Alzheimer's Research & Therapy

RESEARCH

Open Access

Effects of the novel phosphodiesterase 9 inhibitor irsenontrine on CSF proteomics profile in Aβ^{+} and Aβ^{-} dementia with Lewy bodies patients

Marlies Oosthoek[1*], Sjors G.J.G. In 't Veld[1,2], Lisa Vermunt[1,3], Yuanqing Ye[4], Manon van Ingen[1], Marleen Koel-Simmelink[1], Everard G.B. Vijverberg[3], Satya Saxena[4], Steven Hersch[4], Michael Irizarry[4], Pallavi Sachdev[4] and Charlotte E. Teunissen[1]

Abstract

Background Irsenontrine (E2027) is a potent and selective PDE9 inhibitor that increases cellular cGMP, essential for glutamatergic synaptic function, and was investigated as symptomatic treatment for dementia with Lewy bodies (DLB). A recent Phase 2 study suggested differences in clinical responses in irsenontrine-treated DLB patients depending on amyloid (Aβ) co-pathology. In this post-hoc CSF proteomic sub-study, we evaluated how protein levels and biological pathways changed from baseline in DLB participants with and without amyloid co-pathology (Aβ^{+} and Aβ^{-}). We hypothesized that the treatment with irsenontrine affects specific protein pathways, which is attenuated in Aβ+ DLB patients.

Methods The CSF proteome was measured using proximity extension assay (PEA) proteomics in all available paired baseline and week 9 CSF samples from a trial with irsenontrine. This included 9 Aβ^{+} and 6 Aβ^{-} DLB patients (CSF Lumipulse Aβ42/40 cut-off 0.057) with a mean ± sd age of 76 ± 6 years and 67% were male. We determined the treatment effect stratified by amyloid status using paired Wilcoxon tests on the protein levels ($p < 0.05$) and individual pathway enrichment KEGG pathway z-scores ($p < 0.05$).

Results Following irsenontrine treatment, CSF protein level changes were present in both groups (DLB Aβ^{-} 10 up; 32 down; DLB Aβ^{+} 16 up; 27 down). More pathways were affected in DLB Aβ^{-} (5 pathways), compared to DLB Aβ^{+} (3 pathways), and several of these were related to the mechanism of action, including the upregulation of the Pantothenate and CoA biosynthesis pathway (upregulated in Aβ^{+} and Aβ^{-}), and downregulation in the GABAergic synapse pathway (DLB Aβ^{-} only).

Conclusion This global proteomics analysis in this DLB clinical trial suggests that, despite the small number of treated participants, there were changes in CSF proteins related to the mechanism of action, as indicated by the upregulation of pantothenate and CoA biosynthesis pathway, which relates to glutamate metabolism. In addition,

*Correspondence:
Marlies Oosthoek
m.c.oosthoek@amsterdamumc.nl

Full list of author information is available at the end of the article

more pathways that were associated with the mechanism of irsenontrine were affected in the DLB Aβ^{-} vs. Aβ^{+} group, supporting differential effects based on presence or absence of amyloid co-pathology.

Trial registration information The clinical trial was registered at clinicaltrials.gov with NCT03467152 at March 16th 2018. Patient enrollment started on the 4th of May 2018. Trial protocol and statistical analysis plan can be accessed on clinicaltrials.gov.

Keywords Dementia with Lewy bodies, Co-pathology, Phosphodiesterase 9 inhibitor, Proteomics

Background

Dementia with Lewy bodies (DLB) is the second most common cause of dementia [1]. DLB is pathologically characterized by the aggregation of α-synuclein in neurons or neurites, known as Lewy Bodies or Lewy neurites, respectively [2]. Clinical symptoms include progressive cognitive decline, recurring visual hallucinations, REM sleep disorder, and parkinsonism (bradykinesia, rest tremor, or rigidity) [3]. Most countries have no symptomatic treatments to reduce cognitive symptoms available for DLB, while off-label prescriptions may include cholinesterase inhibitors [4, 5]. However, the efficacy of these drugs is limited and there is a need for improved symptomatic drugs.

Irsenontrine (E2027) is a phosphodiesterase 9 (PDE9) inhibitor that improved cognitive functioning in preclinical models [6, 7]. PDE9 converts cyclic guanosine monophosphate (cGMP) to its inactive form 5'-GMP, and inhibition of PDE9 results in increased levels of cGMP [6, 7]. cGMP functions as a second messenger and activates protein kinase G (PKG) [8]. This in turn phosphorylates CREB (pCREB), a transcription factor involved in synaptic plasticity and long-term memory. The phosphorylation of CREB leads to synthesis of proteins and dendritic spines required for memory function and synaptic plasticity, such as brain derived neurotrophic factor (BDNF) and the regulation of the incorporation of AMPA-selective glutamate receptor 1 (GluA1) [9–12].

One of the major hurdles in the treatment of DLB is that it is highly heterogeneous and often presents with significant AD co-pathology [13, 14]. Co-pathology may reduce the efficacy of a treatment, and this is hypothesized to be the case for irsenontrine. While irsenontrine had no effect on cognition in DLB in a phase 2 study, a post-hoc analysis suggested a potential treatment response on cognitive and clinical parameters (MoCa and CIBIC+ scores) in amyloid negative DLB patients [15]. Amyloid β (Aβ) interferes with the activation of pCREB on several different levels in the pathway, which might be the reason for the better efficacy with irsenontrine in Aβ^{-} DLB patients [15, 16]. We hypothesize that different pathways are affected, in the presence or absence of AD co-pathology, and that these effects can be elucidated using CSF proteomics. Such differences may provide possible insights into the mechanism of action of the drug and aid in future patient selection.

Methods

Clinical study design and eligibility criteria

The study was a multicenter, single-arm Phase 2a study evaluating the effect of 50 mg of irsenontrine administered once daily for 12 weeks (NCT03467152). The trial assessed safety, pharmacodynamics, and pharmacokinetics in patients with DLB and Parkinson disease-dementia (PDD) in four diagnostic cohorts (DLB with (DLB Aβ^{+}) and without (DLB Aβ^{-}) and PDD with (PDD Aβ^{+}) and without (PDD Aβ^{-}) amyloid co-pathology based on the ratio of plasma concentration of Aβ42/Aβ40 < 0.092 (based on the C2N Precivity assay). For the purpose of this sub-study, amyloid co-pathology was determined based on CSF Lumipulse Aβ42/40 cut-off 0.057 [17, 18]. Patients were eligible when aged between 50 and 85, they met criteria for probable DLB or probable PDD and had an MMSE score of ≥ 14 and ≤ 26. Patients receiving acetylcholinesterase inhibitors or memantine had to be on a stable dose for the last 12 weeks. Patients receiving PD medication had to be on a stable dose for at least 4 weeks.

Biomarker and clinical outcomes as defined in the trial

The primary endpoint was the change from baseline in CSF cGMP levels at 9 weeks, indicating positive target engagement and was measured at baseline and end of treatment for 16 participants. Exploratory endpoints included clinical outcomes, including the MoCa and Mini Mental State Exam (MMSE) at 12 weeks of treatment as well as plasma and CSF biomarkers measured at baseline and after 9 weeks of treatment. Plasma biomarkers included Aβ42/40 ratio measured using the Precivity assay (C2N, USA) and p-tau181 (singleplex), NFL, and GFAP (NFL and GFAP duplex) measured using the Simoa HD-X analyzer (Quanterix, Billerica, Massachusetts, USA). CSF Neurogranin (NRGN) was measured with ELISA (EUROIMMUN ELISA, Lübeck, Germany), and NFL was measured using the Simoa HD-X analyzer (Quanterix, Billerica, Massachusetts, USA). CSF Aβ42, Aβ40, pTau181, and tTau were measured at baseline and after 9 weeks using Lumipulse (Fujirebio Gent, Belgium) and the ratio was calculated. CSF cGMP was measured as target engagement marker using a validated liquid

chromatography method applying tandem mass spectrometry. For the purpose of this study, amyloid positivity was based on the CSF Aβ42/40 ratio using a cut-off of < 0.057 [17, 18].

CSF proteomics sub-study patient selection

For this post-hoc CSF proteomics DLB sub-study evaluating the effect of irsenontrine, we selected participants with a diagnosis of DLB, known CSF amyloid status, and a baseline sample available ($n = 18$). There were 15 participants with baseline and week 9 samples available. In addition, we selected a group of cognitively unimpaired (CU) individuals from the Amsterdam Dementia Cohort (ADC), who were included as controls ($n = 17$). The CU group was age and sex matched to the trial participants and had a normal AD CSF biomarker levels (Aβ42 < 813 pg/mL, pTau181 > 51 pg/mL, and tTau > 375 pg/mL, tTau/Aβ ratio > 0.46, positivity was based on the ratio) [19–21]. The CSF biomarker levels of the CU individuals were determined using commercially available kits (ELISA Innotest Aβ42, hTAUAg, phospho-Tau(181P) (Fujirebio, Gent, Belgium). Drift correction was applied where necessary and the levels were transformed to Lumipulse in the comparison with the DLB group [22].

CSF proteomics sub-study measurements

In both the ADC and trial samples, CSF was obtained by lumbar puncture and collected in polypropylene tubes. Samples were centrifuged at *2000 x g* for 10 min at room temperature. Aliquots were stored in polypropylene tubes at -80 °C until analysis. Control samples and DLB samples were measured in the same run to reliably compare the proteomic profiles.

Proteomics was performed using antibody-based proximity extension assay (PEA) technology with the Olink Explore 3072 panel. In brief, upon binding of the antibody pairs to the protein, the oligonucleotide-strands connected to the antibody form a PCR chain reaction. This combination allows for specific and sensitive protein measurements. The protein measurements are expressed as normalized protein expression (NPX) values. A standardized QC pipeline was applied as we described earlier [23–26]. Only if protein levels were detectable in 85% or more of the sample, they were included in the analyses. Forty-two proteins were excluded because the assay did not meet the batch-release quality-control criteria as defined by Olink. Resulting in a total 1720 proteins to be included in the final analyses.

Statistical analysis

Data was analyzed using R version 4.4.3 Group differences in demographics were determined using t-tests (DLB vs. CU). Fisher's exact test was used to evaluate the sex distribution stratified per amyloid status.

All group comparisons were performed with Wilcoxon signed-rank tests. Baseline comparisons (DLB vs. CU, $A\beta^+$ DLB vs. CU, and $A\beta^-$ DLB vs. CU) were done with regular Wilcoxon tests. Baseline vs. week 9 effects were analyzed with paired Wilcoxon signed rank tests.

To determine target engagement, the CSF cGMP levels were compared using the paired Wilcoxon signed-rank test. The analysis was stratified per amyloid status. The absolute change per participant was calculated by subtracting the baseline value from the week 9 value. The following formula was applied to determine the percent change: *absolute change/baseline value * 100%*. The Wilcoxon signed-rank test was used to determine the difference in absolute and percent change stratified by amyloid status.

To quantify pathway activity per sample, we performed a sample-level enrichment analysis based on z-scores, applying previously described methods [27]. Pathway-specific protein sets were derived from the KEGG database [28], in total 200 pathways were included in the analyses. For each sample, the median NPX value of all detected proteins in each pathway was compared to the distribution of medians from 10,000 randomly sampled protein sets of the same number of proteins from the same sample. Z-scores were calculated per sample and pathway. This method takes advantage of the large number of reliably measured proteins ($n = 1720$). Enrichment scores were used for downstream statistical testing.

Results

General results of the trial

Irsenontrine was safe and well-tolerated in DLB and PDD and achieved robust pharmacodynamic responses in all subgroups [15, 29]. The results did not support a differential pharmacodynamic effect (CSF cGMP) of irsenontrine based on amyloid status. however, a trend toward larger CSF cGMP % changes from baseline in DLB $A\beta^-$ vs. DLB $A\beta^+$ was observed when amyloid status was defined based on CSF cut-point of 0.0571 [29].

Baseline participant characterization

Baseline proteomics data was available for 18 DLB patients and 17 CU controls were included in this follow-up study. DLB and CU groups had a mean age of 74 (sd ± 6) and 73 (sd ± 5) years respectively (Table 1). Approximately two-thirds of the individuals were male in the DLB and CU groups. Stratifying by amyloid status, $A\beta^+$ patients had a mean age of 76 (sd ± 6) years and $A\beta^-$ patients had a mean age of 75 (sd ± 4) years. All of the $A\beta^-$ patients were male, and just over half of the $A\beta^+$ patients were male ($p = 0.1033$). The $A\beta^+$ and $A\beta^-$ DLB patients had a mean baseline MMSE score of 21 ($A\beta^+$ sd ± 4; $A\beta^-$ sd ± 3).

Table 1 Patient characteristics

	CONTROL ($n=17$)[1]	DLB ($n=18$)[2]	DLB Aβ$^+$ ($n=9$)[3]	DLB Aβ$^-$ ($n=6$)[3]
Age (sd)	73 (5)	74 (6)	76 (6)	75 (4)
Sex (M%)	11 (65%)	12 (67%)	5 (56%)	6 (100%)
MMSE (sd)	29 (2)	21 (4)	21 (4)	21 (3)
CSF Aβ42/40 (sd)	NA	0.056 (0.0184)	0.045 (0.0099)***	0.075 (0.0143)***
CSF pTau181 (sd)	40.4 (15.1)	83.8 (67.9)	97.3 (64.9)*	39.7 (16.9)*
CSF tTau (sd)	320 (59)	556 (423)	645 (407)*	277 (100)*
CSF NRGN (sd)	NA	464 (334)	555 (323)**	222 (58.2)**
CSF DDC (IQR)	-2.74 (-3.14 - -2.43)	-1.57 (-2.03 - -1.30)	-1.66 (-2.19 - -1.30)	-1.43 (-1.68 - -0.95)
CSF CRH (IQR)	0.988 (0.613–1.28)	0.284 (-0.48–0.91)	0.573 (0.18–1.20)**	-0.595 (-1.10 - -0.45)**
CSF MMP3 (IQR)	-1.16 (-1.63 - -1.04)	-1.20 (-1.60 - -0.98)	-1.22 (-1.60 - -1.16)	-1.19 (-1.50 - -1.02)
CSF ABL1 (IQR)	-1.46 (-1.56 - -1.37)	-1.23 (-1.48 - -0.68)	-0.814 (-1.38 - -0.55)*	-1.50 (-1.64 - -1.30)*
CSF MMP10 (IQR)	-1.91 (-2.21 - -1.44)	-1.52 (-1.75 - -0.98)	-1.25 (-1.62 - -0.06)*	-1.57 (-1.64 - -1.49)*
CSF THOP1 (IQR)	-0.18 (-0.50–1.12)	-0.21 (-0.47–0.24)	0.09 (-0.42–0.59)	-0.49 (-0.66 - -0.17)

Control participants were selected from the Amsterdam Dementia Cohort and had normal AD biomarkers. DLB participants were selected from study 203. Baseline NPX values are shown for six biomarkers characteristic of DLB, as reported previously (Del Campo et al., [25]), and are presented as median (IQR)

Differences between Aβ + and Aβ – DLB patients were assessed using Wilcoxon signed-rank tests (*$p<0.05$; **$p<0.01$; ***$p<0.001$)

[1] Only 13 controls had absolute pTau181 and tTau data available. NRGN and Ab42/40 data was not available for controls

[2] Sixteen of the 18 DLB participants had MMSE and CSF biomarker data available

[3] Fifteen of the 18 DLB patients had amyloid status and baseline and end of treatment proteomic data available

Baseline characterization of CSF proteomics of DLB patients

Baseline protein levels were compared between DLB patients and CU individuals to characterize the DLB patients and allow a benchmark comparison of their proteomic profile to published data of other DLB cohorts [25, 30–34]. Two proteins were significantly downregulated and 54 were significantly upregulated in DLB patients following FDR correction (Fig. 1A). The most strongly upregulated proteins include C3 ($q<0.0001$; FC = 1.4748), DDC ($q=0.0027$; FC = 1.1755), COL4A1 ($q=0.0094$; FC = 0.8924). The two downregulated proteins were GAPDH ($q=0.0145$; FC=-1.1940) and CKMT1B ($q=0.0213$; FC=-1.8121).

Next, baseline characteristics of the Aβ$^+$ and Aβ$^-$ DLB patients were investigated. The selection of Aβ$^+$ patients based on CSF Aβ42/40 ratio, not surprisingly, also resulted in higher pTau181 ($p=0.0176$) and tTau ($p=0.0360$) levels in this group (Supplemental Fig. 1A-C). Moreover, the Aβ$^+$ DLB patients had higher neurogranin levels ($p=0.0076$) compared to Aβ$^-$ DLB patients (Table 1). Recent literature shows that a set of 6 markers can distinguish between DLB and AD (DDC, CRH, MMP3, ABL1, MMP10, and THOP1) [25]. Here, we show that the protein levels of the overall DLB group followed a pattern characteristic of DLB when compared to the CU group as seen in previous literature. The DLB patients had high DDC and MMP10 levels and lower CRH levels compared to the CU group (Table 1). When stratifying by amyloid status, Aβ$^+$ DLB patients follow a more AD-like pattern with relatively higher ABL1 and MMP10 levels and less decrease CHR levels compared to Aβ$^-$ DLB patients (Table 1), similar to what was observed in the larger cohorts when comparing AD vs. DLB [25]. Reviewing the 56 proteins that were significantly different in the analyses comparing DLB patients with CU individuals in the current trial cohort, these findings indicate that the trial participants, both Aβ$^+$ as well as Aβ$^-$ patients, have a CSF proteomic profile consistent with DLB (Fig. 1B). However, the Aβ$^+$ patients also show positive AD biomarkers.

Treatment response per amyloid status

A direct marker of target engagement for irsenontrine are the cGMP levels in the CSF. Increased levels of cGMP, indicate target engagement. Here, we first investigated treatment response stratified by amyloid status for the participants of this sub study. All patients included in this analysis ($n=16$) showed an increase in CSF cGMP in both Aβ$^+$ and Aβ$^-$ DLB patients (Fig. 2A). Purely focusing on the absolute change, there was no difference between Aβ$^+$ and Aβ$^-$ DLB patients (Fig. 2B; FC=-0.445; $p=0.7925$). However, focusing specifically on the percent change, there is a larger percental increase in Aβ$^-$ DLB patients (Fig. 2C; FC = 73.1; $p=0.0312$), indicating a greater pharmacodynamic treatment response in Aβ$^-$ DLB patients. The larger percent increase can also partially be attributed to the fact that baseline cGMP levels are relatively lower in Aβ$^-$ DLB patients, although not significant (FC=-0.24; $p=0.2198$).

To evaluate the biological difference in treatment response per amyloid status, the main study goal, differential protein expression between the baseline and week 9 was determined stratified by amyloid status. In the Aβ$^-$ DLB patients, levels of 32 proteins were decreased and of 10 proteins were increased following treatment (Fig. 2D). In the Aβ$^+$ DLB patients, levels of 26 proteins were decreased and those of 17 proteins were increased

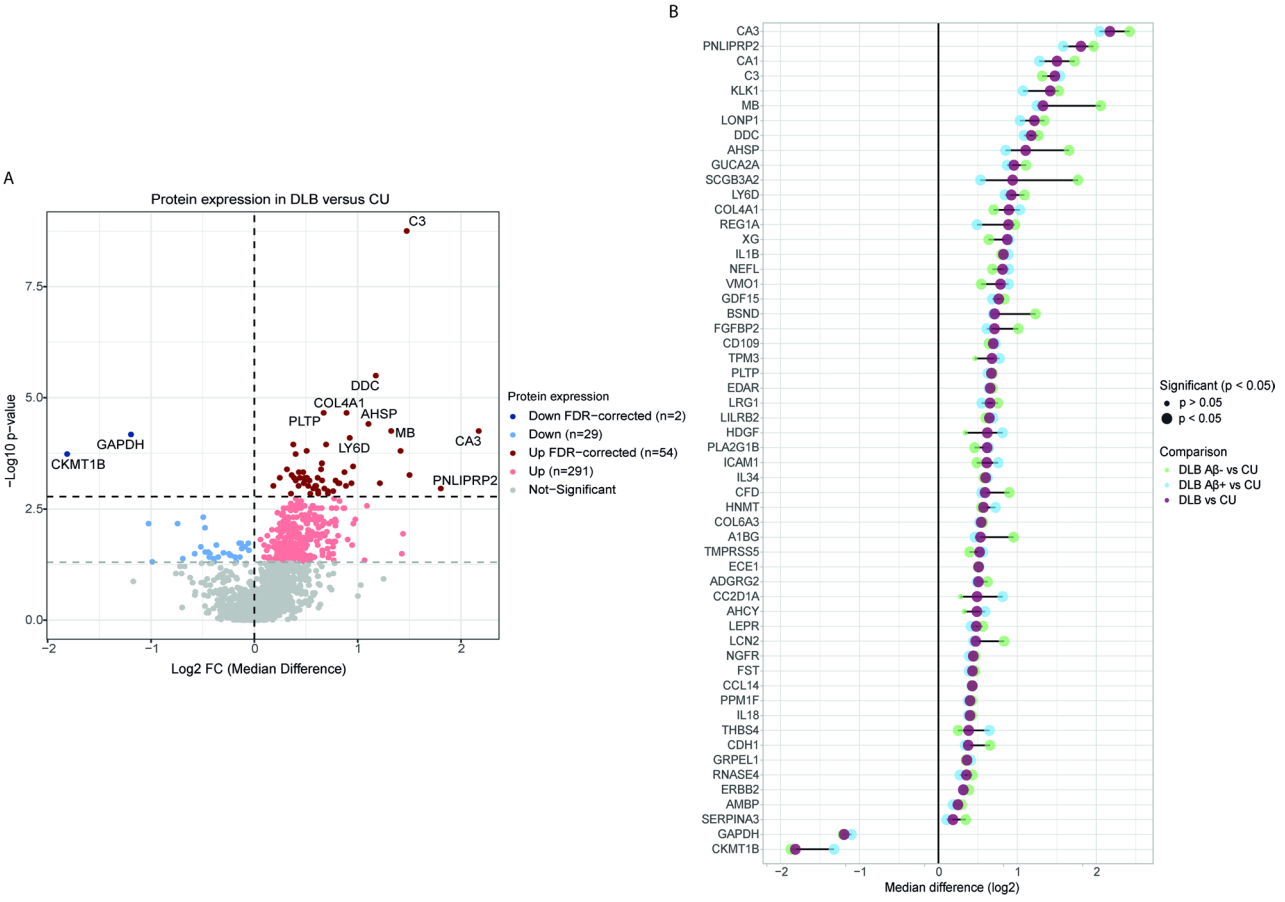

Fig. 1 Differential protein expression in DLB patients compared with CU individuals. **A** Median protein expression differences between DLB patients ($n = 18$) and cognitively unimpaired (CU) individuals ($n = 17$). Significance was assessed using Wilcoxon signed-rank tests. The grey dashed line marks nominal significance ($p < 0.05$), and the black dashed line marks significance after false discovery rate (FDR) correction ($q < 0.05$). **B** Proteins significantly altered in DLB vs. CU are shown for the overall DLB group (maroon) and stratified by amyloid status (Aβ − green; Aβ + blue). Dot size reflects the level of significance in each comparison (DLB overall, Aβ−, Aβ+) relative to CU, based on Wilcoxon signed-rank tests

following treatment (Fig. 2E). Only three proteins were overlapping in both $A\beta^+$ and $A\beta^-$ namely DNM1, CTSV, and KLK13. Levels of DNM1 were decreased in both groups, while levels of CTSV were decreased in $A\beta^+$ and increased in $A\beta^-$ patients. Levels of KLK13 were increased in $A\beta^+$ and decreased in $A\beta^-$ DLB patients. Of the 56 proteins that were differentiated in DLB patients vs. CU, only 2 proteins were affected following treatment. Both proteins, LONP1 and REG1A, were only affected in $A\beta^+$ DLB patients and were closer to levels observed for CU individuals.

Pathway analysis

A total of eight KEGG pathways were affected as indicated by pathway enrichment scores following irsenontrine treatment. A larger number of pathways was changed following treatment in the $A\beta^-$ (5 pathways) compared to the $A\beta^+$ (3 pathways) DLB group. There was only one pathway overlapping between the two groups: the pantothenate and CoA biosynthesis pathway score was increased in $A\beta^-$ ($p = 0.013$) and $A\beta^+$ ($p = 0.0273$) DLB patients (Fig. 3A). In $A\beta^-$ DLB patients, the phosphatidylinositol signaling system ($p = 0.013$) and the apoptosis ($p = 0.013$) pathway scores were increased following treatment (Fig. 3B-C). The GABAergic synapse ($p = 0.013$), virion – herpesvirus ($p = 0.013$), and taste transduction pathway ($p = 0.013$) scores were decreased in $A\beta^-$ DLB patients following treatment (Fig. 3D-F). In the $A\beta^+$ DLB patients, the mitophagy ($p = 0.0078$) and salivary secretion ($p = 0.0195$) pathway scores were reduced following treatment, while no pathway scores were increased in $A\beta^+$ DLB patients (Fig. 3G-H).

Discussion

In this study, we investigated the effect Irsenontrine on the CSF proteome of DLB patients and the difference in biological response to this treatment in the presence or absence of amyloid co-pathology. The percentage increase in CSF cGMP was larger in the post hoc subgroup of $A\beta^-$ DLB patients. The proteomic study was

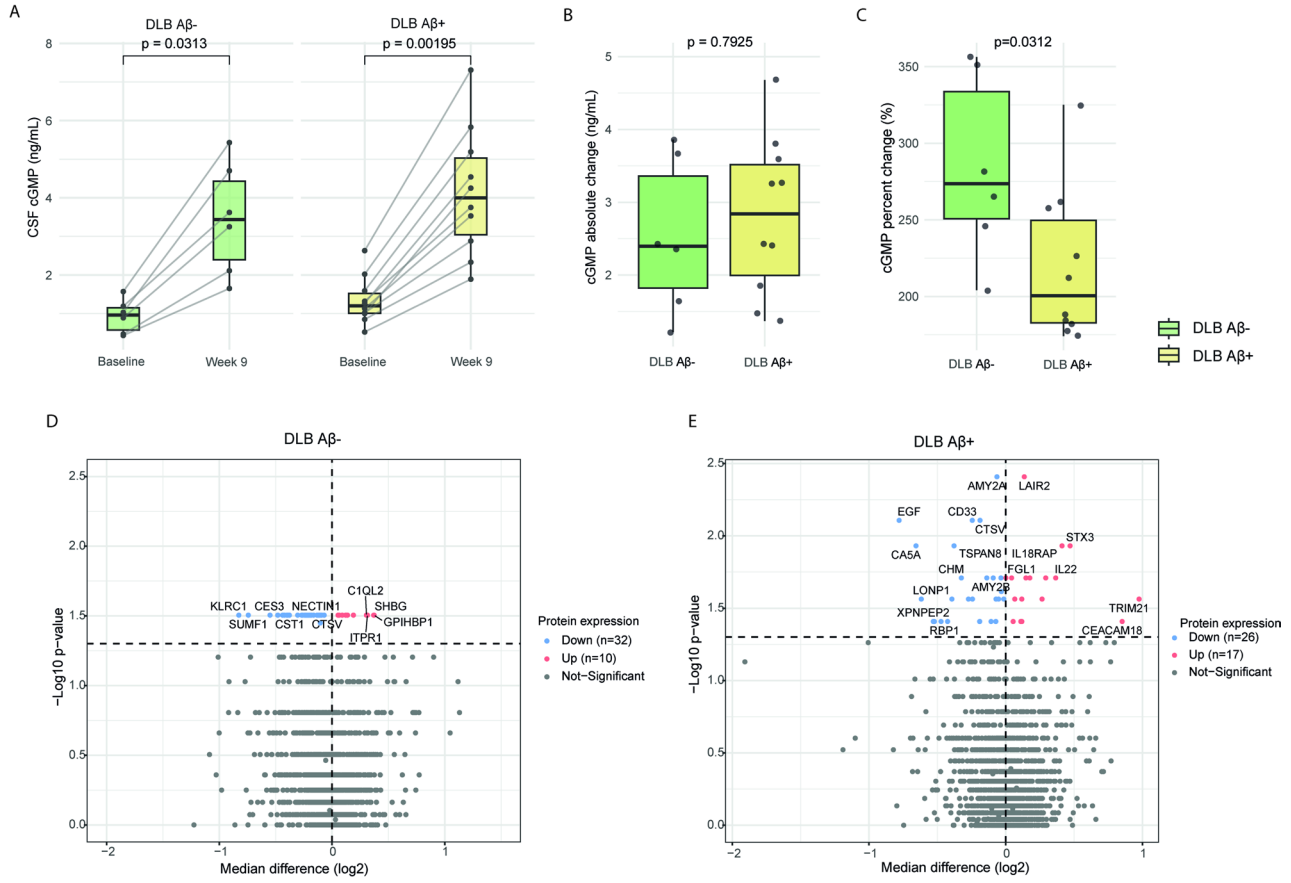

Fig. 2 Differential treatment response stratified by amyloid status. **A** CSF cGMP levels (ng/mL) in Aβ- (green) and Aβ+ (yellow) patients. Paired Wilcoxon signed-rank tests were used to compare baseline vs. end-of-treatment (EOT) values. **B** Absolute change in CSF cGMP between baseline and EOT, stratified by amyloid status. Differences between Aβ- and Aβ + patients were assessed with Wilcoxon signed-rank tests. **C** Percent change in CSF cGMP, calculated as *(absolute change / baseline) × 100%*. Comparisons between amyloid groups were performed with Wilcoxon signed-rank tests. **D**, **E** Differential protein expression in Aβ- (**D**) and Aβ+ (**E**) patients, shown as volcano plots. Significance was assessed with paired Wilcoxon signed-rank tests. Proteins with increased expression are shown in red, decreased in blue. BSDN had a median difference of ~ −4 in Aβ- patients ($p = 0.4375$) but is not displayed for clarity. Significance thresholds: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

used to better understand this difference and generate new hypothesis regarding affected pathways. We first confirmed that typical DLB-related CSF protein abnormalities, such as DDC and IL18 [25], were detected in both $A\beta^+$ and $A\beta^-$ DLB groups. Secondly, the differential protein abundance profiles following the treatment differed between the $A\beta^+$ and $A\beta^-$ DLB patients. This was also confirmed by differential representation of the KEGG pathways following treatment with irsenontrine, distinct pathways were affected based on AD co-pathology status and less co-pathology led to more pathways being affected. Combined this suggests that amyloid co-pathology attenuates the treatment response of irsenontrine and that AD biomarkers may be a useful screening strategy for a future clinical trial investigating irsenontrine.

Irsenontrine inhibits PDE9, elevates cGMP, and engages the PKG-CREB axis to support glutamatergic synaptic plasticity. Preclinical work shows neuronal GluA1 phosphorylation and memory improvement with irsenontrine via cGMP elevation [11]. In this CSF proteomics analysis, pathway-level pharmacodynamic signatures consistent with this mechanism of action of irsenontrine emerged. The pantothenate and CoA biosynthesis pathway scores were increased in both $A\beta^+$ and $A\beta^-$ DLB patients following irsenontrine treatment, suggesting improved metabolic capacity relevant to synaptic function. Pantothenate, also known as vitamin B5, is required for the synthesis of Coenzyme A (CoA), which has many metabolic functions and is essential for the production of acetylcholine [35]. Although cGMP and CoA are involved in different processes, cGMP signaling can affect fatty acid metabolism in which CoA is involved [36]. It has been shown that patients with Parkinson's disease dementia and Alzheimer's dementia have lower levels of pantothenate, which can result in reduced CoA levels and reduced tricarboxylic acid (TCA) cycle functioning [37, 38]. The reduced function of the TCA cycle

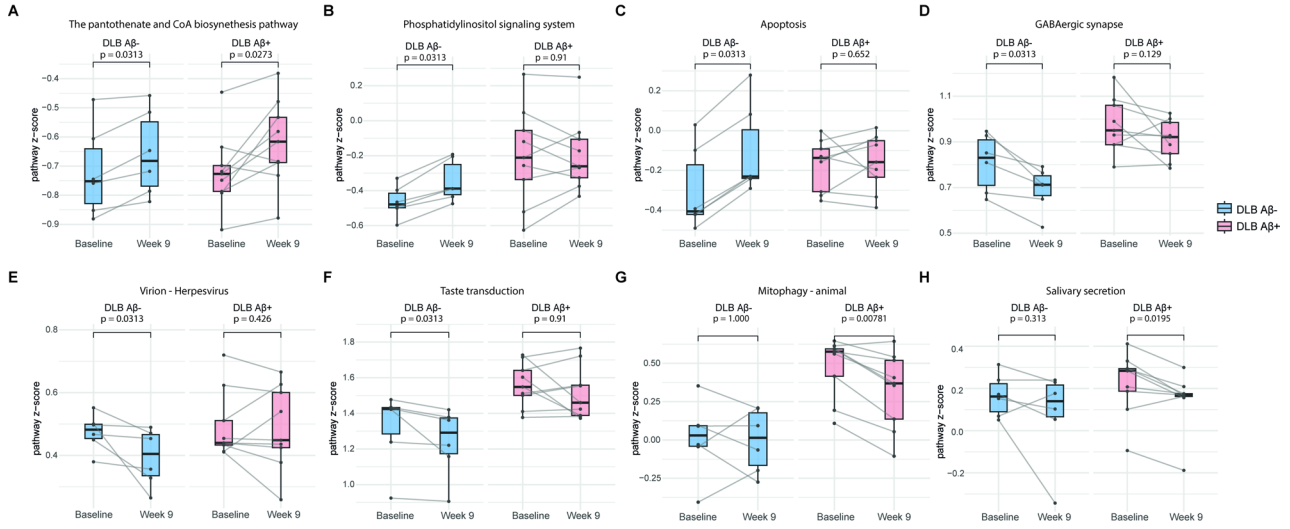

Fig. 3 Differential treatment response on a pathway level per amyloid status. Pathway level z-scores per sample were calculated using sample-level enrichment analysis. Protein sets were derived from the KEGG pathways, for each sample, the median NPX value of proteins within a pathway was compared to the distribution of medians from 10,000 randomly sampled protein sets of equal size, and z-scores were calculated. Paired Wilcoxon signed-rank tests were applied to determine the treatment response on a pathway level (200 KEGG pathways), stratified by amyloid status (Aβ-:blue; Aβ+ pink). Only significant pathways are shown: the pantothenate and CoA biosynthesis pathway (**A**), phosphatidylinositol signaling system (**B**), apoptosis pathway (**C**), the GABAergic synapse pathway (**D**), the virion - herpesvirus pathway (**E**), the taste transduction (**F**), mitophagy - animal pathway (**G**), and the salivary secretion pathway (**H**). Significance thresholds: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

has been reported in Lewy Body Diseases (LBD) and may be indicative of mitochondrial dysfunction [39]. Moreover, impairment of the cholinergic pathways is a core pathology in DLB [40]. Increased activity of this pathway could therefore be beneficial and indicative of improved overall energy metabolism and cholinergic function.

The Phosphatidylinositol (PI) signaling system and apoptosis pathway scores were increased only in $A\beta^-$ DLB patients, as shown by the pathway analysis. PI, a phospholipid, is a building block for phosphoinositides, which are involved in cell signaling and cell membrane physiology [41, 42]. Interestingly, these molecules are also involved in the process of apoptosis, the third pathway increased only in $A\beta^-$ DLB patients. Previous research has shown that the NO-cGMP pathway, which is activated by irsenontrine [11], can induce apoptosis in healthy neuronal cells when overstimulated [43]. However, it has also been shown that stimulation of the cGMP/PKG pathway, which is targeted by irsenontrine [11], has an anti-apoptotic effect in astrocytes following ischemia [44]. Thus, the increase in the apoptotic pathway shown here could be an effect of a more chronic over-stimulation compared to the acute setting of stroke. Polyphosphoinositides are also regulators in Ca^{2+} signaling [42], which is also involved in the cGMP-PKG-pCREB pathway. This increase could thus also be directly related to the effect of irsenontrine on cGMP and Ca^{2+}. Potentially, both the increase observed in the PI signaling and apoptosis are related to the same pharmacodynamic effect.

The GABAergic synapse pathway score was lowered in $A\beta^-$ DLB patients following treatment. This pathway represents a key inhibitory synapse in the central nervous system [45]. GABAergic function can have complex consequences: depending on the subtype of the neuron, as well as the receiving receptors, the effects can vary greatly [46]. GABAergic neurons located in the hippocampus have been stipulated to be involved in memory and learning [47, 48]. Irsenontrine affects neurotransmission through the cGMP pathway. It has been shown that increased cGMP levels can inhibit GABA receptors, however, this is cell dependent [49, 50]. This indicates that irsenontrine can have an effect on the excitability of neurons, due to reduced inhibitory effect of GABA. Although the exact mechanism underlying the effect on the GABAergic pathway remains speculative, these findings support the effect of irsenontrine on neurotransmission. Aβ impairs the GABAergic pathway, resulting in an excitatory/inhibitory imbalance [51, 52]. GABAergic synapse pathway decreased in Ab- DLB patients following treatment is therefore consistent with a shift toward excitatory transmission when the cGMP cascade is intact in the absence of amyloid co-pathology.

The mitophagy pathway score decreased only in $A\beta^+$ DLB patients, suggesting differential mitochondrial quality control in the presence of amyloid co-pathology. Mitophagy—the process of degrading dysfunctional mitochondria—is essential for maintaining neuronal health, as mitochondria play a critical role in neurotransmission [53]. This finding represents the second

mitochondrial-related pathway affected by treatment, the first being pantothenate and CoA biosynthesis. Together, these changes point to a treatment effect on mitochondrial function, particularly in $A\beta^+$ DLB. Increased activity in the pantothenate/CoA pathway may reflect improved energy metabolism, which could reduce the need for mitophagy by limiting mitochondrial damage. Collectively, these observations support a biologically plausible link between irsenontrine's cGMP-driven synaptic mechanisms and benefit in $A\beta^-$ DLB, while highlighting how amyloid co-pathology may attenuate certain neurotransmission-related effects.

Lastly, the goal of the CSF proteomics analysis was to characterize the DLB patients enrolled in this trial compared to controls and relate this to larger cohorts from literature. Here, we showed that DDC, a novel hallmark of Lewy body related movement disorders involved in the production of dopamine, was one of the proteins that was most upregulated in DLB patients enrolled in this trial compared to the control cohort, consistent with what has been shown previously and characteristic of Lewy body diseases (LBD) [25, 30–33]. IL18, an inflammatory marker, has also been shown to be upregulated in DLB versus controls in a previous study [34]. NEFL was significantly upregulated in DLB compared to CU individuals, similar results (on a trend level) were observed on a larger cohort [32, 33]. Similarly, other markers (FCER2, MMP10, CRH, GH), although not significantly different between DLB patients enrolled in the trial and the control cohort likely due to the small sample size, showed a similar direction as previously described [25]. Overall, these results indicate that the DLB patients included in this trial are a representative group of the overall DLB population. Moreover, we show that both $A\beta^+$ and $A\beta^-$ patients follow a DLB specific pattern at baseline. Because of this, we propose that the difference in treatment response in the two subgroups would primarily be driven by the underlying amyloid co-pathology.

In the current study, we demonstrate that proteomics can support exploratory analyses within relatively small clinical trial cohorts. The main limitations of this study are the small sample size, particularly when stratified per amyloid status, and the lack of a placebo group. As a result, we were unable to apply multiple-testing corrections in the baseline vs. week 9 analyses, thereby increasing the risk of false positives. Moreover, the severity of AD co-pathology can also influence the effects of co-pathology on treatment efficacy. In the current study we cannot purely base AD co-pathology status based on the Aβ42/40 ratio. However, pTau or tTau can also inform on the staging of disease. The sample size of the current study is too small to investigate this further. Nonetheless, the broad scope of proteomics and pathway analysis allow for the generation of novel hypotheses that can be explored in future clinical trials.

Conclusion

The current findings suggest that co-pathology has an effect on the overall irsenontrine response, visible in more proteins and pathways being affected in DLB patients without AD co-pathology, suggesting that DLB patients without amyloid co-pathology might respond better to treatment. The impact of co-pathology in clinical trials has been previously recognized with the implication for precision medicine that biomarkers can improve patient selection [25, 54, 55]. Here, we show that there is a differential effect of irsenontrine on pathways related to its anticipated mechanism of action. Specifically, in the $A\beta^-$ DLB patients pathways related to cell signaling and neurotransmission were affected. In the $A\beta^+$ DLB patients, pathways related to the mitochondria were more affected. It could be useful to focus future clinical trials with irsenontrine on $A\beta^-$ DLB patients.

Abbreviations

Aβ	Amyloid β
BDNF	Brain derived neurotrophic factor
cGMP	Cyclic guanosine monophosphate
CSF	Cerebrospinal fluid
CU	Cognitively unimpaired
DLB	Dementia with Lewy bodies
E2027	Irsenontrine
GluA1	AMPA-selective glutamate receptor 1
LBD	Lewy body diseases
MMSE	Mini Mental State Exam
PDD	Parkinson disease-dementia
PDE9	Phosphodiesterase 9
PEA	Proximity extension assay
PI	Phosphatidylinositol
PKG	Protein kinase G
pTau	Phosphorylated Tau
TCA	Tricarboxylic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-026-02065-w.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

M.O., S.G.J.G.I.V., L.V., P.S. and C.E.T. designed the study. Y.Y. and M.v.I. contributed to the statistical analysis and figures. L.V. and E.G.B.V. recruited participants and collected data and samples. M.K.S. and L.V. prepared samples for proteomic and biomarker analyses. S.S., S.H., M.I. designed and contributed to the clinical trial. M.O. and L.V. drafted the manuscript. P.S. and C.E.T. were major contributors to the writing of the manuscript. All authors read and contributed to the revisions and editing of this manuscript.

Funding and conflicts

The clinical trial was funded by Eisai Inc. The post-hoc proteomics sample analyses, data analyses, and manuscript preparation were a shared effort between Amsterdam UMC and Eisai Inc.

Data availability
This clinical trial dataset is not publicly available. Qualified researchers can apply for collaboration projects and federated analyses.

Declarations

Ethics approval and consent to participate
The trial was conducted in accordance with ICH guidelines and the ethical principles of the Declaration of Helsinki. The trial was approved by an independent local ethical committee at each participating country/center. Ethical approval of the sub-study was provided by a local review board of the Amsterdam UMC. All study participants or a representative provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Neurochemistry Laboratory, Department of Laboratory Medicine, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, De Boelelaan 1117, Amsterdam 1081 HV, The Netherlands
[2]Translational AI in Laboratory Medicine, Department of Laboratory Medicine, Vrije Universiteit Amsterdam, Amsterdam UMC, De Boelelaan 1117, Amsterdam 1081 HV, The Netherlands
[3]Alzheimer Center, Department of Neurology, Vrije Universiteit Amsterdam, Amsterdam UMC, De Boelelaan 1117, Amsterdam 1081 HV, The Netherlands
[4]Eisai Inc, Nutley, NJ, USA

Received: 5 February 2026 / Accepted: 20 April 2026
Published online: 24 April 2026

References

1. Soysal P, Tan SG. The prevalence and co-incidence of geriatric syndromes in older patients with early-stage Alzheimer's disease and dementia with Lewy bodies. Aging Clin Exp Res. 2021;33:2599–603. https://doi.org/10.1007/s40520-020-01774-y.
2. Attems J, et al. Neuropathological consensus criteria for the evaluation of Lewy pathology in post-mortem brains: a multi-centre study. Acta Neuropathol. 2021;141:159–72. https://doi.org/10.1007/s00401-020-02255-2.
3. McKeith IG, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology. 2017;89:88–100. https://doi.org/10.1212/wnl.0000000000004058.
4. Azhar L, Kusumo RW, Marotta G, Lanctôt KL, Herrmann N. Pharmacological Management of Apathy in Dementia. CNS Drugs. 2022;36:143–65. https://doi.org/10.1007/s40263-021-00883-0.
5. Hershey LA, Coleman-Jackson R. Pharmacological Management of Dementia with Lewy Bodies. Drugs Aging. 2019;36:309–19. https://doi.org/10.1007/s40266-018-00636-7.
6. Ando M, Kotani S, Watanabe N, Fukushima T. [P3–043]: PRECLINICAL CHARACTERIZATION OF E2027, A NOVEL PHOSPHODIESTERASE 9 INHIBITOR. Alzheimer's Dement. 2017;13:946–P946. https://doi.org/10.1016/j.jalz.2017.06.1856.
7. Ando M, P3-062: Effect of repeated administration of e, a novel Phosphodiesterase-9 inhibitor, on cyclic GMP levels in rat cerebrospinal fluid. 2027,. *Alzheimer's & Dementia* 14, P1088–P1088 (2018). https://doi.org/10.1016/j.jalz.2018.06.1418
8. Francis SH, Busch JL, Corbin JD. cGMP-Dependent Protein Kinases and cGMP Phosphodiesterases in Nitric Oxide and cGMP Action. Pharmacol Rev. 2010;62:525–63. https://doi.org/10.1124/pr.110.002907.
9. Tropea MR, et al. Nitric oxide/cGMP/CREB pathway and amyloid-beta crosstalk: From physiology to Alzheimer's disease. Free Radic Biol Med. 2022;193:657–68. https://doi.org/10.1016/j.freeradbiomed.2022.11.022.
10. Bollen E, et al. Improved Long-Term Memory via Enhancing cGMP-PKG Signaling Requires cAMP-PKA Signaling. Neuropsychopharmacology. 2014;39:2497–505. https://doi.org/10.1038/npp.2014.106.
11. Ishihara Y, et al. A novel selective phosphodiesterase 9 inhibitor, irsenontrine (E2027), enhances GluA1 phosphorylation in neurons and improves learning and memory via cyclic GMP elevation. Neuropharmacology. 2025;273:110428. https://doi.org/10.1016/j.neuropharm.2025.110428.
12. Ando M, et al. E2027 (irsenontrine), a phosphodiesterase 9 inhibitor, enhances cholinergic function when combined with donepezil hydrochloride. Eur J Pharmacol. 2025;1006:178126. https://doi.org/10.1016/j.ejphar.2025.178126.
13. van de Beek M, 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:e1262–72. https://doi.org/10.1212/wnl.0000000000200048.
14. Beach TG et al. Clinicopathological Heterogeneity of Lewy Body Diseases: The Profound Influence of Comorbid Alzheimer's Disease. *medRxiv* (2024). https://doi.org/10.1101/2024.08.30.24312864
15. IrizarryM et al. Results of a Phase 2/3 Placebo-Controlled, Double-Blind, Parallel-Group, Randomized 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 BodiesClinical Trials on Alzheimer's disease (2022).15th Conference Clinical Trials Alzheimer's Disease, November 29–December 2, 2022, San Francisco, CA, USA: Posters (Clinical Trial Alzheimer's Disease). J Prev Alzheimers Dis. 2022;9(Suppl 1):51–248. https://doi.org/10.14283/jpad.2022.97.
16. Teich AF, et al. Synaptic Therapy in Alzheimer's Disease: A CREB-centric Approach. Neurotherapeutics. 2015;12:29–41. https://doi.org/10.1007/s13311-014-0327-5.
17. Leuzy A, et al. Robustness of CSF Aβ42/40 and Aβ42/P-tau181 measured using fully automated immunoassays to detect AD-related outcomes. Alzheimer's Dement. 2023;19:2994–3004. https://doi.org/10.1002/alz.12897.
18. Dumurgier J, et al. Cerebrospinal fluid amyloid-β 42/40 ratio in clinical setting of memory centers: a multicentric study. Alzheimers Res Ther. 2015;7:30. https://doi.org/10.1186/s13195-015-0114-5.
19. van der Flier WM, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. J Alzheimers Dis. 2014;41:313–27. https://doi.org/10.3233/jad-132306.
20. van der Flier WM, Scheltens P. Amsterdam Dementia Cohort: Performing Research to Optimize Care. J Alzheimers Dis. 2018;62:1091–111. https://doi.org/10.3233/JAD-170850.
21. Duits FH, et al. The cerebrospinal fluid Alzheimer profile: Easily said, but what does it mean? Alzheimer's Dement. 2014;10:713–e723712. https://doi.org/10.1016/j.jalz.2013.12.023.
22. Tijms BM, et al. Unbiased Approach to Counteract Upward Drift in Cerebrospinal Fluid Amyloid-β 1–42 Analysis Results. Clin Chem. 2018;64:576–85. https://doi.org/10.1373/clinchem.2017.281055.
23. van der Ende EL, et al. CSF proteomics in autosomal dominant Alzheimer's disease highlights parallels with sporadic disease. Brain. 2023;146:4495–507. https://doi.org/10.1093/brain/awad213.
24. del Campo M, et al. CSF proteome profiling across the Alzheimer's disease spectrum reflects the multifactorial nature of the disease and identifies specific biomarker panels. Nat Aging. 2022;2:1040–53. https://doi.org/10.1038/s43587-022-00300-1.
25. del Campo M, et al. CSF proteome profiling reveals biomarkers to discriminate dementia with Lewy bodies from Alzheimer´s disease. Nat Commun. 2023;14:5635. https://doi.org/10.1038/s41467-023-41122-y.
26. Hok-A-Hin YS et al. Large-scale CSF proteome profiling identifies biomarkers for accurate diagnosis of Frontotemporal Dementia. *medRxiv*, 2024.2008.2019.24312100 (2024). https://doi.org/10.1101/2024.08.19.24312100
27. Wanggou S, et al. Sample Level Enrichment Analysis of KEGG Pathways Identifies Clinically Relevant Subtypes of Glioblastoma. J Cancer. 2016;7:1701–10. https://doi.org/10.7150/jca.15486.
28. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28:27–30. https://doi.org/10.1093/nar/28.1.27.
29. Sachdev P, et al. The effects of the novel phosphodiesterase 9 (pde9) inhibitor e2027 (irsenontrine) on csf cgmp, additional csf and plasma biomarkers, and clinical outcomes in amyloid positive and amyloid negative patients with dementia with lewy bodies (dlb) and parkinson's disease dementia (pdd). 15th Conference Clinical Trials Alzheimer's Disease, November 29–December 2, 2022, San Francisco, CA, USA: Posters (Clinical Trial Alzheimer's Disease). J Prev Alzheimers Dis. 2022;9(Suppl 1):51–248. https://doi.org/10.14283/jpad.2022.97.

30. Appleton E, et al. DOPA-decarboxylase is elevated in CSF, but not plasma, in prodromal and de novo Parkinson's disease. Transl Neurodegener. 2024;13:31. https://doi.org/10.1186/s40035-024-00421-0.
31. Paslawski W, et al. Large-scale proximity extension assay reveals CSF midkine and DOPA decarboxylase as supportive diagnostic biomarkers for Parkinson's disease. Transl Neurodegener. 2023;12:42. https://doi.org/10.1186/s40035-023-00374-w.
32. Pereira JB, et al. DOPA decarboxylase is an emerging biomarker for Parkinsonian disorders including preclinical Lewy body disease. Nat Aging. 2023;3:1201–9. https://doi.org/10.1038/s43587-023-00478-y.
33. Rutledge J, et al. Comprehensive proteomics of CSF, plasma, and urine identify DDC and other biomarkers of early Parkinson's disease. Acta Neuropathol. 2024;147:52. https://doi.org/10.1007/s00401-024-02706-0.
34. Higginbotham L et al. Multiplex Proteomics of Lewy Body Dementia Reveals Cerebrospinal Fluid Biomarkers of Disease Pathology and Progression. *bioRxiv*, 2025.2006.2010.658994 (2025). https://doi.org/10.1101/2025.06.10.658994
35. Ismail N, et al. Vitamin B5 (d-pantothenic acid) localizes in myelinated structures of the rat brain: Potential role for cerebral vitamin B5 stores in local myelin homeostasis. Biochem Biophys Res Commun. 2020;522:220–5. https://doi.org/10.1016/j.bbrc.2019.11.052.
36. García-Villafranca J, Guillén A, Castro J. Involvement of nitric oxide/cyclic GMP signaling pathway in the regulation of fatty acid metabolism in rat hepatocytes. Biochem Pharmacol. 2003;65:807–12. https://doi.org/10.1016/S0006-2952(02)01623-4.
37. Scholefield M, et al. Substantively Lowered Levels of Pantothenic Acid (Vitamin B5) in Several Regions of the Human Brain in Parkinson's Disease Dementia. Metabolites. 2021;11. https://doi.org/10.3390/metabo11090569.
38. Xu J, et al. Cerebral deficiency of vitamin B5 (d-pantothenic acid; pantothenate) as a potentially-reversible cause of neurodegeneration and dementia in sporadic Alzheimer's disease. Biochem Biophys Res Commun. 2020;527:676–81. https://doi.org/10.1016/j.bbrc.2020.05.015.
39. Rajkumar AP, et al. Postmortem Cortical Transcriptomics of Lewy Body Dementia Reveal Mitochondrial Dysfunction and Lack of Neuroinflammation. Am J Geriatric Psychiatry. 2020;28:75–86. https://doi.org/10.1016/j.jagp.2019.06.007.
40. Okkels N, et al. Cholinergic changes in Lewy body disease: implications for presentation, progression and subtypes. Brain. 2024;147:2308–24. https://doi.org/10.1093/brain/awae069.
41. Posor Y, Jang W, Haucke V. Phosphoinositides as membrane organizers. Nat Rev Mol Cell Biol. 2022;23:797–816. https://doi.org/10.1038/s41580-022-00490-x.
42. Frere SG, Chang-Ileto B, Di Paolo G. Role of phosphoinositides at the neuronal synapse. Subcell Biochem. 2012;59:131–75. https://doi.org/10.1007/978-94-007-3015-1_5.
43. Fiscus RR. Involvement of Cyclic GMP and Protein Kinase G in the Regulation of Apoptosis and Survival in Neural Cells. Neurosignals. 2002;11:175–90. https://doi.org/10.1159/000065431.
44. Takuma K, et al. Anti-apoptotic Effect of cGMP in Cultured Astrocytes: INHIBITION BY cGMP-DEPENDENT PROTEIN KINASE OF MITOCHONDRIAL PERMEABLE TRANSITION PORE*. J Biol Chem. 2001;276:48093–9. https://doi.org/10.1074/jbc.M108622200.
45. Rivera J, Sharma B, Torres MM, Kumar S. Factors affecting the GABAergic synapse function in Alzheimer's disease: Focus on microRNAs. Ageing Res Rev. 2023;92:102123. https://doi.org/10.1016/j.arr.2023.102123.
46. Krueger-Burg D. Understanding GABAergic synapse diversity and its implications for GABAergic pharmacotherapy. Trends Neurosci. 2025;48:47–61. https://doi.org/10.1016/j.tins.2024.11.007.
47. Heaney CF, Kinney JW. Role of GABAB receptors in learning and memory and neurological disorders. Neurosci Biobehavioral Reviews. 2016;63:1–28. https://doi.org/10.1016/j.neubiorev.2016.01.007.
48. Giachino C, et al. GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. Development. 2014;141:83–90. https://doi.org/10.1242/dev.102608.
49. Barnstable CJ, Wei J-Y, Han M-H. Modulation of synaptic function by cGMP and cGMP-gated cation channels. Neurochem Int. 2004;45:875–84. https://doi.org/10.1016/j.neuint.2004.03.018.
50. Wexler EM, Stanton PK, Nawy S. Nitric Oxide Depresses GABA < sub>A Receptor Function via Coactivation of cGMP-Dependent Kinase and Phosphodiesterase. J Neurosci. 1998;18:2342–9. https://doi.org/10.1523/jneurosci.18-07-02342.1998.
51. Carello-Collar G, et al. The GABAergic system in Alzheimer's disease: a systematic review with meta-analysis. Mol Psychiatry. 2023;28:5025–36. https://doi.org/10.1038/s41380-023-02140-w.
52. Bi D, Wen L, Wu Z, Shen Y. GABAergic dysfunction in excitatory and inhibitory (E/I) imbalance drives the pathogenesis of Alzheimer's disease. Alzheimer's Dement. 2020;16:1312–29. https://doi.org/10.1002/alz.12088.
53. Wang S, et al. The mitophagy pathway and its implications in human diseases. Signal Transduct Target Therapy. 2023;8:304. https://doi.org/10.1038/s41392-023-01503-7.
54. Scott GD et al. Fluid and Tissue Biomarkers of Lewy Body Dementia: Report of an LBDA Symposium. *Front Neurol* 12, 805135 (2021). https://doi.org/10.3389/fneur.2021.805135
55. Gibson LL, Abdelnour C, Chong J, Ballard C, Aarsland D. Clinical trials in dementia with Lewy bodies: the evolving concept of co-pathologies, patient selection and biomarkers. Curr Opin Neurol. 2023;36:264–75. https://doi.org/10.1097/wco.0000000000001173.

Publisher's Note