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Short report

Effect of sodium phenylbutyrate and taurursodiol on plasma concentrations of neuroinflammatory biomarkers in amyotrophic lateral sclerosis: results from the CENTAUR trial

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ABSTRACT

Background An oral sodium phenylbutyrate and taurursodiol combination (PB and TURSO) significantly reduced functional decline in people living with amyotrophic lateral sclerosis (ALS) in the CENTAUR trial. Biomarkers linking clinical therapeutic effect with biological changes are of high interest in ALS. We performed analyses of neuroinflammatory biomarkers associated with ALS in the literature, including YKL-40 (also known as chitinase-3-like protein 1), chitinase 1 (CHIT1) and C reactive protein (CRP), in plasma samples collected in CENTAUR.

Methods Log10-transformed plasma biomarker measurements were analysed using a linear mixed-effects model. Correlation between paired biomarker concentrations and ALS Functional Rating Scale-Revised (ALSFRS-R) total scores was assessed via Pearson correlation coefficients.

Results By week 24, geometric least squares mean YKL-40 plasma concentration decreased by approximately 20% (p=0.008) and CRP by 30% (p=0.048) in the PB and TURSO versus placebo group. YKL-40 (r of –0.21; p<0.0001) and CRP (r of –0.19; p=0.0002) concentration correlated with ALSFRS-R total score. CHIT1 levels were not significantly different between groups.

Conclusions YKL-40 and CRP plasma levels were significantly reduced in participants with ALS receiving PB and TURSO in CENTAUR and correlated with disease progression. These findings suggest YKL-40 and CRP could be treatment-sensitive biomarkers in ALS, pending further confirmatory studies.

Trial registration number https://clinicaltrials.gov/study/NCT03127514

INTRODUCTION

Safety and efficacy of an oral, fixed-dose sodium phenylbutyrate and taurursodiol combination (PB and TURSO) in amyotrophic lateral sclerosis (ALS) were evaluated in the multicentre phase 2 CENTAUR trial encompassing randomised placebo-controlled and open-label extension phases.[1–3] The randomised phase primary end point was met, with PB and TURSO significantly slowing functional decline as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) over 24 weeks compared with placebo.[2] Changes in plasma phosphorylated neurofilament heavy chain and neurofilament light chain (NfL) concentrations were evaluated as a secondary outcome and showed no between-group differences.[2 4] Plasma samples were prospectively collected from CENTAUR participants for potential future biomarker analyses.[4]

Neuroinflammatory biomarkers may add value beyond other biomarkers for assessing disease progression and therapeutic response in ALS. Chitinases, a class of hydrolases expressed by activated microglia and astrocytes, have emerged as potential prognostic biomarkers in ALS given prominence of glial activation in the neuroinflammatory response.[5] ALS is also characterised by a peripheral immune response that includes chitinase expression by activated myeloid cells, largely in response to cytokines.[5 6] PB and TURSO was previously shown to significantly reduce concentration of the chitinase YKL-40 (also known as chitinase-3-like protein 1) in cerebrospinal fluid (CSF) compared with placebo in adults with mild cognitive impairment and mild to moderate Alzheimer's disease (AD) dementia in a 24-week phase 2, multicentre, randomised trial (PEGASUS).[7] While most YKL-40 data in ALS are also in CSF, limited data in blood (mainly serum)[5 8] prompted our interest in evaluating plasma YKL-40 in an interventional trial setting.

Here, we describe results of exploratory post hoc analyses of neuroinflammatory biomarkers, including YKL-40, chitinase 1 (CHIT1) and the systemic inflammatory biomarker C reactive protein (CRP), in plasma samples from participants with ALS from CENTAUR. Results of these analyses were presented at the 2022 Annual Northeast Amyotrophic Lateral Sclerosis Consortium Meeting.[4]

METHODS

Detailed methods for the CENTAUR randomised phase (NCT03127514) are reported elsewhere.[1 2] CENTAUR enrolled adults with definite ALS (revised El Escorial criteria[9]) ≤18 months from symptom onset and slow vital capacity >60% predicted. Participants were randomised 2:1 to receive PB and TURSO (3 g PB/1 g TURSO) or matching placebo by mouth or feeding tube for 24

Table 1 Plasma biomarker concentrations*

Biomarker	Baseline†		Week 12‡		Week 24‡		P value§
	PB and TURSO (n=81)	Placebo (n=45)	PB and TURSO (n=81)	Placebo (n=45)	PB and TURSO (n=81)	Placebo (n=45)	
YKL-40	32.7 (1.98)	31.5 (1.69)	31.6 (1.05)	35.2 (1.06)	31.4 (1.06)	38.8 (1.08)	0.008
CHIT1	35.4 (3.85)¶	40.5 (2.23)	33.5 (1.12)	35.8 (1.12)	31.5 (1.13)	35.9 (1.14)	0.094
CRP	1686.7 (3.10)	2029.1 (4.58)	1817.9 (1.11)	2185.6 (1.12)	1833.6 (1.14)	2650.2 (1.18)	0.048

*All concentrations expressed in ng/mL.
†Geometric mean (SD) concentration.
‡Geometric least-squares mean (SE) concentration (log10-transformed[8]) from a random-slope, shared-baseline, linear mixed-effects model adjusted for age and ALSFRS-R slope interacting with time.
§For comparison between PB and TURSO group versus placebo group, calculated using shared-baseline, mixed-effects model.
¶Excludes one participant who had a non-positive value for CHIT1 concentration at baseline, as non-positive values cannot be log-transformed.
ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CHIT1, chitinase 1; CRP, C reactive protein; PB and TURSO, sodium phenylbutyrate and taurursodiol; YKL-40, chitinase-3-like protein 1.

weeks. Continuation of stable-dose riluzole and/or edaravone was permitted.

Blood samples were drawn at baseline and every 6 weeks thereafter through week 24 (or early discontinuation) to obtain plasma and stored in the NEALS biorepository.[10] YKL-40, CHIT1 and CRP immunoassays were conducted in a blinded manner using 0.5-mL plasma samples. YKL-40 and CHIT1 assays were developed by the Bowser Laboratory at the Barrow Neurological Institute, while the CRP assay was from Meso Scale Discovery (MSD). All assays were performed within the Clinical Laboratory Improvement Amendments–certified laboratory at *n*Vector (previously Iron Horse Diagnostics) on the MSD platform and demonstrated a <5% coefficient of variation (CV) for intra-assay and interassay quality controls and standards; the average CV across all samples was <4%.

As for all prespecified efficacy outcomes in CENTAUR, the participant population for these analyses was the modified intention-to-treat (mITT) population (ie, all participants who received ≥1 dose of study medication and had ≥1 postbaseline ALSFRS-R assessment). Specifically, participants with ≥1 post-baseline plasma sample collected over the 24-week randomised phase were included. First, log10-transformed plasma biomarker measurements[8] were analysed using a random-slope, shared-baseline, linear mixed-effects model adjusted for age and ALSFRS-R slope (ie, rate of ALSFRS-R total score change from symptom onset) interacting with time. Geometric least squares (LS) mean biomarker concentrations were calculated for each treatment group; given paucity of data at other time points (weeks 6 and 18), results for only weeks 12 and 24 are presented. Second, change-from-baseline analyses that did not assume a shared baseline were performed for all biomarkers. Geometric mean ratios from a mixed model for repeated measures without linear trend assumption were also calculated for weeks 12 and 24. Finally, Pearson correlation coefficients were calculated to assess the correlation between plasma concentrations of YKL-40, CHIT1 and CRP paired with ALSFRS-R total score and between concentrations of each biomarker paired with ALSFRS-R slope.

RESULTS

Of 135 participants in the mITT population (PB and TURSO, n=87; placebo, n=48), 126 (PB and TURSO, n=81; placebo, n=45) had plasma samples available for these analyses. Summary baseline biomarker concentrations are shown in table 1. Additional baseline characteristics for this population are summarised in online supplemental table 1 and were generally similar to those in the overall mITT population.[2]

Geometric LS mean YKL-40 plasma concentration was 10% lower (ratio, 0.90; 95% CI 0.83 to 0.97) at week 12 and approximately 20% lower (ratio 0.81; 95% CI 0.69 to 0.94) at week 24 in the PB and TURSO versus placebo group (p=0.008; table 1). Furthermore, YKL-40 concentration correlated with ALSFRS-R total score (r of –0.21; p<0.0001) and ALSFRS-R slope (r of 0.11; p=0.034). Change-from-baseline analyses also showed significant reduction in plasma YKL-40 concentration in the PB and TURSO versus placebo group (p=0.002; figure 1).

Geometric LS mean CHIT1 plasma levels were not significantly different between treatment groups (table 1). CHIT1 concentration did not correlate with ALSFRS-R total score (r of –0.05; p=0.326) or slope (r of 0.10; p=0.061). Change-from-baseline analyses showed no significant differences between groups (p=0.247; online supplemental figure 1). Geometric LS mean CRP concentration was 17% lower (ratio 0.83; 95% CI 0.69 to 1.00) at week 12 and approximately 30% lower (ratio 0.69; 95% CI 0.48 to 1.00) at week 24 in the PB and TURSO versus placebo group (p=0.048; table 1). CRP concentration correlated with ALSFRS-R total score (r of –0.19; p=0.0002) and slope (r of 0.21; p<0.0001). Change-from-baseline analyses showed significant reduction in plasma CRP concentration in the PB and TURSO versus placebo group (p=0.018; online supplemental figure 2).

DISCUSSION

In our analyses of neuroinflammatory biomarkers in participants with ALS from CENTAUR, PB and TURSO significantly reduced plasma concentrations of YKL-40 and CRP but not CHIT1 relative to placebo over the 24-week randomised phase, with reductions observed as early as week 12. YKL-40 and CRP concentration further correlated significantly with disease progression as measured by the ALSFRS-R, the most widely used functional end point in ALS trials[11] and the primary efficacy outcome in CENTAUR.

CSF YKL-40 concentration has been shown to be elevated in ALS and correlates with clinical measures of disease severity, including the ALSFRS-R, progression rate and survival.[5 12] However, blood-based biomarkers offer the advantages of being more cost- and time-efficient and less invasive than CSF-based biomarkers.[13] While few studies have explored longitudinal blood levels of YKL-40 in ALS,[8] blood YKL-40 levels have been shown to be a treatment-responsive biomarker for multiple sclerosis.[14] Notably, in line with the current analysis, PB and TURSO significantly reduced CSF YKL-40 levels compared with placebo

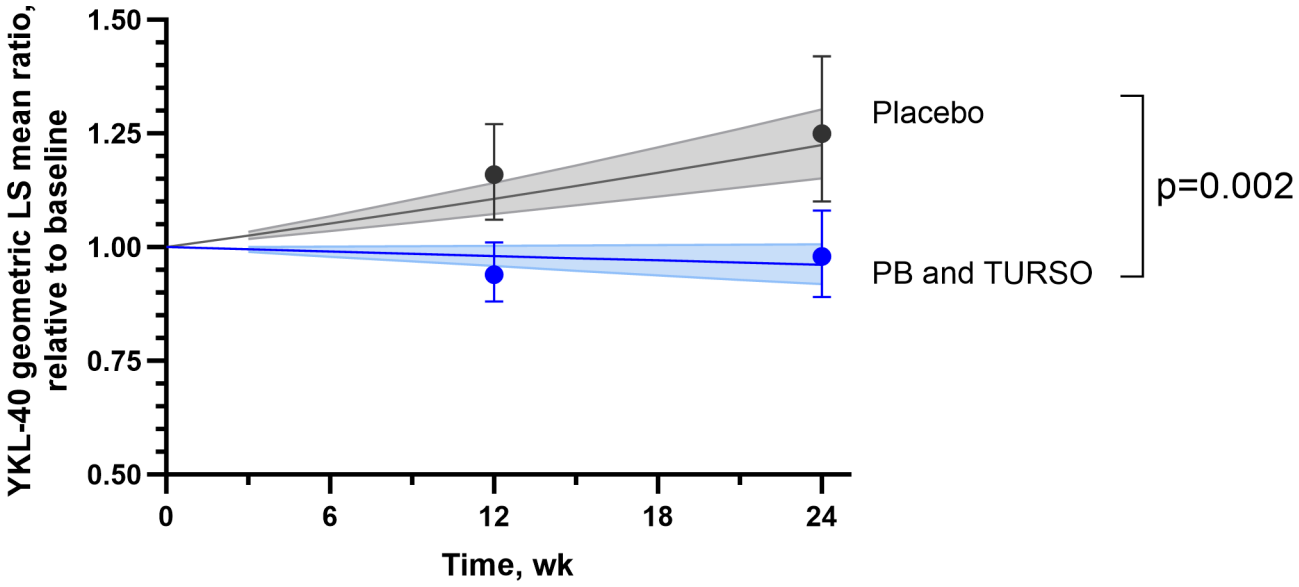

Figure 1 Change in YKL-40 plasma concentration relative to baseline over 24 weeks. Log10-transformed YKL-40 concentration measurements[8] were analysed using a random-slope, shared-baseline, linear mixed-effects model adjusted for age and ALSFRS-R slope interacting with time (coloured lines, with shading reflecting SEs). Geometric mean ratios (solid dots) and 95% CIs (bars) from an MMRM model without linear trend assumption are shown at weeks 12 and 24. ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; MMRM, mixed model for repeated measures; PB and TURSO, sodium phenylbutyrate and taurursodiol; YKL-40, chitinase-3-like protein 1.

in the PEGASUS trial in AD, a condition in which plasma and CSF YKL-40 levels have been shown to modestly correlate.[15]

CRP is a sensitive marker of systemic inflammation that is widely used given its availability and reliability. A systematic review found that CRP was significantly elevated and correlated with disease progression and survival in the majority of studies evaluating this biomarker in ALS, primarily in blood.[6] CRP level was also noted to significantly decrease after riluzole administration in an unblinded surveillance study of people with ALS presenting to a single clinic setting, suggesting CRP may be a potential biomarker for assessing treatment responsiveness in this population.[16] However, CRP may have limitations as a neuroinflammatory biomarker in ALS, including potential for confounding by comorbid inflammatory conditions[6] and the high variability observed for this biomarker.[17] Like YKL-40, CHIT1 has been shown to be elevated in ALS[5 12] but is primarily expressed by circulating cells of myeloid lineage.[5 18] In this analysis, CHIT1 levels did not differ significantly between treatment groups.

In contrast to YKL-40 and CRP in the current analyses, plasma neurofilament levels were not significantly changed with PB and TURSO treatment in CENTAUR.[2] PB and TURSO similarly had no significant effect on CSF NfL levels over a similar duration in the PEGASUS trial in AD.[7] While neurofilaments are a promising biomarker for ALS, the mechanistic linkage to ALS remains under study and other biomarkers may capture different aspects of ALS pathophysiology; though some studies have shown concomitant effects on clinical outcomes and neurofilament levels, others showed clinical effects without changes in neurofilament levels.[2 19 20] Ultimately, translation of the differential effects of PB and TURSO on plasma biomarkers in CENTAUR into the exact mechanism of PB and TURSO in ALS requires further study.

CONCLUSIONS

We demonstrated significantly reduced YKL-40 and CRP plasma levels in participants receiving PB and TURSO in CENTAUR, with lower YKL-40 and CRP concentrations correlating with higher ALSFRS-R total scores and lower rate of ALSFRS-R total score decline through 24 weeks. Analyses of neuroinflammatory biomarkers in the ongoing 48-week phase 3 clinical trial of PB and TURSO in ALS (PHOENIX) are planned to confirm these results. Ultimately, further delineation of the utility of these biomarkers in ALS may provide a tool for evaluating therapies targeting neuroinflammation in ALS trials.

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Contributors MC and SP contributed to conceptualisation and design of the CENTAUR trial. RB, JA, MC and SP participated in acquisition of data. RB, JA and JC performed all statistical analyses. RB and JA provided assay development. JT and LM contributed to drafting of the manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis and critically reviewed interim and final versions of the manuscript.

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Competing interests RB reports receiving laboratory supplies from *n*Vector for the biomarker assays used in these analyses; consulting fees from MT Pharma, RRD International, BrainStorm and Cell Therapeutics unrelated to this manuscript; consulting fees from Amylyx Pharmaceuticals, Inc., related to this manuscript; a pending patent with *n*Vector; and stock options in *n*Vector, AcuraStem and Aural Analytics. JA reports stock options in *n*Vector. LM, JC and JT are full-time employees of and have stock option ownership in Amylyx. MC reports consulting fees from Lilly, Immunity Pharm Ltd, Orion, Cytokinetics, Wave Life Sciences, Takeda, Avexis, Biogen, Helixsmith, Sunovian Pharmaceuticals Inc., Disarm, ALSpharma, RRD International, Transposon, QurAlis, Regeneron Pharmaceuticals, AB Science, Locust

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Patient consent for publication Not applicable.

Ethics approval The CENTAUR trial involved human participants and was approved by a central institutional review board, the Partners Human Research Committee, for all trial sites (no reference number or ID associated). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Deidentified participant data will be made available on reasonable request. Requests for data sharing can be sent to info@amylyx.com.

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Supplementary Online Content

Title: Effect of sodium phenylbutyrate and taurursodiol on plasma concentrations of neuroinflammatory biomarkers in amyotrophic lateral sclerosis: results from the CENTAUR trial

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Supplemental Table 1 Baseline characteristics in the neuroinflammatory biomarkers analysis population*

Characteristic	PB and TURSO (n=81)	Placebo (n=45)
Male, n (%)	56 (69.1)	29 (64.4)
White, n (%)	76 (93.8)	43 (95.6)
Mean (SD) age, y	57.3 (10.41)	57.3 (7.71)
Bulbar onset, n (%)	24 (29.6)	10 (22.2)
Riluzole or edaravone use, n (%)	56 (69.1)	39 (86.7)
Riluzole	53 (65.4)	34 (75.6)
Edaravone	21 (25.9)	22 (48.9)
Both	18 (22.2)	17 (37.8)
Mean (SD) prebaseline ALSFRS-R slope, points/mo†	0.95 (0.431)	0.90 (0.552)
Mean (SD) SVC, percent predicted normal	84.7 (17.99)	84.6 (15.69)
Mean (SD) ALSFRS-R total score, points‡	35.8 (5.76)	36.8 (5.02)
Bulbar score	9.6 (2.36)	9.9 (2.66)
Fine-motor score	8.0 (2.68)	8.2 (2.49)
Gross-motor score	7.5 (2.81)	7.6 (2.65)
Breathing score	10.6 (1.95)	11.0 (1.84)
Mean (SD) upper-limb ATLIS score, percent predicted normal§	55.3 (24.84)	53.3 (24.96)
Mean (SD) lower-limb ATLIS score, percent predicted normal§	57.2 (25.47)	58.2 (25.96)
Mean (SD) total ATLIS score, percent predicted normal§	56.9 (20.61)	55.4 (20.71)
Mean (SD) duration since ALS symptom onset, mo	13.4 (3.92)	13.6 (3.57)
Mean (SD) duration since ALS diagnosis, mo	5.9 (3.44)	6.4 (3.18)
Mean (SD) BMI, kg/m^2	27.0 (4.53)	26.5 (5.99)

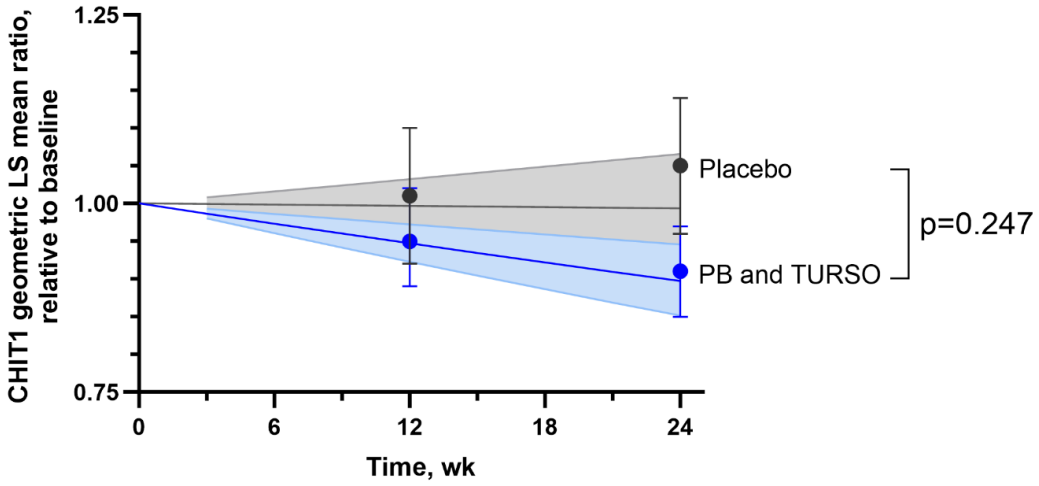
*Consisted of participants within the modified intent-to-treat population with ≥1 postbaseline plasma sample collected over the 24-week randomised phase duration. The modified intent-to-treat population consisted of participants who received ≥1 dose of study medication and had ≥1 postbaseline ALSFRS-R assessment (N=135; PB and TURSO, n=87; placebo, n=48).

†Defined as the rate of change in ALSFRS-R total score from symptom onset to trial baseline.

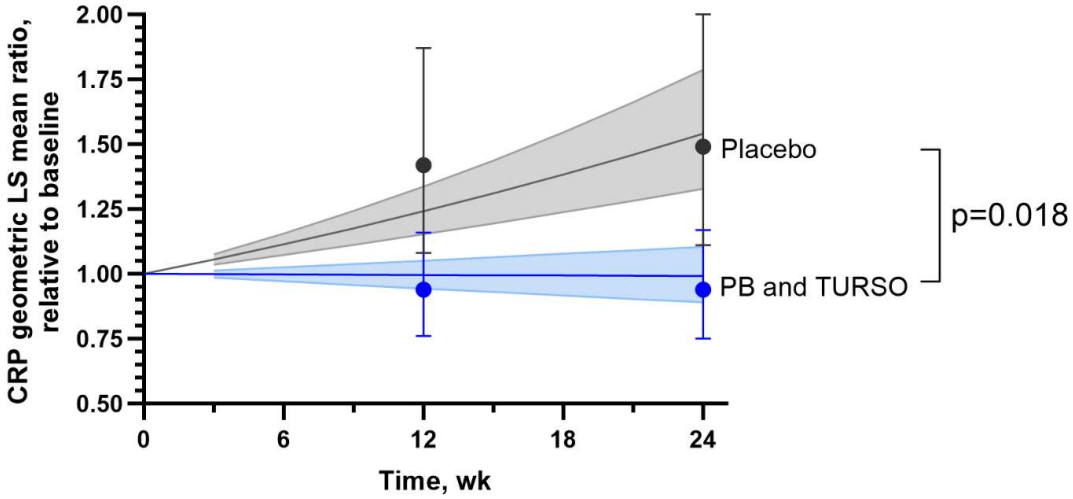
‡The ALSFRS-R measures 12 items in 4 subdomains of function, each scored on a scale from 0–4, with higher scores indicating better function (maximum total score, 48 points; maximum score for each subdomain, 12 points).[1]

§ATLIS values are standardised to the percentage of the predicted normal value based on sex, age, weight and height.[2]

ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ATLIS, Accurate Test of Limb Isometric Strength; BMI, body mass index; PB and TURSO, sodium phenylbutyrate and taurursodiol; SD, standard deviation; SVC, slow vital capacity.



Supplemental Figure 1 Change in CHIT1 plasma concentration relative to baseline over 24 weeks. Log10-transformed CHIT1 concentration measurements[3] were analysed using a random-slope, shared-baseline, linear mixed-effects model adjusted for age and ALSFRS-R slope interacting with time (colored lines, with shading reflecting SEs). Geometric mean ratios (solid dots) and 95% CIs (bars) from an MMRM model without linear trend assumption are shown at weeks 12 and 24. ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CHIT1, chitinase 1; LS, least squares; MMRM, mixed model for repeated measures; PB and TURSO, sodium phenylbutyrate and taurursodiol.



Supplemental Figure 2 Change in CRP plasma concentration relative to baseline over 24 weeks. Log10-transformed CRP concentration measurements[3] were analysed using a random-slope, shared-baseline, linear mixed-effects model adjusted for age and ALSFRS-R slope interacting with time (colored lines, with shading reflecting SEs). Geometric mean ratios (solid dots) and 95% CIs (bars) from an MMRM model without linear trend assumption are shown at weeks 12 and 24. ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CRP, C reactive protein; LS, least squares; MMRM, mixed model for repeated measures; PB and TURSO, sodium phenylbutyrate and taurursodiol.

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