symptom onset to first treatment, use of riluzole, site of onset, baseline slope, baseline ALSFRS-R total score and
  - baseline NfL
- Covariate adjustment of ALS biomarker data is an industry standard.

Results

NurOwn had significant impact on biomarkers across pathway's

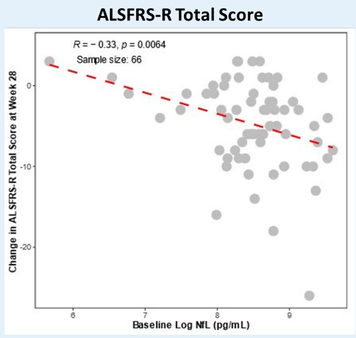
Biomarkers with a significant treatment effect or significant treatment over time effect based on the biomarker change from baseline by pathway*

Primary Biomarker Pathway	Number of Biomarkers	Biomarkers with overall significant treatment effect or treatment by time effect n (%)‡	
		All participants	Baseline >25
Neuroinflammation	16	10 (62.5%)	10 (62.5%)
Neurodegeneration	8	4 (50%)	2 (25.0%)
Neuroprotection	9	8 (88.9%)	8 (88.9%)

‡<0.05, *dependent variable, change in biomarker

Neurofilament levels are prognostic of ALS clinical decline

Participants with greater ALSFRS-R total score decline had higher NfL values at baseline



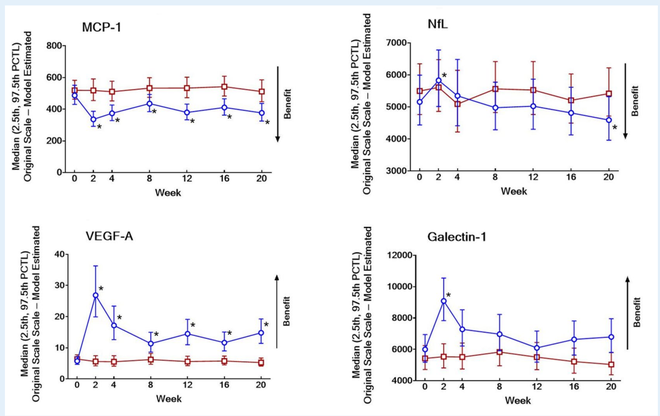
NurOwn Phase 3 trial, placebo data

Longitudinal trajectory of biomarkers generally showed:

- Stable values for placebo treated participants
- Large changes in biomarkers following NurOwn treatment, some quickly (VEGF, 369% increase), others over time (NfL, 11% decrease)

Analyses of biomarkers adjusting for baseline disease characteristics were generally very similar to those not adjusting

- NfL is an exception, placebo values were highly variable
- Adjusting for covariates, which were significant in the model, resulted in treatment difference (p<0.05)



Stepwise variable regression model selected 3 biomarkers, spanning pathways, significantly contributed to the prediction of clinical outcomes with NurOwn treatment

- **Baseline NfL**, *Neurodegenerative biomarker*
- **Baseline LAP/TGFβ1**, *Anti-Inflammatory biomarker*
- **Change in Galectin-1** at week 20, *Neuroprotective biomarker*

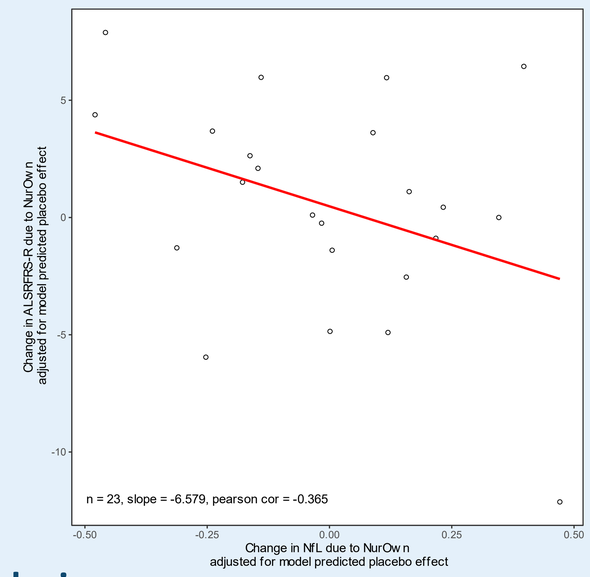
Adjusting model with baseline disease characteristics reveals: **Baseline disease characteristics and biomarker terms have significant impact on clinical outcome**

Type 3 Test for Overall Effect

Source	p-value#
Baseline ALSFRS-R	<.0001
Baseline Slope	<.0001
Time since symptom onset to first treatment	<.0001
Use of Riluzole [No]	<.0001
Site of onset [Limb]	0.0006
Baseline LAP (TGF-β1)	0.0468
Baseline NfL	<.0001
Change Galectin-1	0.4720

#p-value is from a linear regression with response variable post-treatment piecewise slope, and all terms above as the covariates.

Causal Inference: Reductions in NfL is associated with less functional loss following NurOwn treatment, r=-0.365



Conclusions

- Treatment with NurOwn significantly elevates markers of neuroprotection, and lowers markers of neuroinflammation and neurodegeneration over time compared to placebo in all trial participants
- Statistical modeling identifies 3 biomarkers that predicts clinical outcome with NurOwn observed in the BCT-002, including 2 baseline biomarkers (NfL, LAP/TGFβ1). NfL has also been found to be prognostic of ALS disease progression in other trials[3,4].
- Baseline disease covariates, pre-specified for analysis of all efficacy data, combined with biomarkers provide significant impact on the analysis of clinical data, with both types of information proving to be important to adjust for when analyzing clinical data
- Causal Inference model using natural disease progression model shows NurOwn treatment effect. Reduction in NfL during the trial following NurOwn treatment is associated with less disease progression, r=-0.365
- Overall conclusions and relevance to ALS drug development:
  - Analysis provides further the evidence of the importance of NfL as a prognostic and predictive biomarker for ALS.
  - Treatment driven NfL reductions are associated with better outcomes
  - The addition of baseline disease covariates to NfL, and inflammatory biomarker LAP/TGFβ1 more fully explains participants clinical response observed in the trial.

References

1. Cudkowicz, M, Lindborg SR, Goyal N, et al. Musc Nerve, Jan 2022, Supplemental File & Erratum Musc Nerv Aug 2022
2. FDA, Patient-Focused Drug Development Guidance Public Workshop, Methods to Identify What is Important to Patients & Select, Develop, or Modify Fit-for-Purpose Clinical Outcomes Assessments at 9 (Oct. 2018)
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