Supplementary Online Content

Cudkowicz ME, Lindborg SR, Goyal NA, Miller RG, Burford MJ, Berry JD, et al. A Randomized Placebo-Controlled Phase 3 Study of Mesenchymal stem cells induced to secrete high levels of neurotrophic factors in Amyotrophic Lateral Sclerosis

This supplementary material has been provided by the authors to give readers additional information about their work.

EMETHODS

MSC-NTF Cell Preparation

Prior to treatment, 80 to 100 mL of bone marrow was aspirated bilaterally from multiple punctures of the iliac crest of the pelvic bone (~5 mL from each puncture) from every participant as per standard institutional procedures. Mesenchymal stem cells from participants randomized to the MSC-NTF treatment group were isolated, propagated, and cryopreserved. In advance of each treatment, autologous MSC were thawed and induced to become MSC-NTF cells (NurOwn). Manufacturing was conducted at the Cell Manipulation Core Facility at Dana-Farber Cancer Institute, Boston, MA and the Center for Biomedicine & Genetics at City of Hope, Duarte, CA manufacturing sites. Cells were differentiated using proprietary cell culture methods. Participants in the placebo group received Dulbecco's Minimal Essential Medium which did not contain any cells.

MSC-NTF was provided in a ready-to-use participant-personalized treatment package consisting of one 5 mL syringe containing freshly harvested autologous cultured MSC-NTF (approximately 125×10^6 cells) or placebo. Harvested bone marrow was transported to, and MSC expansion and MSC-NTF production was completed under cGMP at, one of two national cell manufacturing facilities.

Statistical Methodology

For the primary endpoint, participants who discontinued were assumed to continue progressing at the rate of disease progression from baseline through last available assessment. A logistic regression model adjusted for covariates of baseline ALSFRS-R total, duration from onset-of-symptoms to first treatment, site of onset, riluzole use at baseline, and ALSFRS-R slope pre-treatment was used to test the hypothesis of an odds ratio of one between treatments.

The responder analysis defined by ≥100% ALSFRS-R slope improvement leveraged the same methodology and statistical model as the primary endpoint. Change from baseline in ALSFRS-R and SVC were analyzed using a mixed effect repeated measures (MMRM) model. The null hypothesis of no difference between treatment groups was tested using a model with treatment group, visit, and the primary model covariates as main effects and the treatment-by-visit interaction. An unstructured covariance-structure was used to model within-participant errors. The CAFS score was used as the dependent variable in an analysis of covariance model with treatment as a fixed effect with covariates from the primary model.

Analyses similar to the ALSFRS-R total score were also conducted for the sub-scale scores for the Bulbar, Gross Motor, Fine Motor, Gross and Fine motor combines and Respiratory domains. Hypothesis testing was performed using mixed model repeated measures (MMRM) with the change in ALSFRS-R score from baseline as the dependent variable and treatment group, visit, baseline ALSFRS-R score, duration from onset of symptoms to first treatment, site of onset (Limb vs Limb & Bulbar), Riluzole use and ALSFRS-R slope pre-treatment as main effect and the interaction between treatment group and visit.

The protocol was amended in March 2020 to only perform SVC when permitted, following resolution of COVID-19 per institutional guidance. COVID-19 pandemic hospital restrictions resulted in rates of missing participant SVC data of 57.9% MSC-NTF and 61.7% placebo patients at week 28, therefore subgroup analysis were not conducted on SVC data.

Handling of missing ALSFRS-R data due to deaths or discontinuations were pre-specified in the SAP. Sensitivity analyses were performed using the method of multiple imputations under assumptions of missing at random and missing not at random. In addition to the pre-specified multiple imputation methods, a Joint Longitudinal-Survival Mixed Effects Model (Guo and Carlin[1]), was applied to the analysis of longitudinal ALSFRS-R score data following the database lock to address observed patterns of deaths and missing data.

AEs were coded using MedDRA and summarized by system organ class and preferred term and in addition by severity and relationship to study treatment. An adverse event is considered a TEAE if the start date/time of the adverse event was on or after the date/time of initiation of cell treatment or if the severity worsened after the initiation of treatment. Changes in suicidal behavior were summarized in shift tables. In addition to tabulating the number of AEs leading to death due to disease progression and due to any cause, the Kaplan-Meier Estimate of the event-free probability is reported with the accompanying 95% confidence intervals (CI) and p-value, both derived from a Cox proportional hazards model adjusted for covariates from the primary efficacy model.

Biomarker Sample Collection

Cerebrospinal fluid (CSF) samples were collected a total of 7 times by lumbar puncture prior to each of three administrations of cells, two and four weeks following the first treatment, and four weeks following subsequent treatments for a total of seven serial collections. The last CSF biomarker sample was collected at 20 weeks following the first treatment. Levels of vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein-1 (MCP-1) in the CSF were detected with a highly sensitive, customized ProcartaPlex multiplex immunoassay (Thermo

Fisher Scientific, Waltham, MA). The assay was thoroughly validated by matrix evaluation including spike recovery, parallelism, and sample stability. The concentrations of neurofilament light chains (NfL) were assessed with the use of the Simoa NF-light assay (Quanterix, Billerica, MA).

REFERENCES

1. Guo X, Carlin BP. Separate and Joint Modeling of Longitudinal and Event Time Data Using Standard Computer Packages. *Am Stat.* 2004;58(1):16-24. doi: 10.1198/0003130042854

SUPPLEMENTARY TABLES

Table S1 Safety Results, Common (>10% in either group) TEAE

	MSC-NTF (N=95) n (%)	Placebo (N=94) n (%)
Procedural pain	50 (52.6)	34 (36.2)
Headache	45 (47.4)	32 (34.0)
Back pain	42 (44.2)	24 (25.5)
Fall	29 (30.5)	34 (36.2)
Procedural headache	31 (32.6)	30 (31.9)
Post-lumbar puncture syndrome	22 (23.2)	29 (30.9)
Nausea	16 (16.8)	18 (19.1)
Post-procedural complication	16 (16.8)	7 (7.4)
Pain in extremity	16 (16.8)	11 (11.7)
Musculoskeletal pain	15 (15.8)	8 (8.5)
Muscular weakness	11 (11.6)	12 (12.8)

Abbreviation: TEAE = treatment-emergent adverse event.

Table S2 Primary Endpoint, Responder Analysis Accounting for Missing Data

	Primary Endpoint, All participants			
ALSFRS-R Baseline Score	**% response MSC-NTF**	**% response Placebo**	**Delta**	**p-value**
SAP Pre-specified Model	32.6	27.7	4.9	0.453
SAP Pre-specified Model, MAR Data	33.7	26.6	7.1	0.251
SAP Pre-specified Model, MNAR Data	34.7	26.6	8.1	0.189

Abbreviations: ALSFRS-R = ALS Functional Rating Scale Revised; MAR = Missing at Random; MNAR = Missing Not at Random; SAP = Statistical Analysis Plan.

Note: Hypothesis testing performed using logistic regression adjusted for baseline ALSFRS-R Total Score, duration from onset of symptoms to first treatment, site of onset (Limb vs Limb & Bulbar), Riluzole use at baseline and ALSFRS-R slope pre-treatment were used to test the hypothesis of an odds ratio of 1 between the two treatment groups.

Table S3 presents results from a pre-specified methods to assess the impact of missing data on the analysis of the secondary endpoint change from baseline to Week 28 in the ALSFRS-R.

Table S3 ALSFRS-R Change from Baseline Accounting for Missing Data

	ALSFRS-R Change from Baseline to 28 weeks, All participants			
	LS Mean MSC-TF	**LS Mean Placebo**	**delta**	**p-value**
SAP Pre-specified Model	-5.52	-5.88	0.37	0.693
SAP Pre-specified Model, MAR Data	-5.37	-5.78	0.41	0.628
SAP Pre-specified Model, MNAR Data	-5.23	-5.71	0.48	0.560

Abbreviations: ALSFRS-R = ALS Functional Rating Scale Revised, LS = Least squares; MAR = Missing at Random; MNAR = Missing Not at Random; SAP = Statistical Analysis Plan.

Note: Hypothesis testing was performed using mixed effects model repeated measures (MMRM) with the change in ALSFRS-R score from baseline as the dependent variable and treatment group, visit, baseline ALSFRS-R score, duration from onset of symptoms to first treatment, site of onset (Limb vs Limb & Bulbar), Riluzole use and ALSFRS-R slope pre-treatment as main effect and the interaction between treatment group and visit.

Table S4 presents results from a post-hoc model that explores the joint impact of survival and missing data on the treatment effect, for the endpoint change from baseline to Week 28 in the ALSFRS-R.

Table S4 ALSFRS-R Change from Baseline, Supplemental Model to Account for Deaths and Missing Data

	ALSFRS-R Change from Baseline to 28 weeks, All participants			
	Average Change MSC-NTF	**Average Change Placebo**	**delta**	**Posterior probability[a]**
SAP Pre-specified Model	-5.52	-5.88	0.37	0.693
Joint Model, Post-hoc analysis	-5.75	-6.29	0.53	0.672

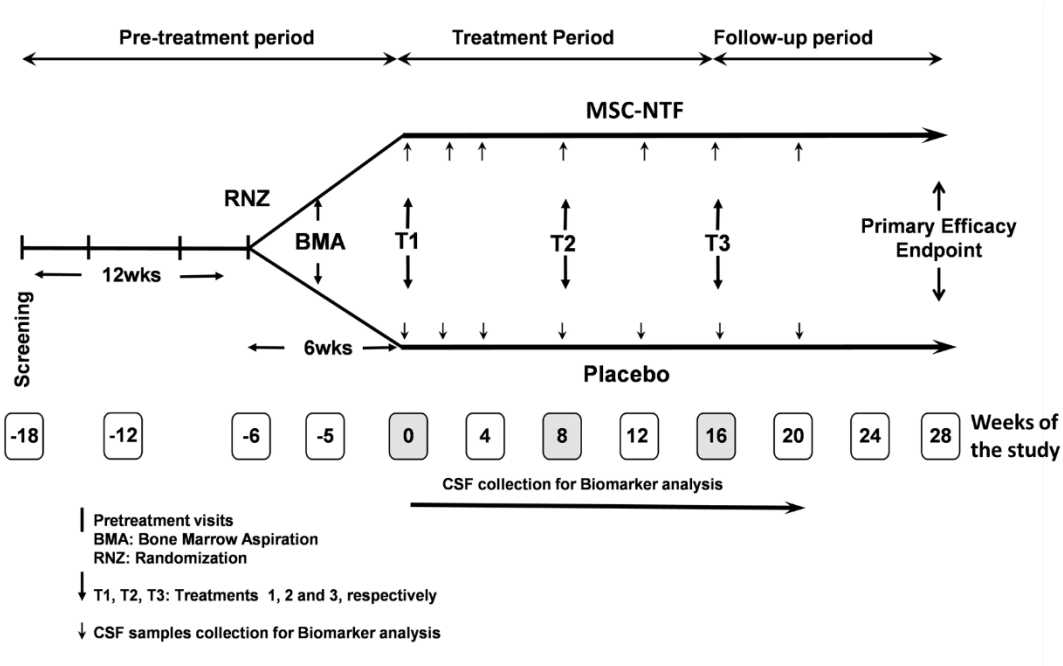
Abbreviations: ALSFRS-R = ALS Functional Rating Scale Revised; LS = Least squares.

[a] Represents the posterior probability that MSC-NTF > placebo. For a relative comparison to a p-value one should look at 1-Posterior Probability.

Across all pre-specified and post-hoc analyses, the treatment difference between MSC-NTF and placebo as captured by the average change in ALSFRS-R from baseline to 28 weeks is consistent. Participants treated with MSC-NTF consistently have more function preserved than participants treated with placebo.

SUPPLEMENTARY FIGURES

Figure S1 Study Schematic

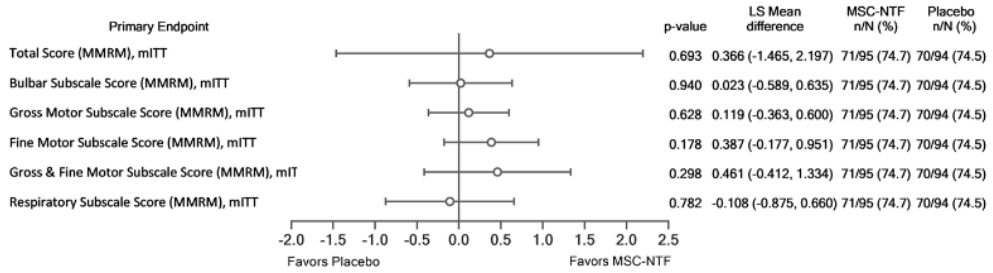


Abbreviations: BMA = Bone marrow aspiration; CSF = Cerebrospinal fluid; RNZ = Randomization.

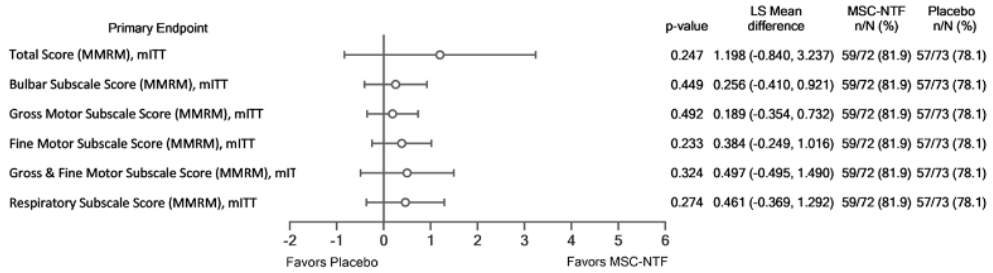
ALSFRS-R Subscale scores are presented in Figure S2 for the full mITT population (Figure S2A), along with analyses with participants with baseline >25 (Figure S2B) and ≥31 (Figure S2C). Results across these analyses are relatively consistent with the greatest difference noted on the Respiratory Subscale. In Figure S2A, which includes all participants including those with the most advanced ALS disease at baseline (<25), the respiratory subscale score favors placebo in contrast to Figure S2B and S2C which examined participants that were above the observed mean at baseline where MSC-NTF was visibly better than placebo on the respiratory subscale (nominal p-value p=.018, Figure S2C).

Figure S2 ALSFRS-R Total and Subscale Scores, Change from Baseline to Week 28, mITT Population

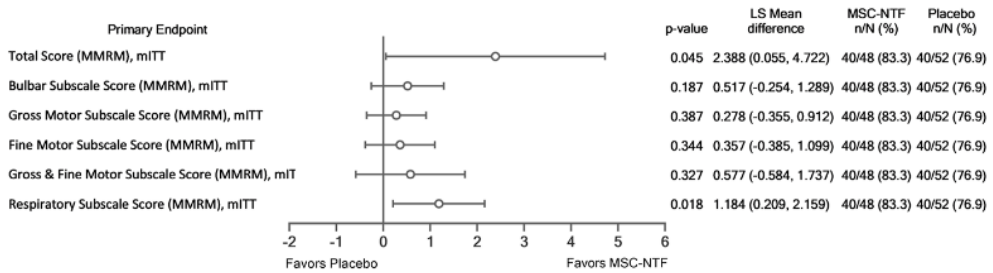
A. ALSFRS-R Total and Subscale Scores, Change from Baseline to Week 28, mITT Population



B. ALSFRS-R Total and Subscale Scores, Change from Baseline to Week 28, Participants with Baseline ALSFRS-R Score >25



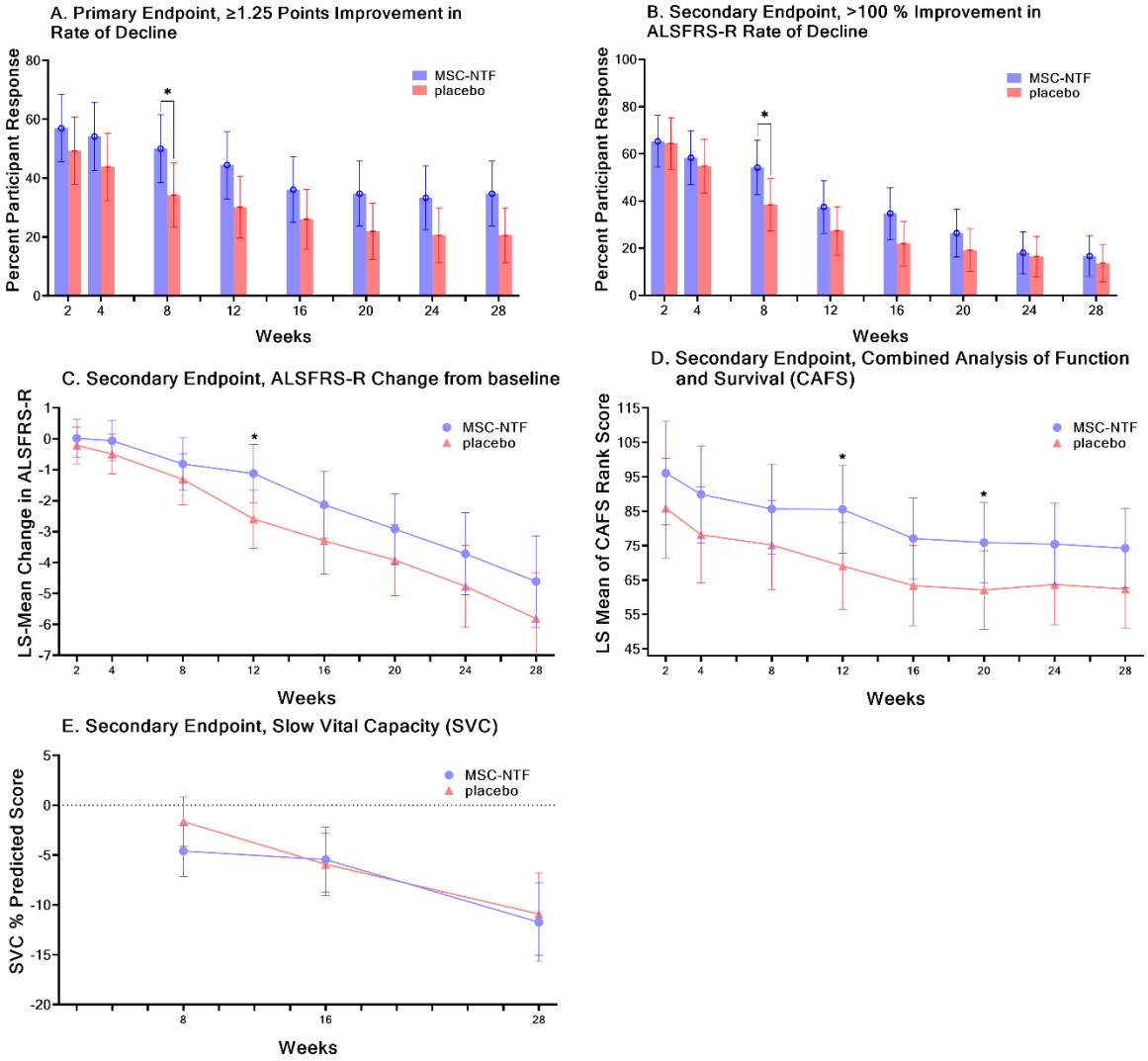
C. ALSFRS-R Total and Subscale Scores, Change from Baseline to Week 28, Participants with Baseline ALSFRS-R Score ≥ 31



Abbreviations: ALSFRS-R = ALS Functional Rating Scale Revised; LS = Least squares; mITT = Modified Intention-to-Treat; MMRM = Mixed Effects Model Repeated Measures.

***Note*:** Hypothesis testing was performed using mixed model repeated measures (MMRM) with the change in ALSFRS-R score from baseline as the dependent variable and treatment group, visit, baseline ALSFRS-R score, duration from onset of symptoms to first treatment, site of onset (Limb vs Limb & Bulbar), Riluzole use and ALSFRS-R slope pre-treatment as main effect and the interaction between treatment group and visit.

Figure S3 Efficacy in Participants with ALSFRS-R Baseline Score >25



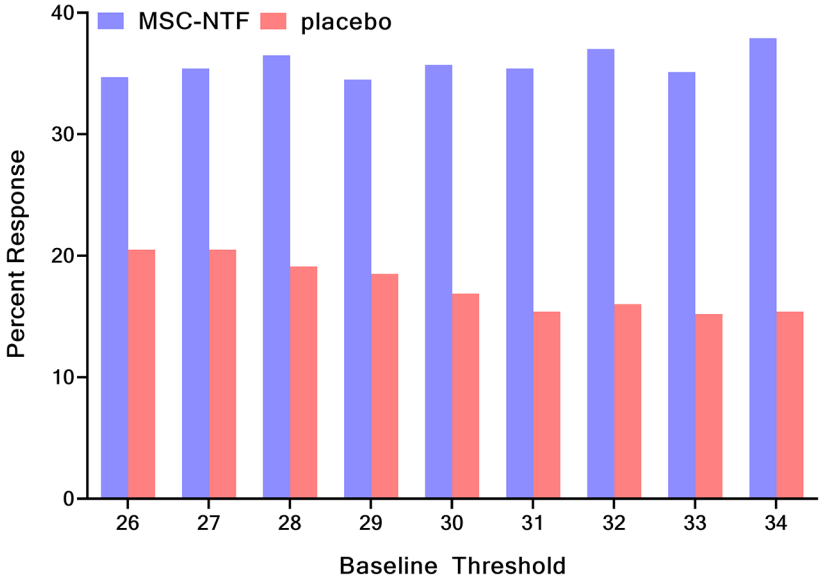
Abbreviations: ALSFRS-R = Revised ALS Functional Rating Scale; CAFS = Combined Analysis of Function and Survival; SVC = Slow vital capacity.

***Note*:** Efficacy measured in participants with ALSFRS-R score >25 at baseline over the course of the trial. (A) percentage of participants with at least 1.25 points/month improvement (95% CI) in ALSFRS-R score; (B) percentage of participants with 100% improvement (95% CI) in ALSFRS-R slope; (C) mean change from baseline in ALSFRS-R score (95% CI); (D) mean combined analysis of function and survival (CAFS) score (95% CI); and (E) mean change from baseline in slow vital capacity (SVC) % predicted score (95% CI).

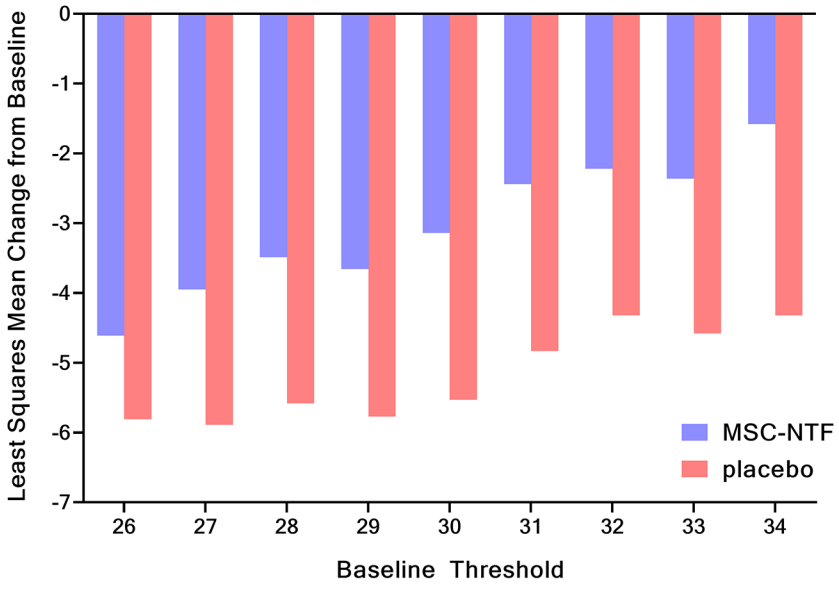
95% CI for response calculated based on normal approximation to the binomial distribution; 95% CI for least square means change in ALSFRS-R score, CAFS, SVC % predicted score. *$p < 0.05$.

Figure S4 Efficacy Analyses Across ALSFRS-R Baseline Thresholds

A. Primary Endpoint, Analysis of Responders Across ALSFRS-R Baseline Thresholds



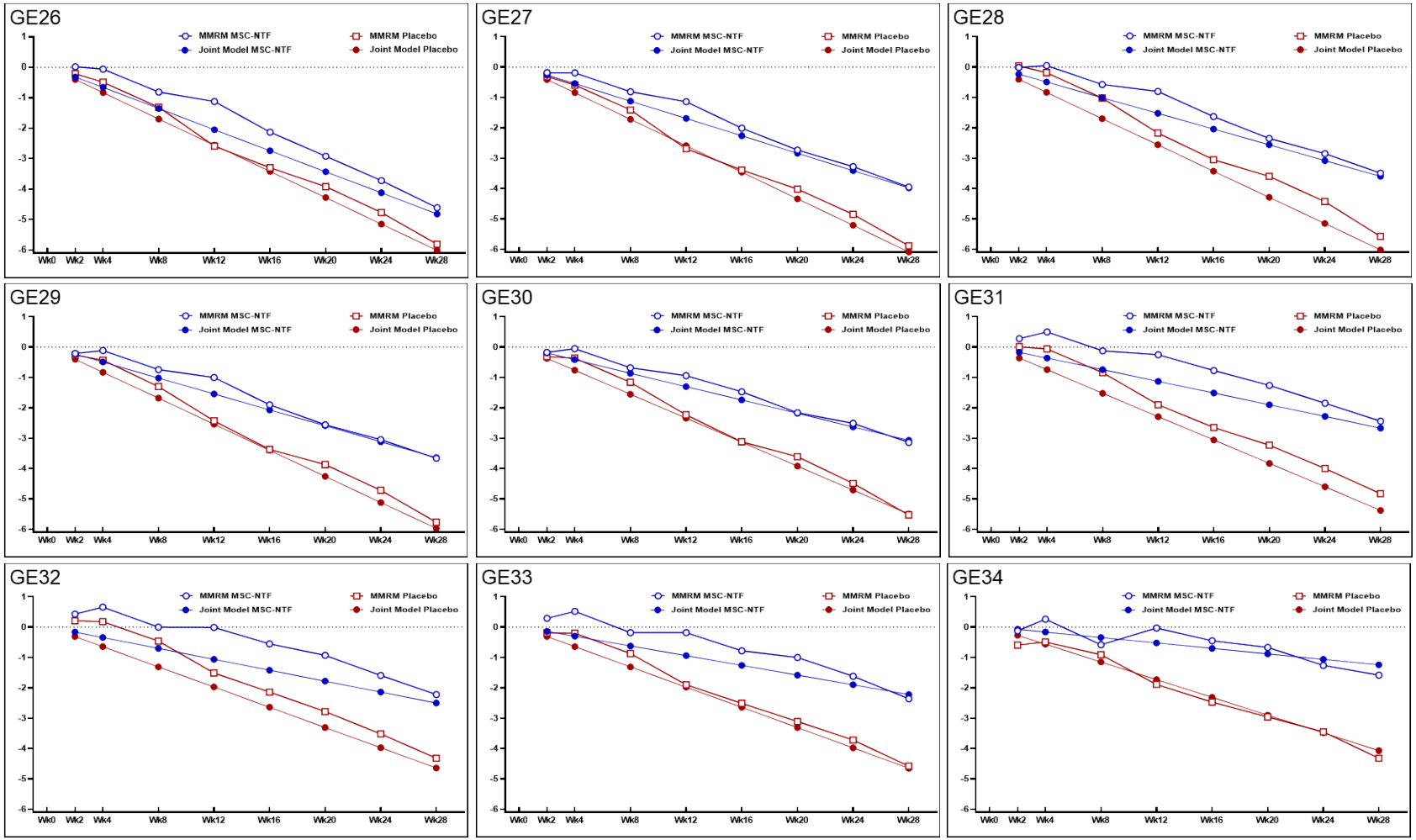
B. ALSFRS-R LS Mean Change from Baseline Across ALSFRS-R Baseline thresholds



Abbreviations: ALSFRS-R = Revised ALS Functional Rating Scale; LS = Least squares; SE = Standard error.

Notes: (A) Primary Endpoint: percentage of participants with at least 1.25 points/month improvement across ALSFRS-R Baseline Thresholds; (B) Secondary Endpoint: ALSFRS-R MMRM Change from baseline to week 28 across ALSFRS-R Baseline Thresholds

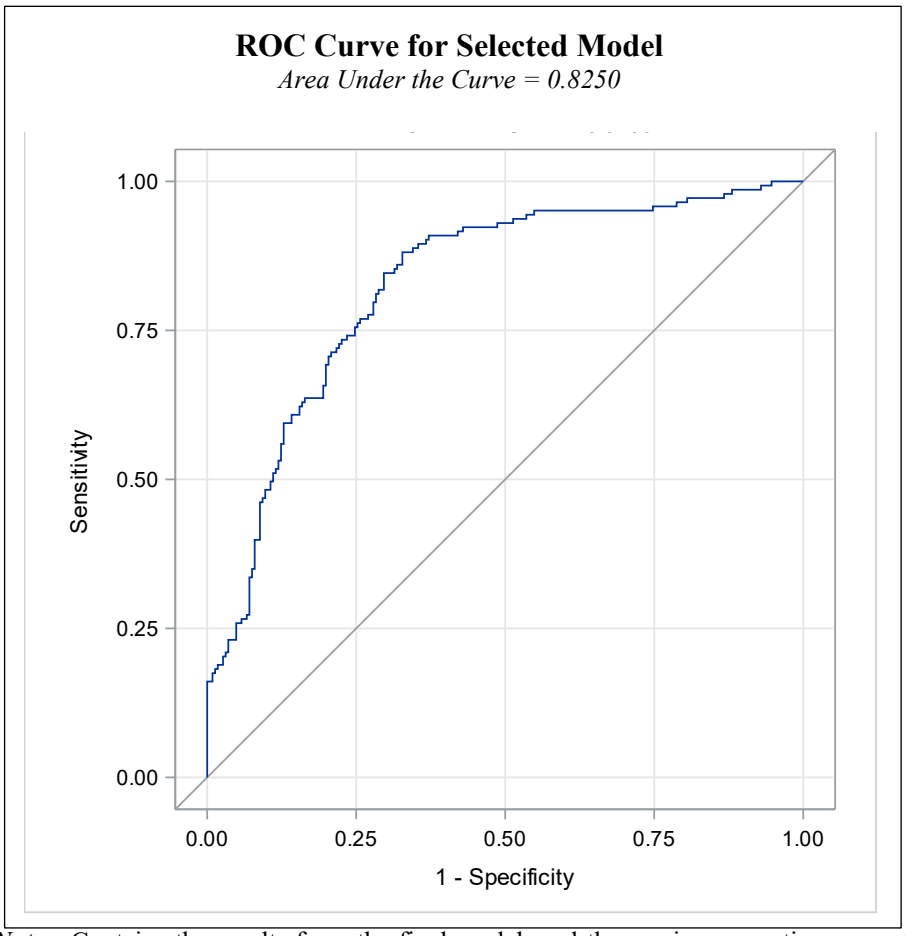
Figure S5 ALSFRS-R Change From Baseline by MMRM and Joint Models at Various Baseline ALSFRS-R Thresholds



Abbreviations: ALSFRS-R = ALS Functional Rating Scale Revised; GE = Greater or Equal to; MMRM = Mixed Effects Model Repeated Measures; PW = Piecewise.

Note: Each panel graphs the results for participants with baseline ALSFRS-R score greater than or equal to the particular number, i.e., GE36=data for participants with baseline score ≥36. In all panels, MSC-NTF is labeled in blue, placebo in red. MMRM: pre-specified SAP analysis, graphed with an open circle for MSC-NTF and open square for placebo. The joint model is graphed using a filled circle for MSC-NTF, open circle for placebo.

Figure S6 ROC Curve for the final stepwise forward regression model with the primary endpoint and the dependent variable



***Note*:** Contains the results from the final model, and the receiver operating curve (ROC) allowing a view into the model fit. The model terms are summarized in Table 5.

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