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RESEARCH ARTICLE

Biological effects of sodium phenylbutyrate and taurursodiol in Alzheimer's disease

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Abstract

INTRODUCTION: Sodium phenylbutyrate and taurursodiol (PB and TURSO) is hypothesized to mitigate endoplasmic reticulum stress and mitochondrial dysfunction, two of many mechanisms implicated in Alzheimer's disease (AD) pathophysiology.

METHODS: The first-in-indication phase 2a PEGASUS trial was designed to gain insight into PB and TURSO effects on mechanistic targets of engagement and disease biology in AD. The primary clinical efficacy outcome was a global statistical test combining three endpoints relevant to disease trajectory (cognition [Mild/Moderate Alzheimer's Disease Composite Score], function [Functional Activities Questionnaire], and total hippocampal volume on magnetic resonance imaging). Secondary clinical outcomes included various cognitive, functional, and neuropsychiatric assessments. Cerebrospinal fluid (CSF) biomarkers spanning multiple pathophysiological pathways

in AD were evaluated in participants with both baseline and Week 24 samples (exploratory outcome).

RESULTS: PEGASUS enrolled 95 participants (intent-to-treat [ITT] cohort); cognitive assessments indicated significantly greater baseline cognitive impairment in the PB and TURSO (n = 51) versus placebo (n = 44) group. Clinical efficacy outcomes did not significantly differ between treatment groups in the ITT cohort. CSF interleukin-15 increased from baseline to Week 24 within the placebo group (n = 34). In the PB and TURSO group (n = 33), reductions were observed in core AD biomarkers phosphorylated tau-181 (p-tau181) and total tau; synaptic and neuronal degeneration biomarkers neurogranin and fatty acid binding protein-3 (FABP3); and gliosis biomarker chitinase 3-like protein 1 (YKL-40), while the oxidative stress marker 8-hydroxy-2-deoxyguanosine (8-OHdG) increased. Between-group differences were observed for the Aβ42/40 ratio, p-tau181, total tau, neurogranin, FABP3, YKL-40, interleukin-15, and 8-OHdG. Additional neurodegeneration, inflammation, and metabolic biomarkers showed no differences between groups.

DISCUSSION: While between-group differences in clinical outcomes were not observed, most likely due to the small sample size and relatively short treatment duration, exploratory biomarker analyses suggested that PB and TURSO engages multiple pathophysiologic pathways in AD.

KEYWORDS

Alzheimer's disease, amyloid beta, biomarkers, fixed-dose combination, mild cognitive impairment, neurodegeneration, sodium phenylbutyrate and taurursodiol, tau

Highlights

- Proteostasis and mitochondrial stress play key roles in Alzheimer's disease (AD).
- Sodium phenylbutyrate and taurursodiol (PB and TURSO) targets these mechanisms.
- The PEGASUS trial was designed to assess PB and TURSO effects on biologic AD targets.
- PB and TURSO reduced exploratory biomarkers of AD and neurodegeneration.
- Supports further clinical development of PB and TURSO in neurodegenerative diseases.

1 | BACKGROUND

Multiple risk factors and pathophysiologic processes drive the signature plaque and tangle pathologies, neuronal dysfunction, synaptic loss, neurodegeneration, and dementia of Alzheimer's disease (AD).[1,2] AD is distinguished by the presence of amyloid beta (Aβ)—containing extracellular plaques and paired-helical filament tau-containing intracellular neurofibrillary tangles and dystrophic neurites. Accumulated evidence indicates these pathological lesions emerge along with disturbances in multiple cellular and molecular pathways, including mitochondrial dysfunction, elevated endoplasmic reticulum (ER) stress response, inflammation, vascular dysfunction, and altered metabolism.[1–5] As such, a strong rationale exists for therapies targeting multiple pathways simultaneously in AD.

An oral, fixed-dose sodium phenylbutyrate and taurursodiol combination (PB and TURSO) is hypothesized to simultaneously mitigate ER stress and mitochondrial dysfunction. Preclinical studies show that PB and TURSO may individually reduce neuronal death.[6,7] PB is a class I and class II histone deacetylase (HDAC) inhibitor that decreases ER stress response by upregulating chaperone proteins.[8,9] PB was shown to reduce Aβ plaque burden, tau hyperphosphorylation, and hippocampal neurodegeneration and improve cognitive performance measures in murine AD models.[10–13] TURSO has been shown to recover mitochondrial bioenergetic deficits by reducing Bax translocation to the mitochondrial membrane, reducing mitochondrial permeability, and increasing the apoptotic threshold of the cell.[6] In preclinical studies in an APP/PS1 mouse model of AD, TURSO reduced hippocampal and frontal cortex amyloid deposition[14–16] and astrocyte and microglia

activation.[16] The combination of PB and TURSO for 1 month at a 200-mg/kg dose was shown to reduce soluble Aβ (preferentially Aβ42) in an acute, pre-plaque Tg2576 mouse model of AD (data on file, Amylyx Pharmaceuticals, Inc.). ER stress and mitochondrial dysfunction are among several pathogenic mechanisms AD shares with other neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) in which PB and TURSO has also been studied.[17–20]

We conducted a first-in-indication, phase 2a, 24-week, placebo-controlled trial (PEGASUS) evaluating the activity of PB and TURSO in people with AD ranging from mild cognitive impairment (MCI) to moderate stages of dementia. The trial was designed to gain insight into the effects of PB and TURSO on mechanistic targets of engagement and disease biology. As previously presented,[21] PB and TURSO had an acceptable safety profile and was generally well tolerated in PEGASUS; a greater proportion of gastrointestinal adverse events was seen in the PB and TURSO group compared with placebo (similar to the CENTAUR trial in ALS[19]). Here, we report clinical efficacy outcomes and findings from exploratory analyses of biomarkers representing a spectrum of pathophysiologic processes of interest in AD in participants with MCI and mild to moderate AD dementia in PEGASUS.

RESEARCH IN CONTEXT

1. **Systematic review**: Established sources (eg, PubMed) were used to review literature pertaining to mechanisms underlying Alzheimer's disease (AD) pathogenesis. A first-in-indication, proof-of-concept trial (PEGASUS) examined the effects of a sodium phenylbutyrate and taurursodiol combination (PB and TURSO) on mechanistic targets of engagement and disease biology in AD.
2. **Interpretation**: No between-group differences were observed in clinical outcomes, most likely due to the small sample size ($N = 95$) and short treatment duration (24 weeks). In exploratory analyses, administration of PB and TURSO yielded changes in cerebrospinal fluid biomarkers of core AD pathology (ie, amyloid beta and tau), synaptic and neuronal degeneration, gliosis, and DNA oxidation compared with placebo.
3. **Future directions**: Exploratory biomarker analyses from PEGASUS provide preliminary evidence that PB and TURSO engages several pathophysiological pathways in AD, complementing preclinical evidence of biological activity. These findings provide support for further clinical development of PB and TURSO for AD and other neurodegenerative diseases.

2 | METHODS

2.1 | Trial design and oversight

PEGASUS (NCT03533257) was conducted at 10 specialty AD clinical research centers in the United States between September 14, 2018, and November 6, 2020. The trial was conducted in accordance with Good Clinical Practices as defined by the International Conference on Harmonization and with the ethical principles of the Declaration of Helsinki. The Institutional Review Board for each site approved the study. Each participant provided written informed consent.

2.2 | Participants

The trial enrolled adults aged 55 through 89 years, with a diagnosis of "probable AD" or MCI accompanied by biomarkers supporting AD as the likely etiology of cognitive impairment (ie, amyloid positron emission tomography [PET], cerebrospinal fluid [CSF] AD biomarkers [ie, Aβ42, total tau, and phosphorylated tau-181 (p-tau181)], fluorodeoxyglucose-PET, or volumetric magnetic resonance imaging [MRI]). Additional inclusion criteria included a Montreal Cognitive Assessment (MoCA) score of ≥8 and a Geriatric Depression Scale total score of <7. For individuals receiving a cholinesterase inhibitor and/or memantine, these treatments must have been initiated ≥3 months prior to baseline, and the dosing regimen must have remained stable for 6 weeks prior to baseline. Individuals who received any investigational AD therapy within 3 months of screening or any other investigational therapy within 28 days of screening were excluded. Use of any investigational immunotherapy was prohibited beginning 1 year (365 days) prior to the baseline visit and throughout the study.

2.3 | Interventions

Enrolled participants were randomized in a 3:2 ratio to receive PB and TURSO (3 g PB/1 g TURSO per sachet) or matching placebo for 24 weeks. Participants were instructed to consume one sachet of the trial drug dissolved in water once daily for the first week, then twice daily for the remainder of the trial. Further details of study drug characteristics and administration are published.[19]

2.4 | Clinical efficacy outcomes

The primary clinical efficacy outcome was the change from baseline to Week 24 in a global statistical test (GST) combining three endpoints measuring different facets of disease trajectory: (1) cognition, as assessed by the Mild/Moderate Alzheimer's Disease Composite Score (MADCOMS); (2) function, as assessed by the Functional Activities Questionnaire (FAQ); and (3) total hippocampal volume on volumetric MRI. Secondary clinical efficacy outcomes included changes in the following assessments from baseline to Week 24 in hierarchical order: total hippocampal volume, MADCOMS (cognition endpoint of the GST), 14-item Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog14) score, FAQ score (the functional endpoint of the GST), Dementia Severity Rating Scale score, MoCA score, and Neuropsychiatric Inventory Questionnaire score. Further details

regarding the clinical efficacy assessments are provided in the Supplementary Methods.

2.5 | CSF biomarker outcomes

CSF biomarkers spanning multiple pathophysiological processes in AD were measured at baseline and Week 24 as pre-specified exploratory outcomes (Table S1). These included the following: (1) core AD biomarkers, specifically Aβ42/40 ratio, p-tau181, and total tau[2,22]; (2) biomarkers reflecting synaptic and neuronal degeneration, including neurogranin,[22,23] fatty acid binding protein-3 (FABP3),[24] and neurofilament light chain (NfL)[25]; (3) biomarkers associated with gliosis, including YKL-40 (also known as chitinase 3-like protein 1)[26] and glial fibrillary acidic protein (GFAP)[26]; (4) the inflammation-related biomarkers interleukin (IL)-6,[27] IL-8,[28] IL-15,[29] monocyte chemoattractant protein-1/C-C motif chemokine ligand 2 (MCP-1/CCL2),[30] macrophage inflammatory protein-1 beta (MIP1β),[31] and matrix metalloproteinase-10 (MMP-10)[28,32,33]; (5) the oxidative stress marker 8-hydroxy-2-deoxyguanosine (8-OHdG);[34] and (6) metabolic biomarkers including soluble insulin receptor (sIR [insulin resistance])[35] and 24S-hydroxycholesterol (24-OHC [cholesterol turnover]).[36]

CSF samples were collected via lumbar puncture prior to initiating treatment (ie, anytime between the screening visit and up to 7 days prior to the baseline visit) and at the final study visit (Week 24 ± 28 days) or early discontinuation (unless ≤7 days after initiating study drug). Additional details regarding CSF sample collection, handling, and analysis are provided in the Supplementary Methods.

2.6 | Statistical analysis

The prespecified efficacy population in PEGASUS was the intent-to-treat (ITT) cohort, consisting of all randomized participants who received ≥1 dose of study medication, had a baseline assessment, and had ≥1 post-baseline efficacy assessment for the primary efficacy outcome. For the primary clinical efficacy analysis, the GST was calculated for each participant as a mean *z*-score across the three component endpoints. Hippocampal volume was assessed using paired baseline and Week 24 data from the same participant (longitudinal approach). The GST change from baseline was estimated using a mixed model with repeated measures (MMRM) incorporating composite covariates as described in the Supplementary Methods. The same MMRM was used to estimate changes from baseline for all secondary clinical efficacy outcomes. Procedures for handling missing data are summarized in the Supplementary Methods.

Biomarker analyses were performed on the CSF subcohort, a subset of the ITT cohort consisting of participants who completed the study with CSF having been successfully collected at both baseline and Week 24. Differences in essential demographic, clinical, and biomarker variables at baseline between the placebo group and the PB and TURSO group were evaluated using two-tailed *t*-tests or chi-square tests. Demographic and clinical variables included age, sex, education, race, apolipoprotein E ε4 (*APOE* ε4) status, MoCA, and FAQ. Treatment effects were analyzed for each biomarker independently. In addition, to provide a composite assessment of the evaluated inflammatory biomarkers that are produced predominantly by microglia and/or other immune cells (eg, lymphocytes) in the brain and CSF (ie, IL-6,[37] IL-8,[38] IL-15,[39] MCP-1/CCL2,[38] MIP1β,[40] and MMP-10[33]), an inflammatory composite index was calculated by averaging *z*-transformed data for these biomarkers.

For each CSF biomarker, treatment group effects were assessed in two ways. First, within each group, we used paired *t*-tests to determine the change between baseline and Week 24 biomarker concentrations. This approach allowed us to determine the change over 24 weeks of disease progression for each treatment separately. Second, we used linear regression models to compare changes in each biomarker with PB and TURSO versus placebo. This model included change in biomarker level from baseline to Week 24 as the response variable, with treatment group and baseline biomarker levels as explanatory variables. Given the exploratory nature of the biomarker analyses, adjustments for multiplicity were not undertaken, and *p*-values are provided without a claim of significance.

Finally, to evaluate the robustness of findings in biomarkers showing between-group differences in their changes from baseline, we conducted sensitivity analyses that included age, sex, *APOE* ε4 status, and baseline MoCA score as additional explanatory variables. These variables were used as they showed either a significant correlation with biomarker levels in the CSF subcohort as a whole (age) or numerical (though not statistically significant) differences between groups at baseline (sex, *APOE* ε4 status, and baseline MoCA score).

3 | RESULTS

3.1 | Trial participants

A total of 95 participants were enrolled, comprising the ITT cohort, of whom 51 were randomized to PB and TURSO and 44 were randomized to placebo; 96% of participants in the placebo group and 80% in the PB and TURSO group completed the study ($p = 0.028$; Figure 1). Mean cognitive assessment scores indicated a significantly greater baseline level of cognitive impairment among those randomized to PB and TURSO versus placebo (MADCOMS, ADAS-Cog14 total score, and MoCA score, all $p \leq 0.007$; Table S2). Baseline values for all other measures were similar between groups in the ITT cohort.

Twenty-eight participants from the ITT cohort were excluded from the biomarker analyses either because they discontinued the study and did not have the end-of-study lumbar puncture ($n = 12$) or because one or both lumbar punctures were unsuccessful ($n = 16$), leaving 67 participants in the CSF subcohort (PB and TURSO, $n = 33$; placebo, $n = 34$). Baseline demographic and clinical characteristics were generally well matched between the overall ITT cohort and CSF subcohort and between treatment groups within the CSF subcohort (Table 1). Differences between treatment groups in the CSF subcohort were

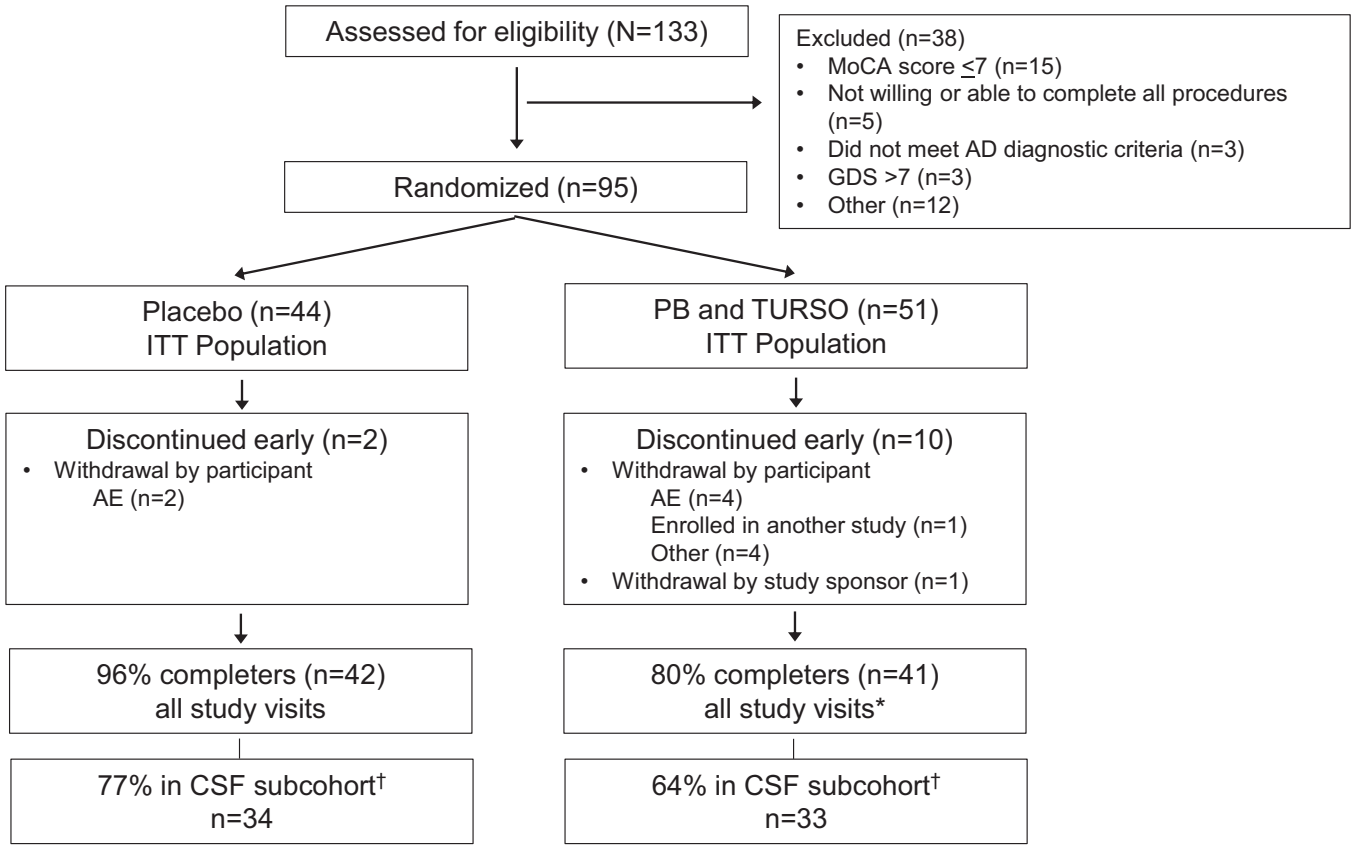

FIGURE 1 PEGASUS trial participant disposition flow diagram. *Includes two randomized participants who completed the study off the drug. †The CSF subcohort consisted of participants who completed the study with successful CSF collection at baseline and Week 24. AD, Alzheimer's disease; AE, adverse event; CSF, cerebrospinal fluid; GDS, Geriatric Depression Scale; ITT, intent-to-treat; MoCA, Montreal Cognitive Assessment; PB and TURSO, sodium phenylbutyrate and taurursodiol.

TABLE 1 Demographic and clinical characteristics of participants at baseline.

Characteristic	Overall		PB and TURSO CSF subcohort ($n = 33$)	Placebo CSF subcohort ($n = 34$)	*p*-value, placebo vs. PB and TURSO statistic
	ITT cohort ($N = 95$)	CSF subcohort ($n = 67$)			
Age, mean (SD), years	70.7(7.5)	70.1 (7.4)	70.1 (7.6)	70.1 (7.3)	$t = 0.02$; $p = 0.99$
Female, %	45.3	40.3	33.3	47.1	$\chi^2 = 1.32$; $p = 0.25$
Education, mean (SD) years	16.1 (2.7)	15.9 (2.8)	16.2 (2.8)	15.7 (2.8)	$t = 0.78$; $p = 0.43$
White race, %	96.8	97.0	97.0	97.1	$\chi^2 = 0$; $p = 0.98$
APOE ε4 positive, %	69.6	69.7	78.1	61.8	$\chi^2 = 2.11$; $p = 0.15$
MoCA score, mean (SD)	17.9 (4.9)	18.4 (5.2)	17.3 (5.4)	19.4 (4.8)	$t = 1.69$; $p = 0.09$
FAQ score, mean (SD)	11.4 (7.3)	10.7 (7.1)	11.3 (7.8)	10.1 (6.3)	$t = 0.72$; $p = 0.48$

Abbreviations: *APOE* ε4, apolipoprotein E gene ε4 allele; CSF, cerebrospinal fluid; FAQ, Functional Activities Questionnaire; ITT, intent-to-treat; MoCA, Montreal Cognitive Assessment; PB and TURSO, sodium phenylbutyrate and taurursodiol; SD, standard deviation.

observed for sex, *APOE* ε4 genotype, and MoCA score, but none were statistically significant. In addition, there were no statistically significant baseline differences between treatment groups within the CSF subcohort for any biomarkers (Table 2). Groupwise baseline biomarker concentrations in the ITT cohort are provided in Table S2.

3.2 | Clinical outcomes

The estimated mean (standard error) change from baseline to Week 24 in the GST (primary efficacy outcome, calculated from the mean *z*-score across the three component endpoints at each time point)

TABLE 2 CSF biomarker levels: within-group and between-group comparisons.

Biomarker	PB and TURSO ($n = 33$)				Placebo ($n = 34$)				Between-group comparison[b] $F_{adj\ BL}$, p-value
	Baseline mean (SD)[a]	Week 24 mean (SD)[a]	Percent change from baseline	Within-group statistics	Baseline mean (SD)[a]	Week 24 mean (SD)[a]	Percent change from baseline	Within-group statistics	
Core AD									
Aβ42/40 ratio	0.054 (0.019)	0.058 (0.016)	+8.47	$t = 1.86$, $p = 0.0729$	0.051 (0.015)	0.048 (0.014)	-5.73	$t = 1.26$, $p = 0.2182$	8.84, 0.0042
p-tau181	113.5 (48.3)	99.1 (42.6)	-12.66	$t = 5.13$, $p < 0.0001$	114.4 (43.7)	113.7 (46.6)	-0.56	$t = 0.28$, $p = 0.7829$	15.33, 0.0002
Total tau	507.1 (190.3)	443.6 (173.8)	-12.54	$t = 5.44$, $p < 0.0001$	512.3 (184.3)	521.1 (217.0)	+1.72	$t = 0.73$, $p = 0.4733$	18.19, <0.0001
Neurodegeneration									
Neurogranin	483.9 (186.2)	403.8 (180.0)	-16.56	$t = 6.25$, $p < 0.0001$	466.5 (206.9)	458.8 (222.2)	-1.67	$t = 0.57$, $p = 0.5755$	14.31, 0.0004
FABP3	3921.8 (1531.2)	3599.5 (1464.3)	-8.22	$t = 3.98$, $p < 0.0004$	3791.3 (1484.4)	3901.3 (1693.0)	+2.90	$t = 1.26$, $p = 0.2168$	12.59, 0.0007
NfL	1336.9 (534.1)	1491.1 (724.1)	+11.53	$t = 1.74$, $p = 0.0918$	1276.7 (511.6)	1335.2 (619.6)	+4.59	$t = 1.11$, $p = 0.2736$	0.9991, 0.3213
Gliosis									
YKL-40	227,358 (79,674)	210,410 (72,345)	-7.45	$t = 3.87$, $p = 0.0005$	233,857 (73,197)	234,359 (72,074)	+0.22	$t = 0.23$, $p = 0.8199$	9.01, 0.0039
GFAP	13,019 (6505)	13,945 (6619)	+7.11	$t = 1.58$, $p = 0.124$	13,512 (7622)	13,977 (8682)	+3.45	$t = 0.47$, $p = 0.641$	0.253, 0.617
Neuroinflammation									
Inflammatory composite index[c]	0.115 (0.726)	0.294 (1.313)	+155.50	$t = 0.97$, $p = 0.3417$	-0.116 (0.467)	0.180 (0.668)	+254.78	$t = 2.36$, $p = 0.0242$	0.0071, 0.933
IL-6	1193.5 (538.1)	1773.8 (2473.1)	+48.62	$t = 1.34$, $p = 0.1907$	1147.6 (442.5)	1718.6 (1826.6)	+49.75	$t = 1.85$, $p = 0.0735$	0.0031, 0.956

(Continues)

TABLE 2 (Continued)

Biomarker	PB and TURSO ($n = 33$)				Placebo ($n = 34$)				Between-group comparison[b] $F_{adj\ BL}$, p-value
	Baseline mean (SD)[a]	Week 24 mean (SD)[a]	Percent change from baseline	Within-group statistics	Baseline mean (SD)[a]	Week 24 mean (SD)[a]	Percent change from baseline	Within-group statistics	
IL-8	26.5 (6.7)	27.2 (11.8)	+2.78	$t = 0.38$, $p = 0.7095$	24.1 (5.9)	25.6 (7.5)	+6.37	$t = 1.72$, $p = 0.0944$	0.0012, 0.973
IL-15	3.28 (1.02)	3.23 (1.03)	-1.38	$t = 0.02$, $p = 0.9879$	2.95 (0.73)	3.20 (0.85)	+8.76	$t = 3.91$, $p = 0.0004$	5.03, 0.028
MCP-1/CCL2	246.5 (83.2)	242.4 (73.8)	-1.67	$t = 0.52$, $p = 0.6067$	241.7 (70.6)	243.1 (63.2)	+0.59	$t = 0.18$, $p = 0.8583$	0.1768, 0.6756
MIP1β	9.71 (3.43)	10.60 (4.69)	+9.10	$t = 2.06$, $p = 0.0482$	9.74 (5.21)	10.19 (4.89)	+4.61	$t = 2.42$, $p = 0.0211$	1.09, 0.300
MMP-10	67.4 (39.3)	60.0 (23.6)	-10.97	$t = 1.47$, $p = 0.1522$	51.9 (19.3)	53.9 (21.3)	+3.82	$t = 1.32$, $p = 0.1948$	0.332, 0.5665
Oxidative stress									
8-OHdG	3.26 (0.78)	3.61 (1.03)	+10.59	$t = 2.92$, $p = 0.0066$	3.20 (0.88)	3.10 (0.94)	-3.04	$t = 0.98$, $p = 0.3346$	8.578, 0.0048
Metabolism									
sIR	4.34 (1.31)	4.34 (1.30)	-0.058	$t = 0.09$, $p = 0.9276$	4.39 (1.25)	4.22 (1.50)	-3.79	$t = 1.12$, $p = 0.2709$	0.7764, 0.3816
24-OHC	7.20 (2.33)	7.01 (1.92)	-2.53	$t = 0.93$, $p = 0.3606$	6.90 (2.52)	6.82 (2.13)	-1.25	$t = 0.37$, $p = 0.7139$	0.0108, 0.9174

Abbreviations: 8-OHdG, 8-hydroxy-2-deoxyguanosine; 24-OHC, 24S-hydroxycholesterol; Aβ, amyloid beta; CSF, cerebrospinal fluid; FABP3, fatty acid binding protein-3; GFAP, glial fibrillary acidic protein; IL, interleukin; MCP-1/CCL2, monocyte chemoattractant protein-1/C-C motif chemokine ligand 2; MIP1β, macrophage inflammatory protein-1 beta; MMP-10, matrix metalloproteinase-10; NfL, neurofilament light chain; PB and TURSO, sodium phenylbutyrate and taurursodiol; p-tau181, phosphorylated tau-181; SD, standard deviation; sIR, soluble insulin receptor; YKL-40, chitinase 3-like protein 1.

[a]Values consist of the mean (SD) concentration in pg/mL, except for the Aβ42/40 ratio and the inflammation composite index.

[b]From a linear regression model with Week-24 biomarker level as the response variable and treatment group and baseline biomarker levels as explanatory variables.

[c]The inflammation composite index was calculated by averaging z-transformed data for IL-6, IL-8, IL-15, MCP-1/CCL2, MIP1β, and MMP-10.

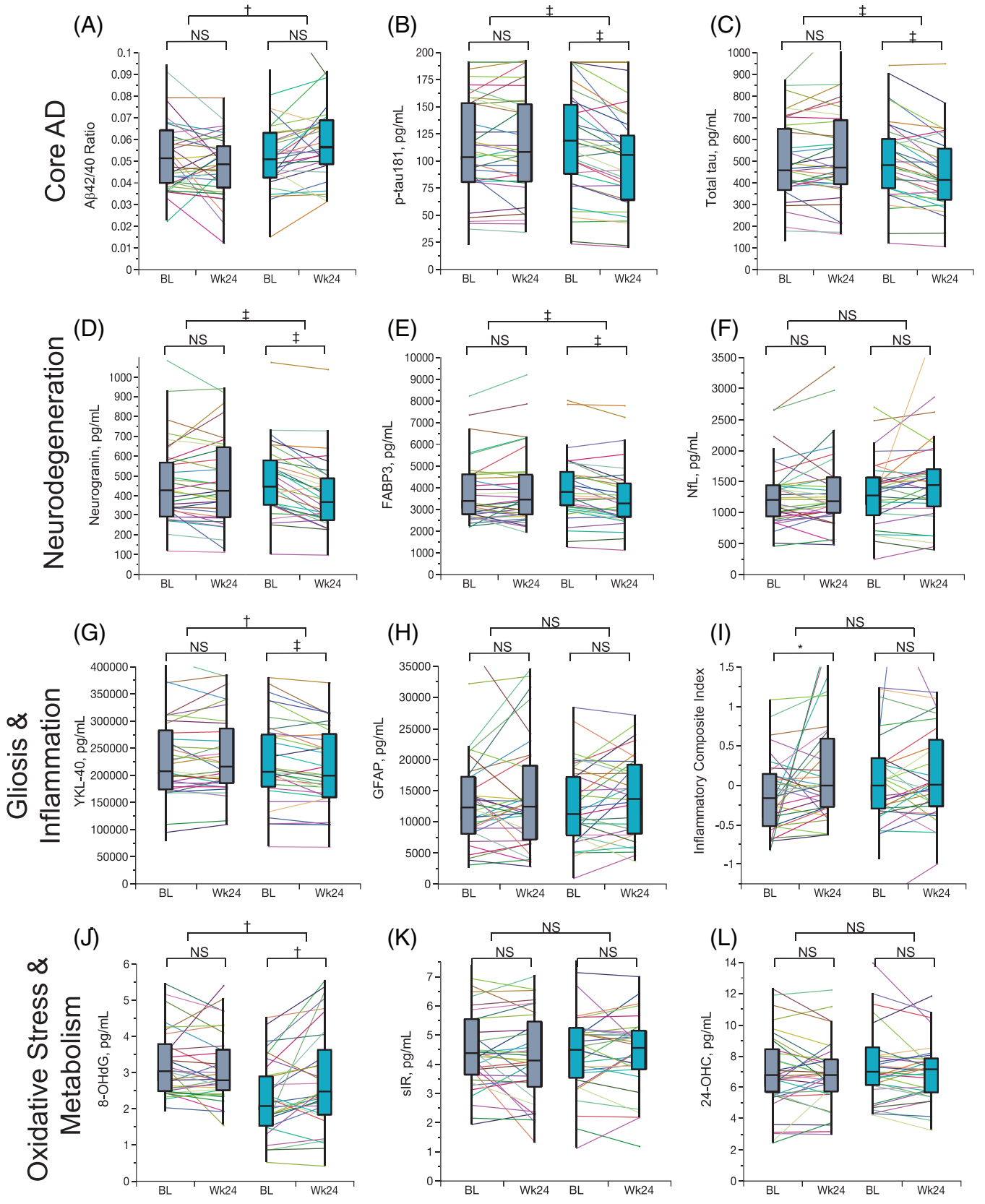

FIGURE 2 CSF biomarker effects. Mean (SD) values and interquartile ranges in the placebo (gray bars on left) and PB and TURSO (blue bars on right) groups are shown at baseline and Week 24 for the following: core AD biomarkers, namely Aβ42/40 (A), p-tau181 (B), and total tau (C); biomarkers of synaptic and neuronal degeneration, namely neurogranin (D), FABP3 (E), and NfL (F); biomarkers of gliosis and inflammation, namely YKL-40 (G), GFAP (H), and an inflammatory composite index (I) consisting of the average of *z*-transformed data for IL-6, IL-8, IL-15, MCP-1/CCL2, MIP1β, and MMP-10; a biomarker of oxidative stress, 8-OHdG (J); and biomarkers of metabolism, namely sIR (K) and 24-OHC (L). 8-OHdG,

did not significantly differ between the PB and TURSO (0.24 [0.05]) and placebo (0.17 [0.05]) groups (between-group difference, 0.07; 95% confidence interval [CI]: −0.08 to 0.21; $p = 0.36$). Between-group differences for secondary clinical outcomes did not achieve statistical significance (Table S3).

3.3 | Biomarkers

CSF biomarker findings are graphically portrayed in Figure 2 and presented in Table 2. Within the placebo-treated group, changes from baseline were observed for the inflammatory composite index ($p = 0.02$) and the component biomarkers IL-15 ($p = 0.0004$) and MIP1β ($p = 0.0211$), all of which increased over the 24-week study duration. Treatment with PB and TURSO yielded reductions in core AD biomarkers p-tau181 ($p < 0.0001$) and total tau ($p < 0.0001$), neurodegeneration biomarkers neurogranin ($p < 0.0001$) and FABP3 ($p = 0.0004$), and gliosis biomarker YKL-40 ($p = 0.0005$). In addition, the oxidative stress marker 8-OHdG increased from baseline to Week 24 in the PB and TURSO group ($p = 0.007$). There was also a trend toward an increase in the Aβ42/40 ratio ($p = 0.07$). No group-wise differences were observed for other biomarkers in the PB and TURSO treatment group.

A regression model directly compared the effects of placebo and PB and TURSO treatment on CSF biomarkers. Compared to the placebo group, the PB and TURSO group exhibited differences in effect on the Aβ42/40 ratio ($p = 0.004$), p-tau181 ($p = 0.0002$), total tau ($p < 0.0001$), neurogranin ($p = 0.0004$), FABP3 ($p = 0.0007$), YKL-40 ($p = 0.004$), IL-15 ($p = 0.028$), and 8-OHdG ($p = 0.005$) (Figure 2 and Table 2).

As a sensitivity analysis, we separately added age, sex, MoCA score, and *APOE* ε4 genotype to the regression models for the seven biomarkers for which between-group differences were observed. None of the variables attenuated the effects of PB and TURSO treatment on these seven biomarkers (Table S4).

4 | DISCUSSION

PEGASUS was a first-in-indication phase 2a trial designed to evaluate the safety and biological activity of PB and TURSO in AD. The trial included individuals along a spectrum of AD severity, from MCI to moderate dementia, and incorporated multiple clinical, neuroimaging, and CSF biomarker endpoints to evaluate proof of concept of PB and TURSO for AD and to inform further clinical development and design of next-phase trials. No between-group differences were seen in clinical outcomes in PEGASUS. Potential reasons for the lack of between-group differences include the small sample size and the short duration of the trial. In addition, there was evidence of greater cognitive impairment in the PB and TURSO group compared with the placebo group at baseline, based on cognitive assessments that were incorporated within the primary GST outcome and as secondary clinical outcomes in the trial. In contrast, exploratory CSF biomarker analyses in PEGASUS suggested PB and TURSO engagement of several pathophysiologic targets in AD, including Aβ, tau, and neurodegeneration, across a sample of individuals with a broad range of disease severity.

PB and TURSO may reduce AD pathologies and rescue neurodegeneration by several mechanisms. Abnormalities in regulation of protein translation, chaperone-mediated protein folding, and protein degradation are well-described features of AD that may be both cause and consequence of Aβ and tau pathologies.[2,3] As an HDAC inhibitor, PB has been shown to upregulate expression of anti-apoptotic genes as well as proteins involved in synaptic function and plasticity.[11,41] PB may also act as a chemical chaperone, binding exposed hydrophobic segments of unfolded proteins, stabilizing protein structure, and reducing ER stress.[9] TURSO is a bile acid that prevents Bax translocation to the outer mitochondrial membrane, stabilizing mitochondrial membranes, inhibiting release of cytochrome C, and limiting activation of caspases, thus decreasing apoptotic cell death.[6] Growing evidence supports amelioration of protein misfolding and ER stress as additional mechanisms underlying the neuroprotective action of TURSO; in the same manner as with PB, these effects appear to be mediated via chemical chaperone activity.[42]

Compared with placebo, PB and TURSO reduced levels of CSF total tau, a general biomarker of neurodegeneration, and p-tau181, a more specific biomarker of AD pathology.[43,44] PB and TURSO also raised the ratio of CSF Aβ42/40. Lower CSF Aβ42/40 ratios have been reported to signify the presence of AD as the underlying cause of dementia and MCI/prodromal dementia.[45] In addition, the CSF Aβ42/40 ratio has shown greater concordance with amyloid PET imaging than Aβ42 alone[46–48] and inversely correlates with cerebral β-amyloid level.[49] PB and TURSO may lower cerebral β-amyloid levels through enhanced mitochondrial activity in microglia, augmenting phagocytosis and Aβ clearance.[50] Further study will be necessary to explore this possibility.

PB and TURSO appear to have synergistic benefits in protecting neurons under severe oxidative stress based on in vitro preclinical studies.[51] This effect may be the basis for the observed reductions in CSF neurogranin and FABP3 in our trial. Neurogranin, a calmodulin-binding, neuron-specific protein involved in synaptic plasticity and regeneration, is present in increased concentrations in the CSF in several diseases including AD, where it is thought to signify synaptic degeneration.[22,23,52] In line with the effects of PB and TURSO observed in our analysis, neurogranin levels positively correlate with p-tau and total tau levels but only weakly correlate with Aβ42 levels in AD, presumably because amyloid plaque burden does not correspond

8-hydroxy-2-deoxyguanosine; 24-OHC, 24S-hydroxycholesterol; Aβ, amyloid beta; AD, Alzheimer's disease; BL, baseline; CSF, cerebrospinal fluid; FABP3, fatty acid binding protein-3; GFAP, glial fibrillary acidic protein; IL, interleukin; MCP-1/CCL2, monocyte chemoattractant protein-1/C-C motif chemokine ligand 2; MIP1β, macrophage inflammatory protein-1 beta; MMP-10, matrix metalloproteinase-10; NfL, neurofilament light chain; NS, not significant; p-tau181, phosphorylated tau-181; SD, standard deviation; sIR, soluble insulin receptor; Wk24, Week 24; YKL-40, chitinase 3-like protein 1. $^{*}p < 0.05$. $^{\dagger}p \leq 0.01$. $^{\ddagger}p \leq 0.001$.

with synapse loss. Because synaptic loss is an early event in the AD continuum, CSF neurogranin is a potentially useful biomarker in the pre-symptomatic stages of AD as well as for monitoring the effects of investigational agents on synaptic integrity in AD trials.[22,23]

FABP3 is a fatty acid-binding protein abundant in the cytoplasm and highly expressed in the brain.[24,53–55] In addition to its utility as a general biomarker of neurodegeneration, FABP3 participates in the uptake, intracellular metabolism, and/or transport of long-chain fatty acids, playing a role in lipid membrane composition.[24,53–57] FABP3 is considered a biomarker of both lipid dyshomeostasis and neuronal membrane disruption. FABP3 elevation has been reported in several AD studies and is thought to indirectly contribute to amyloid plaque formation and facilitate oligomerization of α-synuclein, a suspected mediator of tau hyperphosphorylation.[24,53–58]

We did not observe a between-group difference in change from baseline in NfL, another often cited general neurodegeneration biomarker. While CSF NfL levels are especially elevated in traumatic brain injury, frontotemporal dementia, and ALS, lesser and more variable elevations are described in AD and Lewy body dementia.[59–61] As such, elevation of CSF NfL concentration may be a less reliable indicator of neurodegeneration in AD compared with other biomarkers, particularly in later stages characterized by cognitive decline.[62] For example, longitudinal studies indicate that NfL increases may slow during the symptomatic course of AD, following an initial increase.[62]

Evolving evidence suggests that neuroinflammation plays an early role in AD progression.[63,64] We examined biomarkers primarily produced by microglia and lymphocytes in the brain and CSF (cytokines, chemokines, and MMP-10)[33,37–40] and astrocytes (GFAP and YKL-40), key mediators of neuroinflammation in AD.[64] Among the former group of biomarkers, only the change in IL-15 differed between the PB and TURSO group and placebo group, with the level increasing from baseline to Week 24 in the latter. While no changes in GFAP were observed in our analyses, PB and TURSO decreased CSF levels of YKL-40, a chitinase expressed by astrocytes upon microglial activation.[64] CSF YKL-40 is elevated in the earliest stages of AD, and levels have been shown to correlate with total tau and p-tau181 as well as cortical volume loss and rate of cognitive decline.[64–66] Consistent with our findings in PEGASUS, a significant decrease in plasma YKL-40 was observed over 24 weeks in participants with ALS receiving PB and TURSO versus placebo in the CENTAUR trial.[67]

In the PB and TURSO group, we saw an increase in 8-OHdG, a product of DNA oxidation that correlates with general mitochondrial oxidative metabolism and stress.[34] In prior studies, significantly elevated 8-OHdG levels were observed in the CSF[34,68] and plasma[69] of people with AD compared with healthy age-matched controls. The increase in 8-OHdG in our analysis warrants additional investigation. Reactive oxygen species can have positive effects in neurons for enhancing synaptic plasticity, but when excessively elevated may have detrimental effects on neuronal function and survival.[70] In most reported studies of neurological disease models, TURSO is neuroprotective.[42]

We also examined biomarkers of metabolic pathways that are altered in AD, including insulin resistance (sIR)[35,71] and cholesterol turnover (24-OHC).[36] The value of these biomarkers in AD is not well established. While there is evidence suggesting downregulation of insulin receptors (IRs) in the frontal cortex, hippocampus, and hypothalamus in AD, these findings have not yet been correlated to altered IR levels in CSF.[35] Additionally, while growing evidence suggests that accumulation of oxysterol byproducts of cholesterol oxidation contributes to AD pathogenesis, data regarding 24-OHC activity are conflicting, with evidence indicating both damaging and protective effects; furthermore, inconsistency in 24-OHC levels has been noted in various biological samples including CSF in AD, suggesting it is not a reliable biomarker in AD.[36]

The analyses described herein were limited by the brief duration of the PEGASUS trial and small sample size, which was further limited for the biomarker analyses, which included only those participants who completed the study with successful collection of CSF at both baseline and Week 24. In addition, a lower percentage of participants who were randomized to PB and TURSO completed the study. As previously noted, random between-group baseline imbalance in the level of cognitive impairment may have had a bearing on the clinical outcome results. Strengths of the exploratory biomarker analyses included the rich profiling of CSF biomarkers representing pathophysiological pathways beyond conventional Aβ and tau measures in people with a range of clinical AD severity. The changes in multiple biomarkers and consistency of effect across participants and biomarker categories, even after adjusting for covariates, provide preliminary evidence of biological effect of PB and TURSO in AD. However, these analyses were potentially limited by lack of adjustment for multiplicity, given their exploratory nature. Future trials incorporating a longer duration, larger population, and greater participant diversity may ascertain any correlation between clinical outcomes and biomarker effects in AD, as well as identify those who are most likely to respond to PB and TURSO.

In summary, while no between-group differences were seen in clinical outcomes in PEGASUS, most likely due to limited sample size and trial duration, exploratory CSF biomarker results provided preliminary evidence that PB and TURSO engages AD pathology and pathways of neurodegeneration, synaptic function, gliosis, and oxidative stress. Along with those from preclinical studies showing a biological effect of PB and TURSO in AD models, the findings of our analysis provide support for further clinical development of PB and TURSO for AD and may be used to inform the design of subsequent trials.

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CONFLICT OF INTEREST STATEMENT

S.E.A. was the principal investigator of the PEGASUS trial and contributed to its design, conduct, statistical analyses of the exploratory biomarker assessments, writing and revision of this manuscript. 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S.H. is an employee and owner of Pentara Corporation, which was contracted to perform statistical analyses for the PEGASUS trial, including the clinical efficacy outcomes analyses described in this manuscript; reports consulting fees paid to Pentara from multiple pharmaceutical companies developing therapies for neurodegenerative diseases; and reports data safety monitoring board or advisory board fees from Alzheon, Eisai, and Prothena Biosciences paid to Pentara. J.N.-J. and N.K. are employees of Pentara Corporation. V.J.W. reports consulting fees from Cognito Therapeutics, unrelated to the present manuscript, and an unpaid scientific advisory committee position in Division 40 of the American Psychological Association. J.M.B. reports clinical trial support from the National Institutes of Health, Eli Lilly, Biogen, AbbVie, AstraZeneca, and Roche, unrelated to the manuscript, and consulting fees from Amylyx Pharmaceuticals, Renew Research, Eisai, Eli Lilly, and Labcorp. 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P.D.Y. is the Chief Medical Officer and has stock and stock option ownership in Amylyx Pharmaceuticals. K.L. was an employee of Amylyx Pharmaceuticals from July 2015 through August 2021 and received stock options while employed with Amylyx Pharmaceuticals. B.A.T. and P.K. have no disclosures to report. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

This PEGASUS trial was conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations and International Conference on Harmonization guidance documents pertaining to informed consent. All participants provided written informed consent to participate in the trial and were provided with a copy of the fully executed consent form for their records.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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