Published Ahead of Print on September 1, 2023 as 10.1212/WNL.0000000000207755

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The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

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Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000207755

Association of Plasma Phosphorylated Tau With the Response to Neflamapimod Treatment in Patients With Dementia With Lewy Bodies

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Equal Author Contribution:

Contributions:

Figure Count:

4

Table Count:

3

Search Terms:

[28] Dementia with Lewy bodies, Alzheimer's disease co-pathology, Plasma ptau181, neflamapimod, p38 alpha kinase inhibitor

Acknowledgment:

AscenD-LB Clinical Study investigators: USA: K. Amadeo (U. of Rochester, Rochester, NY), G. Baras (Elite Clinical Research, Miami, FL), K. Bell (Columbia U. Medical Center, New York, NY), C. Bernick (U. of Washington, Seattle, WA), B. Boeve (Mayo Clinic, Rochester, NY), N. Bohnen (Michigan Medicine, Ann Arbor, MI), S. Gomperts (Massachusetts General Hospital, Charlestown, MA), S. Holden (U. of Colorado, Aurora, CO), D. Kaufer (U. of North Carolina, Chapel Hill, NY), S. Kesari (Pacific Neuroscience Institute, Santa Monica, CA), A. Khan (Northwest Clinical Research Center, Bellevue, WA), I. Litvan (UC San Diego Health, San Diego, CA), S. Losk (Summit Research Network, Portland, OR), R. Pahwa (U. of Kansas Medical Center, Kansas City, KS), S. Pugh (Inland Northwest Research, Spokane, WA), J. Quinn (Oregon Health and Science University, Portland OR), A. Ritter (Cleveland Clinic, Las Vegas, NV), B. Shah (UVA Health, Charlottesville, VA), D. Scharre (Ohio State Neurological Institute, Columbus, OH), M. Serruya (Jefferson U. Hospitals, Philadelphia, PA), B. Tousi (Cleveland Clinic, Cleveland, OH); Netherlands: P. Dautzenberg (Brain Research Center (BRC) – Den Bosch, Den Bosch), A.W. Lemstra (Brain Research Center (BRC) – Amsterdam, Amsterdam). The authors are very grateful to all patients and caregivers who participated in this study and to the many staff members at the clinical sites for their dedication and commitment to the clinical study. We also acknowledge the study project teams at Worldwide Clinical Trials and EIP particularly Amanda Gardner, who was the clinical project manager for the study at EIP. In addition, we acknowledge Dr. Sylvie Grégoire for her critical reading of the manuscript. We also acknowledge Inge Verberk, PhD, and Sheriff Bayoumi, MSc at the Clinical Chemistry Laboratory at VUMc for their managing the conduct of analysis of plasma ptau181 levels in patient samples.

Study Funding:

The clinical study and the analysis of plasma ptau181 levels at baseline were funded by EIP Pharma, Inc.

Disclosure:

J. Alam and J. Conway are employees of, and S. Doctrow is an independent contractor to EIP Pharma Inc., a private enterprise that is developing neflamapimod and is the sponsor of the study that is the subject of this manuscript. H-M. Chu is an employee of Anoixis, a private enterprise contracted by EIP Pharma Inc. conduct the statistical analyses. P. Maruff and S. Gomperts report no disclosures relevant to the manuscript. C. Teunissen has a collaboration contract with Quanterix Corporation, the company that provides and markets the ptau181 assay utilized in the current study. Go to Neurology.org/N for full disclosures.

Preprint DOI:

Received Date:
2023-02-06

Accepted Date:
2023-06-21

Handling Editor Statement:
Submitted and externally peer reviewed. The handling editors were Deputy Editor Bradford Worrall, MD, MSc, FAAN and Assistant Editor Andrea Schneider, MD, PhD.

ABSTRACT

Background and Objectives

In a proportion of patients, dementia with Lewy bodies (DLB) is associated with Alzheimer's disease (AD) co-pathology, which is linked to accelerated cognitive decline and more extensive cortical atrophy. The objective herein was to evaluate the relationship between a biomarker of AD co-pathology, plasma tau phosphorylated at residue 181 (ptau181), and the treatment effects of the p38α kinase inhibitor neflamapimod, that targets the cholinergic degenerative process in DLB.

Methods

The AscenD-LB study was a phase 2a randomized (1:1) 16-week placebo-controlled clinical trial of neflamapimod in DLB, the main results of which have been published. After the study was completed (*i.e*., *post-hoc*), pre-treatment plasma ptau181 levels were determined and participants grouped based on a cut-off for AD pathology of 2.2 pg/mL (established in a separate cohort to identify AD from healthy controls). Clinical outcomes for the comparison of placebo with neflamapimod 40mg three-times-daily (TID; the higher, and more clinically active of two doses studied) were analyzed utilizing Mixed Models for Repeated Measures within each sub-group (baseline plasma ptau181 < and ≥2.2 pg/mL).

Results

Pre-treatment plasma ptau181 levels determined in eight-five participants with mild-to-moderate DLB receiving cholinesterase inhibitors; with 45 participants below, and 40 above, the 2.2 pg/mL cut-off at baseline. In the 16-week treatment period, in the comparison of placebo with neflamapimod 40mg TID, for all endpoints evaluated, improvements with neflamapimod

treatment were greater in participants below the cut-off, compared with that in those above the cut-off. In addition, participants below the ptau181 cut-off at baseline showed significant improvement over placebo in an Attention Composite measure (+0.42, 95%CI: 0.07–0.78, p=0.023, d=0.78), the Clinical Dementia Rating Scale Sum of Boxes (–0.60, 95%CI:-1.04,-0.06, p=0.031, d=0.70), the Timed Up and Go test (–3.1 sec, 95%CI:-4.7,-1.6, p<0.001, d=0.74), and International Shopping List Test-Recognition (+1.4, 95% CI: 0.2–2.5, p=0.024, d=1.00).

Discussion

Exclusion of patients with elevated plasma ptau181, potentially through excluding patients with extensive cortical neurodegeneration, enriches for a DLB patient population that is more responsive to neflamapimod. More generally, plasma biomarkers of AD co-pathology at study entry should be considered as stratification variables in DLB clinical trials. NCT04001517 at clinicaltrials.gov.

INTRODUCTION

In dementia with Lewy bodies (DLB), degeneration in the basal forebrain occurs early in the disease process and is prominent.[1, 2] The resulting deficit in cholinergic neurotransmission has been proposed as the reason that DLB patients are more responsive to acetylcholinesterase inhibitors (AChEIs) than those with Alzheimer's disease (AD).[3] While most patients with DLB develop cortical atrophy, those with the least AD co-pathology show relatively less atrophy of the medial temporal lobe,[4] than do patients with AD. Conversely, patients with DLB with evidence of AD pathology show more severe cortical atrophy,[5] including in the medial temporal lobe.[6] In addition, concomitant abnormality of AD-associated biomarkers (*e.g.*, reduced CSF levels of amyloid beta 1-42, elevated CSF phosphorylated tau, and/or PET amyloid signal above threshold), seen in approximately 50% of patients with DLB, impacts the clinical profile, including the rate of cognitive decline,[7-9] and might impact response to AChEIs.[10] However, the practical implications of AD co-pathology on management of patients with DLB in either clinical practice or clinical trials remain to be defined.

An opportunity to understand the relative contribution of basal forebrain cholinergic degeneration, relative to that of AD co-pathology, was afforded by a clinical trial of an investigational drug, neflamapimod, that targets the molecular mechanisms underlying degeneration of cholinergic neurons in the basal forebrain.[11] In preclinical studies,[12] neflamapimod reversed the cholinergic neurodegenerative phenotype in the Ts2 transgenic mouse model of Down Syndrome (DS), an animal model that, along with the developmental defects associated with DS, develops basal forebrain cholinergic degeneration in adulthood. In the same animal model, neflamapimod improved behavioral outcomes associated with cholinergic function, while having only a modest impact on long-term potentiation in the CA1–

CA3 region of the hippocampus. Further, in an exploratory 16-week phase 2a placebo controlled clinical trial, the AscenD-LB study,[12] neflamapimod reduced dementia severity and improved motor function, outcomes associated with basal forebrain cholinergic function in patients with DLB. In addition, the highest dose of neflamapimod studied (40mg TID) improved cognition in that study, with the greatest benefits observed for aspects of attention.

While the AscenD-LB study was ongoing, multiple reports from independent studies were published[13-15] demonstrating that plasma tau phosphorylated either at position 181 (ptau181) or 217 (ptau217) predicted, in patients with preclinical or clinical AD, amyloid plaque status (by either PET or CSF), tau pathology (by PET), and/or cerebral, particularly medial temporal lobe, neurodegeneration and atrophy. Moreover, after the AscenD-LB study was completed, Hall et al[16] reported that in patients with DLB, levels of plasma phosphorylated tau, either ptau181 or ptau217, could be utilized to predict the presence of concomitant AD pathology, assessed by either abnormal tau PET scan in temporal cortex or abnormal CSF Aβ42 /Aβ40 ratio [area under the curve (AUC) > 0.78 and > 0.81,respectively].[16] In addition, plasma ptau181 or ptau231 concentrations were reported to be associated with cognitive decline in a long-term (minimum 5 years' follow-up) longitudinal cohort study in patients with DLB.[17] In the latter study, similarly to the findings of Hall *et al*., the patients with abnormal CSF Aβ42 levels had higher plasma concentrations of both p-tau markers than did those with normal CSF levels and the AUC for plasma ptau181 predicting reduced CSF Aβ42 was 0.62.

Based on these developments in the literature regarding plasma ptau as a biomarker in patients with DLB, as well as hypothesizing that the effects of neflamapimod are dependent on the presence of AD co-pathology (specifically, medial temporal lobe tau pathology and/or neurodegeneration), we determined plasma levels of ptau181 in the stored pretreatment samples

from the AscenD-LB clinical study and re-analyzed the efficacy outcomes after stratifying patients by whether pretreatment plasma ptau181 level was elevated above an *a priori* defined cut-off. In addition to informing on the utility of ptau181 as a marker of responsiveness to neflamapimod, our findings increase understanding of the contribution of AD pathology and cortical neurodegeneration on the clinical expression of DLB.

METHODS

Participants and Study Design

The full design of the AscenD-LB study and its patient inclusion/exclusion criteria, participant characteristics, and main results were described previously.[12] Briefly, it was a 16-week phase 2a clinical study, double-blind and placebo-controlled, in 91 patients with mild-to-moderate (Mini Mental Status Examination, MMSE, between 16 and 28) DLB, also receiving cholinesterase inhibitor therapy. Criteria for mild-to-moderate DLB were consistent with consensus clinical criteria [18]: dementia, with at least one core clinical feature of DLB and demonstrated abnormality in dopamine uptake by DaTscan™ (Ioflupane I123 SPECT). The first participant was enrolled on September 30, 2019 and the last visit was on July 14, 2020.

As previously described[12], the primary outcome measure of the study was a study-specific six-test neuropsychological test battery (NTB) designed to assess the cognitive domains most impacted in DLB: attention (Identification, Detection tests), executive function (Category Fluency, Letter Fluency, One Back accuracy), and visual learning (One Card Learning). Secondary objectives included evaluating effects of neflamapimod effects on the Clinical Dementia Rating Scale, on motor function (assessed by the Timed Up and Go, TUG, test), and on memory [assessed by International Shopping List Test (ISLT), Immediate Recall, Delayed Recall and Recognition Index]. The Clinical Dementia Rating Scale Sum of Boxes (CDR-SB),

designed to assess both cognition and function, scores six domains is obtained by clinicians rating the severity of symptoms across six domains (memory, orientation, judgement & problem solving, community affairs, home & hobbies, and personal care) following a semi-structured interview with the subject and a reliable informant (*e.g*. family member) on a 0–3 scale for each domain (total range 0–18, with a higher score indicating worse dementia). .[19] The ISLT is a validated 12-word verbal list learning test that reports out three scores: Immediate Recall, (scored 0–36, the sum of three immediate recall trials), Delayed Recall (0–12, single recall, 20 to 25 min after initial trials), and Recognition (0–12, accurate recognition of the words in the original trials).[20, 21] The TUG test, measuring functional mobility, monitors the time in seconds that a subject takes to rise from a chair, walk three meters, turn, walk back to the chair, and sit down while turning 180 degrees.[22] The NTB and ISLT were performed at baseline and at weeks 4, 8, and 12 during treatment. The CDR-SB and TUG test were performed at baseline and at weeks 8 and 16. The MMSE was also an outcome measure of the study. However, COVID-19 pandemic restrictions on site visits to the clinical research centers led to more than third of on-study MMSE evaluations being missed or conducted remotely by video, an as-yet unvalidated approach. Because of this limitation, the on-treatment MMSE data were not evaluated in the main reporting of study results,[12] nor evaluated herein.

Patients were randomized via an Interactive Response Technology (IRT, Suvoboda, Inc., Conshocken, PA, USA) 1:1 to either neflamapimod (NFMD) 40mg capsules or matching placebo and then, based on body weight, assigned to either a twice-daily (BID) [weight<80 kg; 40mg BID neflamapimod or placebo BID] or thrice-daily (TID) [weight≥80 kg; 40mg TID neflamapimod or placebo TID] regimen.

The study protocol stated that an analysis based on stratification by an AD-associated plasma biomarker would be conducted. However, at the time, the specific biomarker and appropriate cut-offs for the presence of AD pathology in DLB were not available. Instead, plasma samples were obtained from subjects prior to treatment and stored until such a biomarker became available, with the intent to analyze those samples in the future for whether the AD co-pathology was present.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was registered at clinicaltrials.gov (NCT04001517) on June 28, 2019 and in the EU Clinical Trials Register (EudraCT Number 2019-001566-15) June 26, 2019 and was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Applicable local/central ethics committee or IRB approvals were obtained as previously described[12] and all participants provided written informed consent and were uncompensated. The study was performed, and this manuscript prepared in accordance with ICJME guidelines. The protocol and statistical analysis plan (SAP) may be accessed at https://clinicaltrials.gov/ct2/show/study/NCT04001517.

Plasma ptau181 measurements

The primary study completion date for the AscenD-LB study was July, 2020 and the primary study analyses were completed in September, 2020. Following the report in March, 2021 that plasma ptau181 predicted AD co-pathology in DLB,[16] ptau181 levels in the stored plasma samples were assayed in August 2021 in all patients who had at least one on-study efficacy evaluation (see CONSORT flow diagram, Figure 1). Plasma p-tau181 measurements were performed in the Neurochemistry laboratory at the Amsterdam University Medical Centers, following instructions in the assay kit instructions (p-Tau181 V2 kit, Quanterix, Billerica, MA,

USA) on the Simoa HD-X platform (Quanterix). In-house quality control plasma samples were assayed in duplicate at the start and end of each plate to assess within-run and between-run variations. The assay had been validated internally and, when evaluated in an in-house panel of patients with AD dementia, performed similarly to the other available plasma ptau181 assays to differentiate patients with AD from healthy controls, with an AUC of 0.94 (95% CI: 0.89–0.99) using a cut-off of 2.2 pg/mL.[23]

Statistical Analyses

Because the different tests in the NTB have various scales, each test score was converted to a *z*-score as previously described[12]. This enabled all tests to be equally weighted in the NTB composite *z*-score, which was calculated by averaging the z-scores from the components of the six individual tests. For greater understanding of treatment effects on the cholinergic system, an Attention Composite endpoint was also calculated, comprising only the two tests in the NTB that evaluate information processing speed, that is, the Identification and Detection tests.

Whether baseline characteristics were influenced by AD co-pathology was evaluated by comparing the value for each characteristic in patients with elevated (≥ 2.2 pg/mL) baseline plasma ptau181 to that in those with low (<2.2 pg/mL) plasma ptau181 using a *t*-test without adjustment for multiple comparisons. With respect to evaluating treatment response, as pre-specified in the study protocol, the analyses of all efficacy endpoints (primary, secondary, and exploratory) used a Mixed Model for Repeated Measures (MMRM) analysis method with change from baseline as the dependent variable, with study visit and baseline composite score as covariates[12]. Of note, the MMRM approach includes all timepoints on study (*i.e.*, is not an end-of-treatment analysis), which were at baseline and at weeks 4, 8, and 12 for the NTB, Attention, and ISLT evaluations; and at baseline and at weeks 8 and 12 for the CDR-SB and TUG test. For

each endpoint, the MMRM analysis was conducted separately for participants with baseline plasma ptau181 levels below (<) and greater than or equal to (≥) the cut-off of 2.2 pg/mL. As neflamapimod treatment effects on the cognitive endpoints were confined to the 40mg TID dose group and the objective of this report was to evaluate the association of baseline plasma ptau181 with treatment response, the primary focus of this report is the results of the comparison of NFMD 40mg TID versus all placebo, a comparison that was included in the SAP as a secondary (exploratory) analysis to evaluate dose-response; however, we also conducted and report two sensitivity analyses of the results in patients with baseline plasma ptau181 below the cut-off, in which we compared 40 TID recipients with placebo TID and all neflamapimod (40mg BID and 40mg TID) with all placebo, respectively. As the current analysis was exploratory in nature and formally *post-hoc*, the results are reported as mean difference between placebo and neflamapimod, with 95% confidence intervals. *P*-values are also reported, but to evaluate the strength of the evidence rather than to make inferences regarding efficacy. Cohen's *d* effect size was also calculated for each comparison.

Data availability

Individual participant de-identified data that underlies the clinical trial results reported herein will be provided to investigators whose proposed use has been approved by an independent review committee, upon reasonable request to the corresponding author (JJA). These data will be available beginning 9 months, and ending 36 months, after publication. If/when neflamapimod receives marketing approval, patient-level clinical trial data will be shared through the Vivli data-sharing platform (vivli.org).

RESULTS

Baseline characteristics

Baseline demographic and disease characteristics by treatment group and plasma ptau181 strata (< or ≥ 2.2 pg/mL) are shown in Table 1. Mean (SD) baseline plasma ptau181 was 1.6 (0.4) pg/mL in participants below the cut-off at baseline and 3.4 (1.4) in those above the cut-off. Consistent with the literature, patients with elevated plasma ptau181 (*i.e.*, those with AD co-pathology) had worse cognitive function, with significantly lower MMSE scores [mean 22.0 (SD=3.6) vs. 24.0 (3.4) for ptau181<2.2 pg/mL; p=0.008] and significantly higher CDR-SB scores [mean 5.5 (2.8) vs. 4.5 (2.1) for ptau181<2.2 pg/mL; p=0.048]. The mean NTB-cognitive test battery *z*-scores and ISLT Immediate Recall scores were also numerically lower in participants with baseline ptau181 ≥2.2 pg/mL, and these participants were numerically older than those with baseline ptau181 < 2.2 pg/mL, but the differences were not significant (p=0.13 for NTB, p=0.10 for ISLT; p=0.13 for age). Notably, there were no significant differences in the profiles, with respect to "core features" of DLB (*i.e.*, fluctuating cognition, visual hallucinations, REM sleep disorder, parkinsonism), between the participants above or below the ptau181 cut-off. Within each strata (< or ≥ 2.2 pg/mL), there were no significant differences in any of the characteristics between placebo and neflamapimod 40mg TID recipients (Table 2).

Treatment effects of neflamapimod analyzed by baseline plasma ptau181

To evaluate the potential impact of AD co-pathology on neflamapimod treatment effect, we first conducted plasma ptau181 stratified analyses on the endpoints that demonstrated significant improvement, compared with placebo, in the 40mg TID dose in the results previously reported:[12] the NTB (cognitive test battery), Attention Composite, CDR-SB, and TUG test. When the analyses were thus repeated, taking into account plasma ptau181 levels, the

neflamapimod treatment response (*i.e.*, the difference between neflamapimod 40mg TID and placebo from MMRM analysis) in the patients with baseline plasma ptau181 <2.2 pg/mL appeared to be greater than in those with baseline plasma ptau181 levels ≥2.2 pg/mL (Figures 2 and 3), with significant differences between treatment and placebo that were not observed in patients with the higher baseline plasma ptau181 levels. Specifically, in the patients with baseline ptau181 <2.2 pg/mL, there was significant improvement, compared with placebo, in the MMRM analysis (Table 3) for the Attention Composite (40mg TID-placebo difference=0.42, 95%CI 0.07–0.78, p=0.023; Cohen's d=0.78), CDR-SB (40mg TID-placebo difference=-0.60, 95%CI:-1.04,-0.06, p=0.031; d=0.70), and TUG test (40mg-placebo difference=-3.1 sec, 95%CI:-4.7,-1.6, p<0.001; d=0.74). For the NTB, there also appeared to be a positive treatment effect in patients with baseline ptau181<2.2 pg/mL, and one that was greater than in patients with baseline ptau181≥ 2.2 pg/mL, but with the smaller sample size the 95% confidence interval crosses zero (40mg TID-placebo difference 0.21, 95% CI-0.07, 0.49, p=0.13; d=0.56).

Having observed a neflamapimod treatment effect of greater magnitude in patients with baseline plasma ptau181<2.2 pg/mL on the aforementioned outcome measures, we then conducted analyses with stratification for ptau181 for the ISLT measures, in which there were no discernible treatment effects of neflamapimod 40mg TID in the previously reported main results.[12] As shown in Figure 4, for the ISLT, the difference between placebo and neflamapimod 40mg TID in the patients with baseline plasma ptau181 <2.2 pg/mL was positive (*i.e.*, improved compared with placebo) and appears to be greater than in those with baseline plasma ptau181 levels ≥2.2 pg/mL for ISLT Immediate Recall and for ISLT Recognition. Specifically, when MMRM analysis was confined to patients with ptau181<2.2 pg/mL at baseline, a strong positive trend favoring NFMD 40mg TID was seen for ISLT Immediate Recall (Table 3, difference vs.

placebo=2.1 words 95% CI 0.0, 4.2, p=0.053; d=0.55) and significant positive improvement favoring NFMD 40mg TID for ISLT Recognition (Table 3, difference vs. placebo=1.4 words, 95% CI: 0.2–2.5, p=0.024; d=1.00). There were no significant differences between NFMD 40mg TID and placebo on ISLT Delayed Recall, in either plasma ptau181 strata (Figure 3, Table 2). The pattern of an effect on Immediate Recall and Recognition, without an effect on Delayed Recall, is most consistent with an effect on executive function, which is consistent with the study results overall and a mechanism of action of neflamapimod on the cholinergic system.[12]

In addition to the comparison of 40mg TID vs. all placebo recipients, two sensitivity analyses were performed of the results in patients with plasma ptau181 < 2.2 pg/mL at baseline. First, because the TID dosing (either neflamapimod 40mg or placebo matching capsules) was administered only to patients with weights ≥ 80kg, MMRM analyses of the data from patients with baseline plasma ptau181 < 2.2 pg/ were also conducted to compare the response only in the two higher weight cohorts (*i.e.*, within the patients without elevated plasma ptau181, to compare patients receiving 40mg TID to the patients receiving placebo TID), and the results (eTable 1) were similar to those for the comparison of 40mg TID to all placebo recipients . In the second sensitivity analysis, all neflamapimod recipients (*i.e.*, including patients who received the lower dose in the study of 40mg BID) with baseline plasma ptau181 < 2.2 pg/mL were compared to all placebo recipients with baseline plasma ptau181 < 2.2 pg/mL. Given that in the previously published analysis[12] 40mg BID showed limited to no efficacy, while similar trends favoring neflamapimod were seen, the magnitude of the neflamapimod treatment effect compared with placebo, evaluated by Cohen's d effect size, in this second sensitivity analsysis was lower for all the endpoints (eTable 2; d≤0.4, except for CDR-SB, where d=0.58), compared with the effect

sizes shown in Table 3 (*i.e.*, for the comparison of 40mg TID and placebo in patients with baseline plasma ptau181 < 2.2 pg/mL).

Additional results from this clinical trial have been published[12] and area also available at clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/results/NCT04001517).

DISCUSSION

Data from a study of a novel therapeutic modality directed at cholinergic dysfunction and degeneration in patients with DLB[12] were reorganized and analyzed to account for pretreatment levels of plasma ptau181. The results showed that those patients without elevated plasma ptau181 levels (*i.e.*, below a cut-off value of 2.2 ng/mL), had substantial treatment benefits in attention, dementia severity and motor functional mobility, as well as in memory. The magnitude of these benefits was moderate to large by convention (*i.e.*, d>0.7), rendering group comparisons as statistically significant despite the relatively small sample sizes (n=18–22 for placebo and n=10–11 for neflamapimod, with baseline ptau181 below cut-off). More specifically, patients with DLB and plasma ptau181 levels within normal limits, in this 16-week clinical study of neflamapimod, demonstrated significant improvement over placebo on cognitive tests of attention, the CDR-SB, the TUG test measuring functional mobility, and ISLT-Recognition, a measure of memory retrieval. The finding that neflamapimod, an agent targeting cholinergic degeneration, has a beneficial treatment effect in DLB is consistent with the known prominent cholinergic deficit in the disease,[1, 24] as well as with several recent translational studies reporting that the strongest MRI finding associated with cognitive dysfunction in DLB is atrophy of the nucleus basalis of Meynert.[25-27] Indeed, in relation to the dementia component, the cholinergic system may be the primary site of neurodegeneration in DLB, as basal forebrain cholinergic

degeneration was the major MRI finding in prodromal DLB, with cortical atrophy, starting in the inferior temporal lobe, developing as patients progressed to dementia.[2]

With respect to the association between plasma ptau181 and treatment response it is important to note that, in DLB, such elevated plasma levels of phosphorylated tau are associated with more extensive neurodegeneration[6, 7, 28] and more rapid clinical disease progression.[17, 29] This literature is compatible with our observation that elevated plasma ptau181 is associated with poorer cognitive function and greater dementia severity. These findings are also consistent with prior reports of pathology and/or MRI data indicating that patients with DLB and without AD co-pathology (cortical tau pathology and/or neuritic amyloid plaques) have minimal cortical atrophy, particularly in the medial temporal lobe.[4-6, 30] Accordingly, the readiest explanation for the better outcome in subjects with plasma ptau181 <2.2 pg/mL is that they represent a patient population having less advanced disease, in particular less extensive neuronal death and secondary cortical atrophy which, otherwise, are associated with and potentially result from AD co-pathology. That is, although future work will be required to differentiate between these possibilities, we expect that AD co-pathology is not directly modulating treatment response but, instead, that the patients in our study with ptau181<2.2 pg/mL have less extensive neurodegeneration in the cortex, *i.e.*, less irreversible neuronal loss and fixed deficits, and therefore would be more likely to show functional improvement within a 16-week treatment period. In particular, because the role of the basal forebrain cholinergic system is to modulate tasks conducted in the cortex, and not to perform the tasks themselves, a finding that cortical atrophy would limit treatment response may be expected for an agent, such as neflamapimod, presumed to act on the cholinergic system. The concept that patients with DLB have partly reversible deficits, that are limited by neurodegeneration in those with AD co-pathology, is

consistent with results of a Tau PET study[31] in DLB. In that study, tau binding in DLB patients was not different than in controls, except in those with AD co-pathology (by CSF or amyloid PET), where subtly greater tau binding was evident in the medial temporal lobe and occipital lobes. There were, in addition, reductions in occipital and lateral parietal relative cerebral blood flow in DLB patients, which are potentially reversible, compared with in controls and AD patients, that correlated with cognitive dysfunction.

The notion that medial temporal lobe (*i.e.*, hippocampal) neurodegeneration would limit treatment response is consistent with the preclinical results with neflamapimod, as well as with the clinical findings described herein. Specifically, in DS mice that develop basal forebrain cholinergic degeneration, neflamapimod treatment restored behavioral outcomes associated with the basal forebrain cholinergic system but only modestly improved hippocampal function, evaluated as LTP in the CA1–CA3 region.[12] Of note, it has been reported that the cholinergic degenerative process is reversible in DS mice[32], while in the hippocampus they develop neurodegeneration and fixed structural changes.[33, 34] Our results in the TUG test (Figure 3b) and ISLT-Recognition (Figure 4), showing the most prominent treatment effect differences between plasma ptau181 strata, are instructive in that regard, because both measures would be expected to be influenced by the cholinergic input from the medial septal nucleus of the basal forebrain to the hippocampus.[35]

Recently, results similar to ours with respect to the association of a plasma biomarker of AD-co-pathology on treatment response to a novel agent were reported[36] with the PDE9 inhibitor, irsenontrine; in a 12-week placebo-controlled phase 2 study in DLB, "amyloid negative" patients (evidenced by a high plasma Aβ42/40 ratio) demonstrated a trend (p=0.053) towards improvement in cognition, measured using the Montreal Cognitive Assessment, while

no such trends were evident in "amyloid positive" patients. Together with our results, these findings point to the potential utility of a plasma biomarker for underlying pathology to decrease patient heterogeneity either as a covariate or by excluding patients with a concomitant, and potentially confounding, pathology. Though much work will be required to establish any one measure as a treatment response marker, we would suggest that a plasma biomarker of AD co-pathology be considered as a baseline stratification factor for randomization in future therapeutic clinical trials in DLB. Utilizing stratification for such a biomarker at randomization would be particularly important in phase 2 trials, as the relatively small size of the trials could not otherwise guarantee a balance among treatment groups, and the impact of cortical neurodegeneration on short-term effects may be more prominent than in longer duration clinical trials.

There are several limitations to our report. First, formally, our analysis was conducted *post-hoc*, *i.e.*, after the primary study data were analyzed and reported. However, to temper this, an analysis based on baseline AD co-pathology status by a plasma biomarker was defined in the protocol; in addition, the cut-off was independently developed (against a panel of AD patients and controls)[23] and selected prior to conducting the analysis. Second, after stratification and limiting the treatment effect analysis to those participants receiving the higher neflamapimod dose, the number of patients within the sub-groups are small, inherently requiring a larger study to confirm the results. Third, from a biological perspective, despite the preclinical data showing an effect on the cholinergic system and the supportive clinical associations presented herein and in a prior report,[12] without a specific biomarker or clinical endpoint for cholinergic degeneration and/or function, we cannot be certain that the therapeutic effects of neflamapimod are mediated via acting on cholinergic dysfunction and degeneration. This does not impact the interpretation

that plasma ptau181 is useful to enrich the patient population for treatment responsiveness to neflamapimod; however, it limits the extent that the results can be interpreted, with respect to the role of the cholinergic system in disease expression and progression in DLB.

In conclusion, exclusion of patients with elevated plasma ptau181 levels in a *post-hoc* analysis, potentially by excluding patients with more extensive cortical neurodegeneration, enriches for a DLB patient population that is more responsive to neflamapimod, a therapy directed at cholinergic degeneration. More generally, plasma biomarkers of AD co-pathology at study entry should be considered as stratification variables in DLB clinical trials.

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WNL-2023-000517_etab1 ---http://links.lww.com/WNL/D91

WNL-2023-000517_etab2 ---http://links.lww.com/WNL/D92

References

1. Grothe MJ, Schuster C, Bauer F, Heinsen H, Prudlo J, Teipel SJ. Atrophy of the cholinergic basal forebrain in dementia with Lewy bodies and Alzheimer's disease dementia. J Neurol 2014;261:1939-1948.

2. Kantarci K, Nedelska Z, Chen Q, et al. Longitudinal atrophy in prodromal dementia with Lewy bodies points to cholinergic degeneration. Brain Commun 2022;4:fcac013.

3. Van Der Putt R, Dineen C, Janes D, Series H, McShane R. Effectiveness of acetylcholinesterase inhibitors: diagnosis and severity as predictors of response in routine practice. Int J Geriatr Psychiatry 2006;21:755-760.

4. Watson R, O'Brien JT. Differentiating dementia with Lewy bodies and Alzheimer's disease using MRI. Neurodegen Dis Manage 2012;2:411–420.

5. Hansen LA, Daniel SE, Wilcock GK, Love S. Frontal cortical synaptophysin in Lewy body diseases: relation to Alzheimer's disease and dementia. J Neurol Neurosurg Psychiatry 1998;64:653-656.

6. Ye R, Touroutoglou A, Brickhouse M, et al. Topography of cortical thinning in the Lewy body diseases. Neuroimage Clin 2020;26:102196.

7. Abdelnour C, Ferreira D, Oppedal K, et al. The combined effect of amyloid-beta and tau biomarkers on brain atrophy in dementia with Lewy bodies. Neuroimage Clin 2020;27:102333.

8. Lemstra AW, de Beer MH, Teunissen CE, et al. Concomitant AD pathology affects clinical manifestation and survival in dementia with Lewy bodies. J Neurol Neurosurg Psychiatry 2017;88:113-118.

9. Nedelska Z, Schwartz CG, Lesnick TG, et al. Association of Longitudinal β-Amyloid Accumulation Determined

by Positron Emission Tomography With Clinical and Cognitive Decline in Adults With Probable Lewy Body Dementia. JAMA Network Open 2019;2:e1916439.

10. Graff-Radford J, Boeve BF, Pedraza O, et al. Imaging and acetylcholinesterase inhibitor response in dementia with Lewy bodies. Brain 2012;135:2470-2477.

11. Alam JJ, Nixon RA. Disease-modifying pharmacological approaches to correcting basal forebrain cholinergic neuronal (BFCN) dysfunction and degeneration. Neuropsychopharmacology 2022;47:405-406.

12. Jiang Y, Alam JJ, Gomperts SN, et al. Preclinical and randomized clinical evaluation of the p38alpha kinase inhibitor neflamapimod for basal forebrain cholinergic degeneration. Nat Commun 2022;13:5308.

13. Moscoso A, Grothe MJ, Ashton NJ, et al. Longitudinal Associations of Blood Phosphorylated Tau181 and Neurofilament Light Chain With Neurodegeneration in Alzheimer Disease. JAMA Neurol 2021;78:396-406.

14. Tissot C, A LB, Therriault J, et al. Plasma pTau181 predicts cortical brain atrophy in aging and Alzheimer's disease. Alzheimers Res Ther 2021;13:69.

15. Wang YL, Chen J, Du ZL, et al. Plasma p-tau181 Level Predicts Neurodegeneration and Progression to Alzheimer's Dementia: A Longitudinal Study. Front Neurol 2021;12:695696.

16. Hall S, Janelidze S, Londos E, et al. Plasma Phospho-Tau Identifies Alzheimer's Co-Pathology in Patients with Lewy Body Disease. Mov Disord 2021;36:767-771.

17. Gonzalez MC, Ashton NJ, Gomes BF, et al. Association of Plasma p-tau181 and p-tau231 Concentrations With Cognitive Decline in Patients With Probable Dementia With Lewy Bodies. JAMA Neurol 2022;79:32-37.

18. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology 2017;89:88-100.

19. McDougall F, Edgar C, Mertes M, et al. Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive and Functional Outcomes in a Prodromal Alzheimer's Disease Population. J Prev Alzheimers Dis 2021;8:151-160.

20. Thompson TA, Wilson PH, Snyder PJ, et al. Sensitivity and test-retest reliability of the international shopping list test in assessing verbal learning and memory in mild Alzheimer's disease. Arch Clin Neuropsychol 2011;26:412-424.

21. Bock JR, Russell J, Hara J, Fortier D. Optimizing Cognitive Assessment Outcome Measures for Alzheimer's Disease by Matching Wordlist Memory Test Features to Scoring Methodology. Front Digit Health 2021;3:750549.

22. Nocera JR, Stegemoller EL, Malaty IA, et al. Using the Timed Up & Go test in a clinical setting to predict falling in Parkinson's disease. Arch Phys Med Rehabil 2013;94:1300-1305.

23. Bayoumy S, Verberk IMW, den Dulk B, et al. Clinical and analytical comparison of six Simoa assays for plasma P-tau isoforms P-tau181, P-tau217, and P-tau231. Alzheimers Res Ther 2021;13:198.

24. Duda JE. Pathology and neurotransmitter abnormalities of dementia with Lewy bodies. Dement Geriatr Cogn Disord 2004;17 Suppl 1:3-14.

25. Yoo HS, Jeon S, Cavedo E, et al. Association of beta-Amyloid and Basal Forebrain With Cortical Thickness and Cognition in Alzheimer and Lewy Body Disease Spectra. Neurology 2022;98:e947-e957.

26. Schumacher J, Ray NJ, Hamilton CA, et al. Cholinergic white matter pathways in dementia with Lewy bodies and Alzheimer's disease. Brain 2022;145:1773-1784.

27. Schumacher J, Taylor JP, Hamilton CA, et al. In vivo nucleus basalis of Meynert degeneration in mild cognitive impairment with Lewy bodies. Neuroimage Clin 2021;30:102604.

28. Chen Q, Przybelski SA, Senjem ML, et al. Longitudinal Tau Positron Emission Tomography in Dementia with Lewy Bodies. Mov Disord 2022;37:1256-1264.

29. van de Beek M, Ooms FAH, Ebenau JL, et al. Association of the ATN Research Framework With Clinical Profile, Cognitive Decline, and Mortality in Patients With Dementia With Lewy Bodies. Neurology 2022;98:e1262-e1272.

30. Amin J, Holmes C, Dorey RB, et al. Neuroinflammation in dementia with Lewy bodies: a human post-mortem study. Transl Psychiatry 2020;10:267.

31. Wolters EE, van de Beek M, Ossenkoppele R, et al. Tau PET and relative cerebral blood flow in dementia with Lewy bodies: A PET study. Neuroimage Clin 2020;28:102504.

32. Mufson EJ, Counts SE, Perez SE, Ginsberg SD. Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. Expert Rev Neurother 2008;8:1703-1718.

33. Kurt MA, Kafa MI, Dierssen M, Davies DC. Deficits of neuronal density in CA1 and synaptic density in the dentate gyrus, CA3 and CA1, in a mouse model of Down syndrome. Brain Res 2004;1022:101-109.

34. Lorenzi HA, Reeves RH. Hippocampal hypocellularity in the Ts65Dn mouse originates early in development. Brain Res 2006;1104:153-159.

35. Tsanov M. Basal Forebrain Impairment: Understanding the Mnemonic Function of the Septal Region Translates in Therapeutic Advances. Front Neural Circuits 2022;16:916499.

36. Irizarry M, Lai R, Hersch S, Pinner K, Dhadda S, Kramer L. Results of a phase 2/3 placebo-controlled, double-blind, parallel-group. rendomized study to evaluate the efficacy and safety of 12 week treatment with the phosphodiesterase 9 (PDE9) inhibitor irsenontrine (E2027) in subjects with dementia with Lewy bodies. J Prev Alz Dis 2022;9:S13 (Abstract OC#12).

Table 1. Baseline characteristics by plasma ptau181 status

	Baseline ptau181 < 2.2 pg/mL (N=45)	Baseline ptau181 ≥ 2.2 pg/mL (N=40)	*p*-value for comparison of < vs. ≥ 2.2 ng/mL groups
Age (y)	71.7 (6.4)	73.9 (7.0)	0.13
Male	84%	85%	>0.2
CDR Sum of Boxes	4.5 (2.1)	5.5 (2.8)	0.048
MMSE	24 (3.4)	22 (3.6)	0.008
ISLT-Immediate	15 (6.0)	13 (5.0)	0.10
ISLT -Delayed	4 (2.5)	4 (2.4)	>0.2
ISLT-Recognition	10.4 (1.4)	10.1 (1.8)	>0.2
Timed Up and Go	13 (6.1)	13 (4.1)	>0.2
NTB *z*-score	0.13 (0.76)	-0.12 (0.73)	0.13
Fluctuating cognition	53%	65%	>0.2
Visual hallucinations	58%	58%	>0.2
REM sleep disorder	71%	60%	>0.2
Parkinsonism	80%	83%	>0.2

Legend: Mean (SD), except when shown as percentage. *p*-value determined by *t*-test. For ISLT-delayed, ISLT-recognition and NTB (Neuropsychological Test Battery) *z*-score: missing data for 2 participants with ptau>2.2 pg/mL. Also missing TUG (Timed Up and Go) test data for 1 participant with baseline ptau181≥ 2.2 pg/mL.

Table 2. Baseline characteristics by plasma ptau181 status and treatment group

	Baseline ptau181 < 2.2 pg/mL		Baseline ptau181 ≥ 2.2 pg/mL	
	Placebo (N=23)	Neflamapimod 40mg TID (N=11)	Placebo (N=20)	Neflamapimod 40mg TID (N=9)
Age (y)	70.7 (6.0)	69.3 (5.5)	73.8 (7.5)	75.7 (6.4)
Male	87%	100%	85%	89%
CDR Sum of Boxes	4.3 (2.0)	4.3 (1.7)	6.1 (3.4)	5.2 (2.0)
MMSE	24 (3.8)	25.2 (2.2)	23 (3.6)	21.8 (3.2)
ISLT-Immediate	15 (6.0)	16.4 (4.8)	13 (4.6)	11.1 (3.2)
ISLT -Delayed	5 (2.3)	5.0 (2.4)	4 (1.8)	3.3 (2.2)
ISLT-Recognition	10.7 (1.3)	10.3 (1.4)	9.5 (2.0)	11.0 (1.1)
Timed Up and Go	14 (7.7)	12.8 (4.0)	13 (3.6)	14 (3.8)
NTB *z*-score	0.09 (0.82)	0.22 (0.74)	-0.12 (0.65)	-0.16 (0.71)
Fluctuating cognition	56%	45%	60%	67%
Visual hallucinations	65%	64%	45%	78%
REM sleep disorder	83%	73%	65%	67%
Parkinsonism	87%	82%	85%	100%

Legend: Mean (SD), except when shown as percentage. For ISLT-delayed, ISLT-recognition and NTB (Neuropsychological Test Battery) *z*-score: missing data in 1 placebo participant with ptau>2.2 pg/mL. Also missing TUG (Timed Up and Go) test data in 1 placebo participant with baseline ptau181≥ 2.2 pg/mL. There were no significant differences noted between placebo and neflamapimod 40mg TID groups in any baseline characteristic (p>0.05).

Table 3. Clinical Outcomes in Patients with Baseline plasma ptau181 < 2.2 pg/mL

	Neflamapimod (NFMD) 40mg TID vs. placebo			
	N= NFMD, Placebo	MMRM Analysis Difference (95% CI)	*p*-value	Cohen's *d* Effect size
*NTB**	11,19	0.21 (-0.07,0.49)	0.13	0.56
Attention	11,18	0.42 (0.07,0.78)	0.023	0.78
CDR-SB	11,22	-0.60 (-1.04,-0.06)	0.031	0.70
Time up and Go	11,20	-3.1 (-4.7,-1.6)	<0.001	0.74
ISLT-Immediate	11,22	2.1 (0.0,4.2)	0.053	0.55
ISLT-Delayed	10,21	0.2 (-1.0,2.4)	>0.2	0.15
ISLT-Recognition	10,21	1.4 (0.02,2.5)	0.024	1.0

Figure 1. CONSORT Flow Diagram

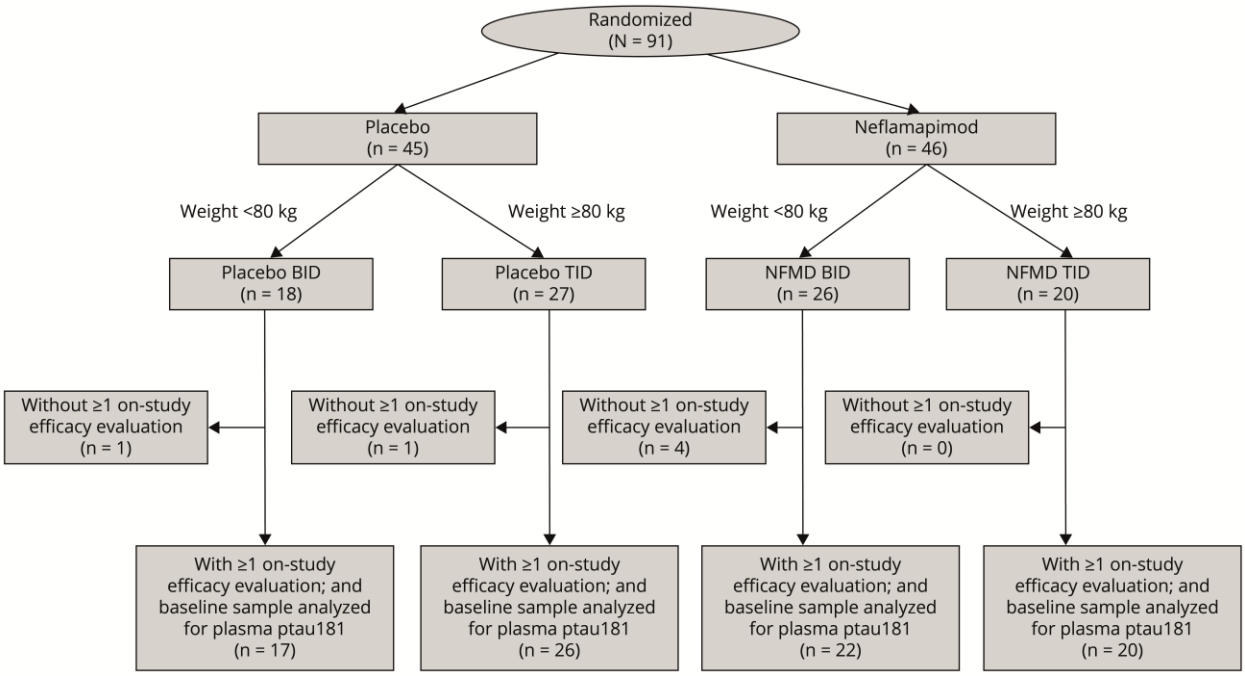


Figure 2. Cognitive outcomes in AscenD-LB Clinical Study stratified by baseline plasma ptau181 status

Mean difference between placebo and neflamapimod 40mg TID, 95% confidence interval, of analysis change from baseline utilizing Mixed Model for Repeated Measures (MMRM) with baseline as a covariate for patients with baseline ptau181 < 2.2 pg/mL (absence of AD co-pathology) or ≥ 2.2 pg/mL (presence of AD co-pathology). Neuropsychological Test Battery (NTB) composed of six tests that individually assess attention, executive function or visuospatial function: computerized tests from the Cogstate® cognitive testing battery (Detection, Identification, One Card Learning, One Back) and two verbal fluency tests (Letter Fluency Test, Category Fluency Test) that were recorded on paper. The Attention composite z-score includes results on the two tests within the NTB that evaluate information processing speed, Detection and Identification. **P<0.05.*

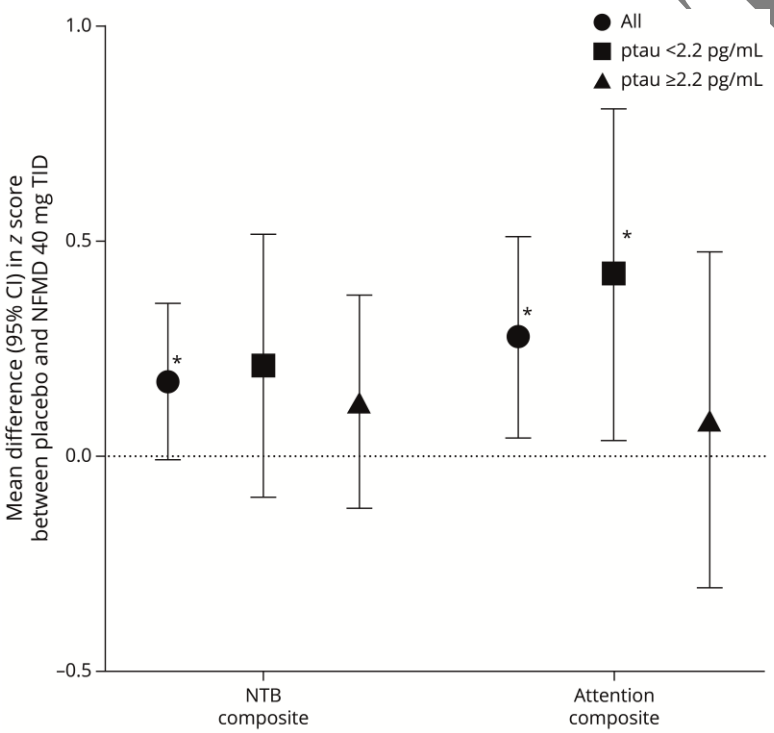


Figure 3. Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) and Timed Up and Go (TUG) test results in AscenD-LB Clinical Study stratified by baseline plasma ptau181 status

Mean difference between placebo and neflamapimod 40mg TID, with 95% confidence interval, of analysis change from baseline utilizing Mixed Model for Repeated Measures (MMRM) with baseline as a covariate for patients with baseline ptau181 < 2.2 pg/mL (absence of AD co-pathology) or ≥ 2.2 pg/mL (presence of AD co-pathology). A. Clinical Dementia Rating Sum of Boxes (CDR-SB) score. B. Time Up and Go Test in seconds. **P<0.05, **P<0.01, ***P<0.001*

AAN's positions on brain health

1	Brain health is a key to neurologic health and a core function of neurology
2	Brain health is foundational to the overall health of communities throughout the United States and worldwide
3	Brain health requires collaboration between the many disciplines that share a mission to promote the prevention of neurologic diseases, optimal mental health, and the well-being of individuals across the lifespan

AAN's brain health goals

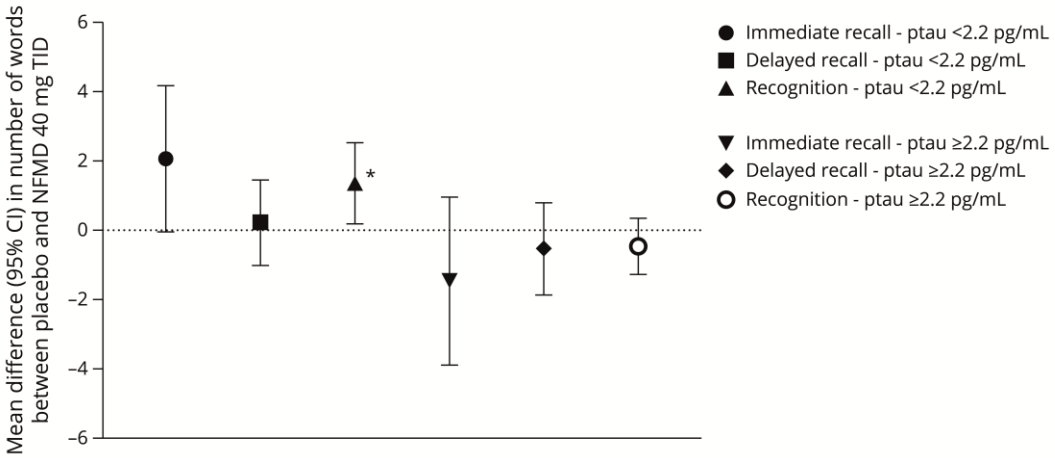
1	Accelerate scientific discovery in brain health through cross-disciplinary collaboration
2	Optimize brain health through the integration of preventive care practices
3	Enhance public and patient engagement to advance public policy in brain health

AAN national brain health vision by 2050

1	Brain health research leads to an accumulation of a critical body of knowledge and scientific breakthroughs
2	Preventive neurology is a thriving cross-disciplinary field that develops new leaders in brain health
3	Evidence-based practice guidelines on brain health for all ages are available and continuously updated
4	A "brain health visit" is a standard of care and part of a "well visit" at every stage across the lifespan
5	Education on brain health across the lifespan is widely available and results in a highly aware and engaged public
6	A National Brain Health Plan is established to guide scientific research, care, and public engagement priorities

Figure 4. International Shopping List Test (ISLT) results in AscenD-LB Clinical Study stratified by baseline plasma ptau181 status

Mean difference between placebo and neflamapimod 40mg TID, 95% confidence interval, of analysis change from baseline utilizing Mixed Model for Repeated Measures (MMRM) with baseline as a covariate for the International Shopping List Test Immediate Recall, Delayed Recall and Recognition for patients with baseline ptau181 < 2.2 pg/mL (absence of AD co-pathology) or ≥ 2.2 pg/mL (presence of AD co-pathology). **P<0.05, **P<0.01*



Association of Plasma Phosphorylated Tau With the Response to Neflamapimod Treatment in Patients With Dementia With Lewy Bodies

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Neurology published online September 1, 2023
DOI 10.1212/WNL.0000000000207755