Proteomic Analyses in the 24-Week PEGASUS Trial Using the Olink Platform: Providing Insight Into the Biologic Activity of Sodium Phenylbutyrate and Taurursodiol in Alzheimer's Disease

Poster #P087

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BACKGROUND

- Current disease-modifying therapies for Alzheimer's disease (AD) are exclusively based on amyloid reduction[1]; however, amyloid deposition is an upstream event in AD pathogenesis,[2] warranting exploration of other therapeutic targets that are further downstream and closer to AD pathogenesis
- AMX0035, an oral, fixed-dose combination of sodium phenylbutyrate and taurursodiol (PB&TURSO), significantly improved cerebrospinal fluid (CSF) biomarker levels in a phase 2, randomized, placebo-controlled trial in AD (PEGASUS; NCT03533257), including biomarkers reflecting[3-5]:
  - Core AD pathology (amyloid beta $[A\beta]_{42}/A\beta_{40}$ ratio, total tau, and phosphorylated tau 181 [p-tau^{181}])
  - Synaptic and neuronal degeneration (neurogranin and fatty acid binding protein-3 [FABP3])
  - Gliosis (YKL-40, also known as chitinase 3–like protein 1)
- PB&TURSO also significantly slowed functional decline, prolonged survival, and reduced plasma YKL-40 compared with placebo in a phase 2 trial in amyotrophic lateral sclerosis[6-9]

OBJECTIVE

- To elucidate the potential neuroprotective mechanisms of PB&TURSO in AD via analysis of broad proteomic changes in participants from PEGASUS and correlations with CSF biomarker changes

ANALYSES

PEGASUS Trial Participants Included in the Proteomic Analyses

- PEGASUS enrolled 95 adults aged 55 to 89 years with mild cognitive impairment or mild to moderate dementia (baseline Montreal Cognitive Assessment score ≥8) and supporting biomarkers of AD pathology[4]
- Participants were randomized to receive PB&TURSO (n=51) or matching placebo (n=44) for 24 weeks[4]
- CSF and plasma samples were prospectively collected at baseline and week 24 for analyses of the biomarkers shown in Table 1[4]
- Sixty-six participants (PB&TURSO, n=32; placebo, n=34) who received ≥1 dose of study medication and completed the study with both CSF and plasma samples having been successfully collected at baseline and week 24 were included in the proteomic analyses

TABLE 1. CSF BIOMARKERS ASSESSED IN PEGASUS[3-5,10,11]

Category	Biomarkers
Core AD pathology	$A\beta_{40}$, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$ ratio, total tau, p-tau^{181}
Synaptic and neuronal degeneration	Neurogranin, FABP3, NfL
Gliosis	YKL-40, GFAP
Neuroinflammation	IL-6, IL-8, IL-15, MCP-1/CCL2, MIP1β, MMP-10
Oxidative stress	8-OHdG
Metabolic	Leptin, sIR, 24-OHC

8-OHdG, 8-hydroxy-2-deoxyguanosine; 24-OHC, 24S-hydroxycholesterol; Aβ, amyloid beta; AD, Alzheimer's disease; 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; p-tau181, phosphorylated tau-181; sIR, soluble insulin receptor; YKL-40, chitinase 3–like protein 1.

Association Between CSF and Plasma Olink Proteins

METHODS: Analyzed correlation between CSF and plasma proteins

- A total of 288 proteins were quantified in CSF and plasma using 3 Olink® protein biomarker assay panels (Olink Proteomics, Uppsala, Sweden):
  - Target 96 Neurology
  - Target 96 Inflammation
  - Target 96 Cardiometabolic
- The associations between corresponding Olink proteins in CSF vs plasma were analyzed using Spearman correlation
- Multiple comparisons were accounted for by adjusting *P* values based on the false discovery rate (FDR)

RESULTS

- At baseline in the pooled placebo and PB&TURSO groups:
  - Forty-four Olink proteins (15.9%; 13 in the Inflammation panel, 15 in the Neurology panel, and 16 in the Cardiometabolic panel) showed significant associations between corresponding CSF and plasma levels; correlation coefficients ranged from 0.331 to 0.782
- Longitudinal change over 24 weeks in the placebo group:
  - There was no significant association between 24-week changes in the CSF and plasma levels for any Olink protein after adjustment for multiple comparisons

Analysis of Treatment Effect on Individual Olink Proteins

METHODS: Compared changes in CSF and plasma Olink proteins between the placebo and PB&TURSO groups in PEGASUS

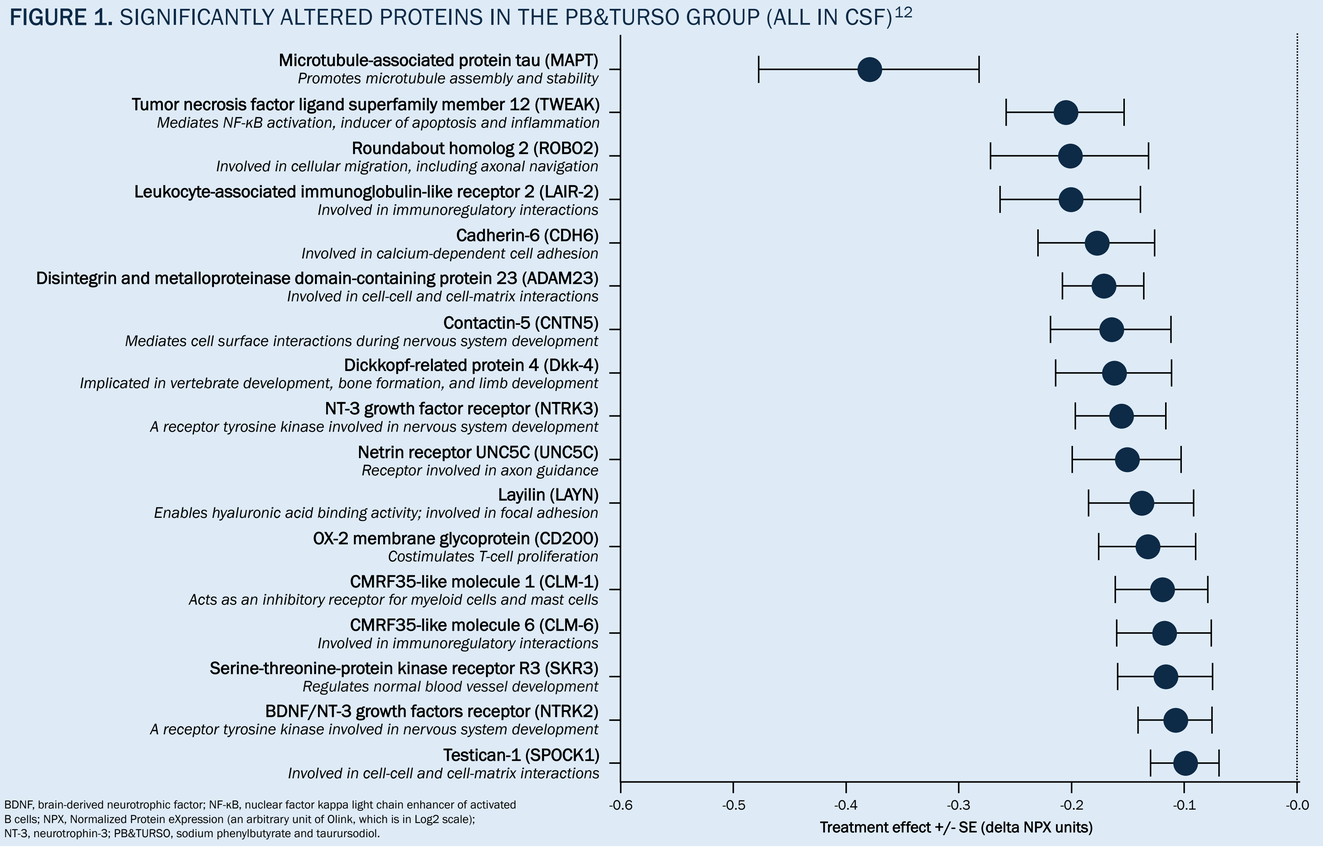
- Changes in each individual CSF and plasma protein level were compared between treatment groups by fitting separate analysis of covariance (ANCOVA) models with change in protein level as the outcome and age, sex, treatment group, and baseline protein level as covariates
- A standardized treatment effect for each protein was calculated by extracting the beta coefficient and *P* value for the treatment group covariate from the ANCOVA model
- FDR correction was applied to *P* values to adjust for multiple comparisons, with CSF and plasma proteins considered as separate "families" for which to adjust
- An enrichment analysis was performed to identify common biological processes and cellular components known among significantly altered OLINK proteins
  - This analysis cross-referenced the OLINK proteins with UniProt databases using the UniprotR package in R to extract the respective. Subsequently, publications directly naming each protein/protein ID were extracted

RESULTS

- A total of 78 Olink proteins (54 CSF and 24 plasma) were nominally altered in the PB&TURSO group compared with the placebo group
- Of these, 17 CSF proteins showed significant changes (all decreased) in the PB&TURSO group compared with the placebo group after correcting for multiple comparisons (**Figure 1**)
  - Enrichment analysis showed that these proteins were primarily involved in cell junction assembly and organization, central nervous system development, neuron migration, and synapse assembly
  - Enriched cellular components included axons, neuron projections, and plasma membranes, among others such as synapses

RESULTS (cont)

FIGURE 1. SIGNIFICANTLY ALTERED PROTEINS IN THE PB&TURSO GROUP (ALL IN CSF)[12]



BDNF, brain-derived neurotrophic factor; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NPX, Normalized Protein eXpression (an arbitrary unit of Olink, which is in Log2 scale); NT-3, neurotrophin-3; PB&TURSO, sodium phenylbutyrate and taurursodiol.

Association Between Changes in Significantly Altered Olink Proteins and CSF Biomarkers

METHODS: Analyzed association between 24-week changes in significantly altered Olink proteins and prespecified CSF biomarkers in PEGASUS

- The association between the 24-week changes in significantly altered proteins in the PB&TURSO group and 24-week changes in levels of prespecified CSF biomarkers (**Table 1**) was analyzed using linear regression, with change in the Olink protein as the outcome and change in the CSF biomarker, age, and sex as covariates

RESULTS

- Of the 17 significantly altered Olink proteins in the PB&TURSO group, 15 were found to be associated with CSF neurogranin; 14 with CSF p-tau^{181}; 13 with CSF total tau (12 using a different CSF total tau assay); 10 with CSF YKL-40; and 9 with CSF 24-OHC
- In all cases, decreases in the Olink panel protein were associated with decreases in the CSF biomarker concentration

CONCLUSIONS

- In the 24-week PEGASUS trial, our analyses of Olink proteins showed treatment effects of PB&TURSO across a variety of biological pathways in AD, with the largest effect in CSF related to tau and neurodegeneration. The results of PEGASUS are being evaluated further in consideration of potential next steps for the development of PB&TURSO in AD
  - PB&TURSO will be evaluated in the tauopathy, progressive supranuclear palsy, in the global phase 3 double-blind, placebo-controlled, multicenter ORION trial. The study is planned to initiate by the end of 2023
- Though exploratory, these analyses provide unique insights into the biological activity of PB&TURSO in AD beyond amyloid reduction and build upon the initial biomarker findings in PEGASUS

AMX0035 is an investigational drug in AD and has not been determined to be safe and effective by any health authority (eg, the EMA, FDA, PMDA, and Health Canada).

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Disclosures

NC received personal fees from Amylyx related to the submitted work. RM, MG, and LM are full-time employees of and may have stock option ownership in Amylyx Pharmaceuticals, Inc. RET is a paid consultant and shareholder in Amylyx Pharmaceuticals, Inc., and was involved with PEGASUS trial design and analyzing de-identified trial results following completion of the trial but not with the execution of the trial.

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