Effects of simufilam on cerebrospinal fluid biomarkers in Alzheimer's disease: A randomized clinical trial

Hoau-Yan Wang
CUNY School of Medicine: The City College of New York CUNY School of Medicine
Zhe Pei
CUNY School of Medicine at The City College of New York: The City College of New York CUNY School of Medicine
Kuo-Chieh Lee
CUNY School of Medicine: The City College of New York CUNY School of Medicine
Yaneicy Gonzalez Rojas
Optimus U Corp
Tamara Doehner
Cognitive Clinical Trials
John Puente
Cognitive Clinical Trials
Patrick Sciara
Cognitive Clinical Trials
Brian Beck
Cognitive Clinical Trials
Evelyn Lopez-Brignoni
IMIC Research
Boris Nikolov
IMIC Research
Carrie Crowley
Cassava Sciences, Inc.
George Ben Thornton
Cassava Sciences, Inc.
Remi Barbier
Cassava Sciences, Inc.
Nadav Friedmann
Cassava Sciences, Inc.
Jeffrey L. Cummings
University of Nevada Las Vegas

Lindsay Burns

lburns@cassavasciences.com

Cassava Sciences, Inc. https://orcid.org/0000-0002-4303-3174

Research

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EDITORIAL NOTE:

20 March, 2024. [In light of concerns](https://www.science.org/content/article/damning-fda-inspection-report-undermines-positive-trial-results-possible-alzheimer-s) about the credibility of claims by Wang and Cassava about their Alzheimer's drug simufilam, it's important for readers to be aware of allegations of data manipulation and the ongoing U.S. criminal probe and SEC scrutiny. While the FDA has allowed ongoing trials to continue, these regulatory and investigative challenges cannot be overlooked. We encourage a balanced view on this preprint considering the potential benefits of the drug alongside the current investigations into Cassava Sciences' research practices.

Abstract

BACKGROUND

Simufilam is a first-in-class drug candidate targeting altered filamin A, a proteopathy in Alzheimer's disease. The primary objective of this Phase 2 clinical trial was to evaluate the effects of simufilam on cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease patients. A secondary objective was to assess cognitive enhancement.

METHODS

In a randomized, placebo-controlled trial conducted across 9 clinical sites in the US, 64 mild-to-moderate Alzheimer's disease patients were randomized to simufilam 50 or 100 mg b.i.d. or placebo for 28 days. Clinical diagnosis was confirmed by CSF total tau/amyloid-beta$_{1-42}$ (Aβ_{42}) > 0.28. Co-primary endpoints were changes in CSF Aβ_{42}, total tau, phospho-tau (P-tau181), neurogranin, neurofilament light chain, and YKL-40. Secondary endpoints included additional CSF biomarkers assessing neuroinflammation and blood brain barrier integrity, and tests of episodic and spatial working memory.

RESULTS

Adjusting for multiplicity of the six co-primary endpoints ($p < 0.008$ versus placebo required for significance), simufilam 50 and 100 mg significantly reduced CSF levels of total tau, hyperphosphorylated tau (P-tau181), neurogranin, neurofilament light chain and YKL-40. Simufilam 50 mg significantly increased CSF levels of Aβ_{42}. On secondary CSF biomarker endpoints, both doses of simufilam significantly reduced IL-6, soluble TREM2 (triggering receptor expressed on myeloid cells-2), HMGB1 (high mobility group box-1), albumin and immunoglobulin G. All but one patient improved from baseline across biomarkers. Simufilam 50 and 100 mg showed effect sizes versus placebo (0.23–0.46) in change from baseline in episodic memory and spatial working memory. Episodic memory improvements correlated most strongly with decreases in P-tau181 ($R^2 = 0.48$). Simufilam was safe and well tolerated. Target engagement was demonstrated by filamin A linkages to nicotinic acetylcholine receptor subtype α7 (α7nAChR) and toll-like receptor 4 (TLR4) in lymphocytes.

CONCLUSIONS

Simufilam was safe and well tolerated and significantly improved eleven CSF biomarkers in patients with Alzheimer's disease, implying biological evidence of disease modification. Simufilam will be further evaluated in large, definitive clinical trials.

TRIAL REGISTRATION:

ClinicalTrials.gov Identifier NCT04079803.

Background

There are no approved treatments to slow the progression of Alzheimer's disease, expected to affect 13.8 million in the U.S. by 2050.[1] Biomarkers may facilitate drug development in Alzheimer's disease by quantifying disease stage, demonstrating target engagement, and supporting disease modification.[2]

Core CSF biomarkers of Alzheimer's disease are amyloid-beta1-42 ($A\beta_{42}$), total tau and phospho-tau181 (P-tau181).[3,4] $A\beta_{42}$ decreases while tau and phosphorylated tau, including P-tau181, increase as disease progresses and cognition declines. Neurogranin and neurofilament light chain, indicating damage to dendrites and axons respectively, are used to track disease progression.[5–7] Interestingly, neurogranin appears specific to Alzheimer's disease.[7] The current clinical trial measured CSF biomarkers in Alzheimer's disease dementia patients to evaluate drug candidate simufilam.

Simufilam represents a novel approach to combat amyloid toxicity and resulting neurodegeneration in Alzheimer's disease. Soluble $A\beta_{42}$ initiates a predominant pathogenic pathway by binding α7 nicotinic acetylcholine receptor (α7nAChR), the only known sub-nanomolar-affinity binding site of soluble $A\beta_{42}$.[8–10] This femtomolar interaction poses enormous competition for any agent aiming to reduce soluble $A\beta_{42}$ interactions. $A\beta_{42}$ binds and signals through this receptor to activate kinases that hyperphosphorylate the protein tau,[10–13] impairing tau's ability to stabilize microtubules. This loss of functional tau is a primary driver of the neuronal degeneration and cognitive impairment in Alzheimer's disease.[14]

Without directly competing with the femtomolar binding of $A\beta_{42}$ to α7nAChR, simufilam disrupts this ultra-high-affinity interaction by binding a critical accomplice to $A\beta_{42}$: an altered conformation of filamin A. Filamin A is an intracellular scaffolding protein that is highly expressed in brain and interacts with over 90 proteins to coordinate signaling processes.[15] $A\beta_{42}$ initiates toxic signaling by binding α7nAChR to recruit filamin A.[16,17] Without directly contacting filamin A, and likely working through α7nAChR and other receptors that link to or recruit filamin A, $A\beta_{42}$ induces the altered conformation of the filamin A protein.[17] Simufilam binds altered filamin A, restores its normal shape and disrupts the aberrant filamin A – α7nAChR linkage, $A\beta_{42}$'s femtomolar binding to α7nAChR and the ensuing toxic signaling that hyperphosphorylates tau.[17,18]

$A\beta_{42}$ also binds the toll-like receptor 4 (TLR4) co-receptor cluster-of-differentiation14 (CD14)[19] to recruit and alter filamin A.[17] The filamin A – TLR4 linkage enables persistent TLR4 activation by $A\beta_{42}$, causing inflammatory cytokine release and neuroinflammation. Simufilam's reversal of the filamin A proteopathy also blocks this $A\beta_{42}$-induced neuroinflammation.[17,18]

In a previous open-label, 28-day trial in patients with mild-to-moderate Alzheimer's disease dementia (NCT03748706), simufilam significantly reduced CSF total tau, P-tau181 and biomarkers of neurodegeneration and neuroinflammation in all patients, with no safety issues.[20] Biomarker reductions implied reduced disease pathophysiology and neurodegeneration, consistent with simufilam's mechanism of action and preclinical data. Based on encouraging prior clinical trial results, we evaluated simufilam in a randomized, double-blind, placebo-controlled Phase 2 trial. We hypothesized simufilam treatment would impact CSF biomarkers and may enhance cognition.

Methods

Patient Population

Patients were 50–85 years old, diagnosed with probable Alzheimer's disease dementia according to National Institute on Aging (NIA)/Alzheimer's Association (AA) criteria and a Mini-Mental State Exam (MMSE) score ≥ 16 and ≤ 26. Diagnosis was confirmed by CSF total tau/$A\beta_{42}$ ≥ 0.28, a ratio selected to exclude dementia due to other causes (0.28 is intermediate between early and late mild cognitive impairment in amyloid-confirmed patients from the Alzheimer's Disease Neuroimaging Initiative[21]). Patients could be receiving acetylcholinesterase inhibitors, memantine and other medications if stable. Chronic opioids, tricyclic antidepressants, monoamine oxidase inhibitors, nicotine therapy (or smokers) were exclusions, as were uncontrolled medical illnesses, other neurodegenerative diseases, or clinically significant laboratory results.

Trial Design

This double-blind, placebo-controlled, trial randomized 64 patients 1:1:1 to placebo or simufilam 50 or 100 mg oral tablets b.i.d. for 28 days. Patients, caregivers, clinic staff, the study sponsor and the laboratory analyzing biomarkers were blind to treatment. A randomization algorithm was generated by an outside vendor using Interactive Response Technology. Doses were selected by body surface area conversion of effective daily doses in mouse efficacy models and prior clinical experience. Sample sizes of 20 per arm were selected based on highly significant changes from baseline in many of the same CSF biomarkers by paired t-test in a prior open-label trial in mild-to-moderate Alzheimer's disease dementia patients.[22]

After initial screening, a second screening visit included a CSF draw and practice cognitive test. Cognitive tests were conducted Days 1 and 28. Blood samples were collected Days 1, 7, 14 and 28, with the Day 28 blood sample following the second CSF draw for CSF/plasma ratios of simufilam. Electrocardiograms and physical examinations were conducted on Days 1 and 28.

Oversight and Settings

This trial was conducted between September 2019 and March 2020 at nine U.S. sites. An independent Data and Safety Monitoring Board approved the protocol and assessed safety mid-study. CSF samples

were analyzed at City University School of Medicine. Plasma was analyzed with a qualified assay at Worldwide Clinical Trials Bioanalytical Sciences. Data were analyzed by a data management and statistics contractor. Data was 100% monitored by independent clinical research associates. No protocol changes were made.

Assessments

Levels of eleven CSF biomarkers in the screening and Day 28 samples were measured. Six CSF biomarkers were designated primary: biomarkers of Alzheimer's disease pathology ($A\beta_{42}$, total tau and P-tau181), neurodegeneration (neurofilament light chain and neurogranin) and neuroinflammation (YKL-40). Also assessed were interleukin-6, soluble triggering receptor expressed on myeloid cells 2 (sTREM2), and high mobility group box 1 (HMGB1). These nine biomarkers were measured using commercial enzyme-linked immunosorbent assay plates and an automated plate reader, with samples assayed in triplicate. CSF albumin and immunoglobulin G assessed blood-brain barrier integrity and were measured by immunoblot with densitometric quantitation. Target engagement was evaluated by measuring filamin A linkages to α7nAChR and TLR4 in patient lymphocytes by co-immunoprecipitation as described.[17]

Cognition was assessed on Day 1 and Day 28 by the Paired Associates Learning (an episodic memory test) and Spatial Working Memory tests of the Cambridge Neuropsychological Test Automated Battery (CANTAB). Both tests increase progressively in difficulty, with errors imputed for levels not reached. Reductions in the total error scores indicate improvement. The CANTAB Reaction Time test assessed psychomotor speed in milliseconds.

Safety was assessed by adverse event monitoring, clinical laboratory tests, electrocardiography, physical examinations and the Columbia-Suicide Severity Rating Scale.

Outcomes

The six co-primary outcome measures were changes in CSF $A\beta_{42}$, total tau, P-tau181, neurogranin, neurofilament light chain, and YKL-40 levels from screening to Day 28. These six biomarkers were prospectively listed in the trial registration. $A\beta_{42}$, total tau and P-tau181 are considered core biomarkers of Alzheimer's disease pathology. Neurogranin and neurofilament light chain are intracellular proteins in dendrites and axons, respectively, that indicate neurodegeneration when found in CSF. YKL-40 is a glycoprotein involved in tissue remodeling after inflammation. Secondary biomarker outcomes included changes in CSF interleukin-6, sTREM2, HMGB1, albumin and immunoglobulin G. Interleukin-6 is an inflammatory cytokine. A marker of microglial-induced inflammation, sTREM2 is the ectodomain of the transmembrane receptor TREM2 that is cleaved and shed by microglia when activated during inflammation.[23] HMGB1 is a damage-associated molecular pattern protein released by necrotic cells and actively secreted by immune cells to further neuroinflammation and neurite damage.[24] Finally, albumin and immunoglobulin G are blood proteins that indicate blood-brain barrier compromise when found in CSF.[25]

Secondary cognitive outcome measures were drug-placebo differences in change from Day 1 to Day 28 in total errors on Paired Associates Learning and Spatial Working Memory tests. The Reaction Time test exploratory outcome was median response time in milliseconds.

The target engagement assay measured changes in filamin A linkages to α7nAChR and TLR4 in patient lymphocytes from Day 1 to Day 28.

Statistical Analysis

The pre-specified analysis for biomarkers was drug-placebo differences in change from baseline, analyzed by the General Linear Model for the analysis of covariance (ANCOVA) with a two-sided 95% confidence interval and baseline CSF measurement as the covariate. Multiplicity of the six co-primary endpoints was addressed by the significance requirement: $p < 0.008$ (i.e., $p < 0.05/6$).

The Full Analysis Set included all subjects with two CSF samples. Although plasma samples were collected at all visits to confirm compliance, the primary analyses were conducted on all patients. The secondary analyses excluded three subjects with no detectable levels of simufilam in plasma at any visit. Percent change from baseline of compliers in active treatment compared to placebo-treated participants was analyzed by the General Linear Model for the ANCOVA.

Lymphocyte biomarkers were analyzed by ANOVA: comparing to each patient's own baseline was considered more appropriate than adjusting for baseline value by ANCOVA, given the range of baseline values. The FLNA – α7nAChR linkage for the 100 mg dose arm versus placebo was the only comparison significantly different by ANOVA but not by ANCOVA.

Tests of cognition were not powered for statistical significance and were therefore evaluated by effect size, a standardized measure of relative size of treatment effect. Effect sizes of 20–25% are considered noteworthy, and a 25% effect size is typically considered clinically meaningful if significance is achieved in later, appropriately powered trials. For cognitive tests, effect sizes for each simufilam dose versus placebo were calculated by Hedge's g, appropriate for groups of 20, and these were identical or nearly identical to those calculated by Cohen's d. For the Paired Associates Learning test, the most and least impaired subjects were excluded by baseline score (≤ 11 or ≥ 54 of 70 total possible errors) prior to calculation of effect size. These cutoffs were employed to remove subjects with very few errors (ceiling effects), as well as subjects who performed so poorly that they may not have understood the task. Effect sizes for spatial working memory included all subjects with detectable plasma simufilam. Reaction time was measured in milliseconds between stimulus onset and response.

Results

Trial Population

Of 115 patients screened, 64 patients enrolled. Twenty-two were randomized to placebo and 21 each to simufilam 50 mg and 100 mg. One participant discontinued for non-medical reasons (Fig. 1). One

completer was excluded from the primary analyses due to a missing Day 28 sample. One patient in the 50 mg arm and two in the 100 mg arm were excluded from the secondary analyses due to no detectable plasma levels of simufilam at return visits. Baseline demographics, MMSE, cognitive assessment scores, concomitant cholinesterase inhibitor or memantine use, and baseline biomarker levels were well balanced between groups (Table 1). CSF/plasma simufilam levels in simufilam arms were 0.29 ± 0.21.

Table 1
Baseline Demographics and Assessments

Demographics and Characteristics	Placebo (N = 22)	Simufilam 50 mg (N = 21)	Simufilam 100 mg (N = 21)
Age, mean (SD)	71.3 (6.68)	69.3 (5.47)	67.1 (8.76)
Female sex, No. (%)	11 (50.0)	12 (57.1)	12 (57.1)
Not white race, No. (%)	3 (13.6)	4 (19.0)	2 (9.5)
Hispanic or Latino ethnicity, No. (%)	9 (40.9)	11 (52.4)	11 (52.4)
CSF total tau/$A\beta_{42}$ ratio (SD)	1.20 (0.55)	1.17 (0.58)	1.08 (0.50)
MMSE, mean (SD)	23.1 (2.78)	22.7 (2.67)	23.0 (2.66)
APOE4 homozygous	1	1	3
APOE4 heterozygous	12	14	10
Taking cholinesterase inhibitor or memantine, No. (%)	8 (36.4)	5 (23.8)	7 (33.3)
Paired Associates Learning total errors, mean (SD)	35.5 (19.65)	36.1 (18.76)	31.0 (20.74)
Spatial Working Memory total errors, mean (SD)	19.0 (7.49)	22.3 (6.64)	22.1 (5.88)
CSF $A\beta_{42}$ pg/mL, mean (SD)	125 (152)	108 (54.8)	117 (51.4)
CSF total tau pg/mL, mean (SD)	104 (32)	101 (17.6)	106 (27.9)
CSF P-tau181 pg/mL, mean (SD)	28.5 (0.73)	29.0 (1.0)	29.7 (1.5)
CSF neurogranin pg/mL, mean (SD)	1200 (365)	1352 (614)	1551 (751)
CSF NfL pg/mL, mean (SD)	161 (42.8)	181 (64.4)	219 (95.3)
CSF YKL-40 pg/mL, mean (SD)	206 (29.5)	194 (26.0)	203 (22.7)
CSF IL-6 pg/mL, mean (SD)	32.5 (1.2)	33.6 (1.7)	33.6 (1.8)
CSF sTREM2, pg/mL, mean (SD)	878 (435)	882 (476)	861 (421)

Demographics and Characteristics	Placebo (N = 22)	Simufilam 50 mg (N = 21)	Simufilam 100 mg (N = 21)
CSF HMGB1, pg/mL, mean (SD)	424 (48.0)	454 (70.6)	446 (67.3)
CSF/plasma albumin ratio, mean (SD)	0.24 (0.03)	0.25 (0.05)	0.25 (0.08)
CSF/plasma IgG ratio, mean (SD)	0.200 (0.07)	0.227 (0.07)	0.217 (0.11)
Lymphocyte filamin A – α7nAChR, Ratio to total filamin A, mean (SD)	0.59 (0.10)	0.66 (0.12)	0.69 (0.11)
Lymphocyte filamin A – TLR4, Ratio to total filamin A, mean (SD)	0.55 (0.10)	0.58 (0.11)	0.60 (0.07)

CSF Biomarker Change from Baseline

The pre-specified primary analysis was change from baseline to Day 28 on six CSF biomarkers ($A\beta_{42}$, total tau, P-tau181, neurogranin, neurofilament light chain and YKL-40) in the drug arms versus the placebo arm. Significance levels were adjusted for multiplicity ($p < 0.05/6$ or $p < 0.008$). Both dose arms showed significant changes from baseline on five of the six primary biomarkers, with the increase in $A\beta_{42}$ in the 100 mg dose arm not significant, likely due to the range of baseline values (Table 2). The secondary analysis of change from baseline with three non-compliers excluded produced similar results to the primary analysis of the full analysis set. Individual patients' Screening and Day 28 values (pg/mL) are shown by spaghetti plots for each treatment arm (Fig. 2).

Table 2
Biomarkers Change from Baseline in pg/mL (SD)

Biomarker	Placebo	Simufilam 50 mg		Simufilam 100 mg	
	(N = 22)	All Patients	Compliers	All Patients	Compliers
		N = 19	N = 18	N = 21	N = 19
CSF $A\beta_{42}$	4.8 (30.9)	16.2 (21.1) p = 0.01	16.9 (21.5) p = 0.006	12.5 (11.9) p = 0.087	13.4 (11.2) p = 0.088
CSF total tau	-3.2 (14.8)	-14.6 (9.6) p = 0.0012	-14.9 (9.8) p = 0.0014	-18.7 (10.4) p = 0.0000	-19.8 (10.3) p = 0.0000
Total tau/$A\beta_{42}$	-0.029 (0.327)	-0.28 (0.27) p = 0.0006	-0.30 (0.27) p = 0.0008	-0.30 (0.22) p = 0.0001	-0.31 (0.22) p = 0.0001
CSF P-tau181	-0.63 (1.8)	-2.4 (1.6) p = 0.002	2.5 (1.6) p = 0.003	-3.1 (1.7) p = 0.005	-3.2 (1.7) p = 0.003
CSF neurogranin	-50.5 (434.0)	-527 (361) p = 0.0005	-531 (371) p = 0.0006	-648 (491) p = 0.0002	-681 (505) p = 0.0002
CSF Neurofilament Light Chain	-10.0 (45.0)	-49.7 (35.5) p = 0.0058	-51.0 (36.1) p = 0.0008	-76.3 (50.6) p = 0.0003	-78.5 (52.9) p = 0.0002
CSF YKL-40	-0.96 (24.2)	-20.4 (17.4) p = 0.0001	-20.9 (17.7) p = 0.002	-22.3 (11.7) p = 0.0001	-23.7 (11.4) p = 0.0001
CSF Interleukin-6	-1.1 (2.0)	-3.3 (1.8) p = 0.011	-3.3 (1.9) p = 0.019	-3.5 (1.8) p = 0.003	-3.7 (1.8) p = 0.0078
CSF sTREM2	-77.3 (510)	-418 (376) p = 0.0005	-424 (386) p = 0.0007	-404 (269) p = 0.0001	-426 (274) p = 0.0002

[a] Units are optical density units of immunoblot bands.

[b] Densitometric quantities of α7nAChR or TLR4 in anti-filamin A precipitates as a ratio to total filamin A.

N.B.: p values are compared to placebo for each biomarker.

Biomarker	Placebo (N = 22)	Simufilam 50 mg		Simufilam 100 mg	
		All Patients N = 19	Compliers N = 18	All Patients N = 21	Compliers N = 19
CSF HMGB1	19.4 (172.3)	-149 (50.3) p = 0.0001	-152 (50.1) p = 0.0001	-140 (51.3) p = 0.0001	143 (51.3) p = 0.0001
CSF albumin[a]	-240 (1620)	-1184 (1707) p = 0.054	-1245 (1735) p = 0.046	-2103 (1774) p = 0.0001	-2292 (1760) p = 0.0001
CSF IgG[a]	-574.8 (2518.32)	-2269 (2176) p = 0.018	-2444 (2097) p = 0.014	-2253 (2414) p = 0.007	-2350 (2517) p = 0.012
Lymphocyte filamin A – α7nAChR[b]	-0.07 (0.19)	-0.22 (0.13) p = 0.014	-0.23 (0.13) p = 0.009	-0.22 (0.16) P = 0.008	-0.24 (0.16) p = 0.005
Lymphocyte filamin A – TLR4[b]	-0.05 (0.18)	-0.19 (0.11) P = 0.011	-0.19 (0.11) p = 0.010	-0.18 (0.13) p = 0.012	-0.19 (0.14) p = 0.010
[a] Units are optical density units of immunoblot bands.					
[b] Densitometric quantities of α7nAChR or TLR4 in anti-filamin A precipitates as a ratio to total filamin A.					
N.B.: p values are compared to placebo for each biomarker.					

CSF Biomarker Percent Change from Baseline

The secondary analysis of percent change from baseline showed significant differences for both dose arms versus placebo on all eleven CSF biomarkers, adjusted for multiplicity for the six primary biomarkers (Fig. 3). P values for change and percent change from baseline were similar, with $A\beta_{42}$ in the 100 mg dose arm the sole comparison that was significant by percent change but not by change, due to the range in baseline values.

Biomarkers of AD Pathology and Neurodegeneration

Low in Alzheimer's disease, CSF $A\beta_{42}$ significantly increased 17% and 14% in the 50 and 100 mg arms, respectively (p = 0.0004 and p = 0.004). CSF total tau decreased 16% and 18% (p = 0.0002 and p = 0.00001) and CSF P-tau181 decreased 8% and 11% (p = 0.002 and p = 0.003) in 50 and 100 mg dose arms compared to placebo, respectively. CSF neurofilament light chain, reflecting axonal damage, decreased 28% and 34% in respective dose arms (p = 0.002 and p = 0.0003). Neurogranin, indicating post-

synaptic damage, significantly decreased 36% and 43% in respective dose arms (p = 0.0004 and p = 0.0001).

Biomarkers of Neuroinflammation

Simufilam treatment significantly decreased four CSF biomarkers of neuroinflammation compared to placebo. YKL-40 decreased 10% and 11% in the 50 and 100 mg arms, respectively (p = 0.0003 and p = 0.0002). Inflammatory cytokine interleukin-6 decreased 10% and 11% in the 50 and 100 mg arms (p = 0.017 and p = 0.007). Indicating reduced microglial activation, sTREM2 decreased 43% and 46% in in the 50 and 100 mg arms (p = 0.0009 and p = 0.0003). Finally, the damage-associated molecular pattern protein HMGB1 decreased 33% and 32% in in the 50 and 100 mg arms (p = 0.0002 and p = 0.0001).

Biomarkers of Blood-Brain Barrier Integrity

Simufilam improved blood-brain barrier integrity, evidenced by lower levels of albumin and immunoglobulin G in CSF. Simufilam 50 and 100 mg significantly decreased CSF albumin by 15% and 29%, respectively (p = 0.04 and p = 0.0001). CSF immunoglobulin G decreased 30% in both drug arms (both p = 0.02).

Validation of Biomarker Analyses

The statistical validation of biomarker data is supported by the placebo dataset: modest changes (-2%, on average) and robust correlations (mean R^2 = 0.96) between all pair combinations among total tau, P-tau181, neurogranin, neurofilament light chain, YKL-40 and IL-6 in change from baseline. Because $A\beta_{42}$ decreases in CSF in Alzheimer's disease as other markers increase, $A\beta_{42}$ movement negatively correlated with changes in those six biomarkers (mean R^2=-0.82 in placebo). Biomarker changes also correlated in simufilam arms (mean R^2 = 0.77, excluding $A\beta_{42}$), indicating that the magnitude of change in individual patients was generally consistent across biomarkers.

Target Engagement

Both α7nAChR and TLR4 receptors and filamin A are present in lymphocytes, allowing assessment of target engagement in patients' lymphocytes. Filamin A linkages to α7nAChR and TLR4 in lymphocytes were significantly reduced 31–34% from baseline in both drug arms ($p \leq 0.01$).

Cognition

On the Paired Associate Learning test assessing episodic memory, patients in the 50 mg arm made on average 5.7 fewer errors on Day 28, patients in the 100 mg arm made 4.5 fewer errors, and placebo patients made 1.5 fewer errors (Fig. 4). These differences represent 0.37 and 0.23 effect sizes for 50 and 100 mg arms, respectively, versus placebo. The most and least impaired subjects were removed by baseline score ($\geq$ 54 and $\leq$ 11 of 70 possible total errors) to eliminate ceiling effects (those with very few errors) and subjects who performed so poorly that they may not have understood the task. Standard

deviations for change from baseline in PAL total errors were 8.5, 13.6, 17.7 for placebo, 50 and 100 mg, respectively.

In Spatial Working Memory, patients in 50 and 100 mg arms made 2.3 and 3.3 fewer errors, respectively, compared to 0.4 in placebo, representing 0.25 and 0.46 effect sizes. Standard deviations for change from baseline in Spatial Working Memory total errors were 7.5, 7.5, 4.7 for placebo, 50 and 100 mg, respectively.

Improvements in episodic memory, correlated most strongly with decreases in P-tau181 (R^2 = 0.48). Interleukin-6, total tau, albumin, neurofilament light chain and YKL-40 also correlated (R^2 values 0.41, 0.37, 0.37, 0.36 and 0.30, respectively).

In reaction time, placebo, 50 and 100 mg arms showed mean (SD) changes from baseline in median reaction time of -11 (57), -19 (38) and 11 (66) milliseconds, respectively.

Safety

Simufilam was safe and well-tolerated. There were no serious adverse events. Adverse events were mostly mild; none caused discontinuation; none were noted likely to be drug related. Total adverse events were 20, 9 and 15 in placebo, 50 and 100 mg arms, respectively. Adverse events that occurred in 3 or more patients were headache (3, 1 and 2), fatigue (2, 1 and 0), nausea (2, 0 and 1), and upper respiratory infection (1, 2 and 2) for placebo, 50 and 100 mg, respectively.

Discussion

In a randomized clinical trial of 64 patients with Alzheimer's disease dementia, simufilam 50 or 100 mg significantly improved multiple biomarkers of Alzheimer's disease, neurodegeneration, neuroinflammation and blood-brain barrier integrity, with no safety issues. Collectively, results of this randomized controlled trial are consistent with the drug's mechanism of action and replicate a prior, open-label study.[20]

Increases in $A\beta_{42}$ and reductions in total tau and p-Tau181 imply reduced Alzheimer's disease pathophysiology. Reduced levels of neurofilament light chain and neurogranin suggest a slower rate of neurodegeneration. The 36% and 43% reductions in neurogranin, considered specific to Alzheimer's disease,[24] additionally suggest reduced disease pathology. Reductions in neuroinflammatory markers YKL-40, interleukin-6, sTREM2 and HMGB1 indicate suppressed neuroinflammation. Because HMGB1 also damages neurites and furthers neuroinflammation,[24] the more than 30% reductions in HMGB1 imply reduced pathogenic drive. Finally, lower CSF albumin and immunoglobulin G indicate improved blood-brain barrier integrity, possibly related to simufilam's suppression of neuroinflammation, as blood-brain barrier breakdown correlates with neuroinflammation and cognitive decline.[25,26] Restoring α7nAChR function by displacing $A\beta_{42}$ from this receptor may also improve blood-brain barrier integrity.[27,28]

Robust statistical correlations between biomarkers in changes from baseline within the placebo arm illustrate the interdependency of biomarkers in Alzheimer's disease and validate the study's biomarker assessments. Strong correlations between biomarkers in changes from baseline within simufilam arms suggest that the filamin A proteopathy is a critical, upstream pathogenic event in Alzheimer's disease.

Reductions in filamin A linkages to α7nAChR and TLR4 in patient lymphocytes, demonstrating target engagement, likely mirror the target engagement of simufilam in brain. Reductions in these filamin A linkages were previously demonstrated in both brain and lymphocytes of simufilam-treated Alzheimer's disease transgenic mice (lymphocytes unpublished), and in postmortem human Alzheimer's disease brain tissue incubated with simufilam.[17]

The small dose-response in this study suggests near saturation of the target protein, anticipated because simufilam, a small molecule, binds the altered conformation of filamin A with ultra-high (580 femtomolar) affinity.[17] Clean safety, a mild dose-response, high (98%) response rate and clear evidence of target engagement collectively suggest 50–100 mg b.i.d. is an optimal dose range.

Effect sizes on tests of episodic and spatial working memory suggest a drug response. Episodic memory improvements correlated best with decreases in levels of CSF P-tau181. Because cognitive decline is not expected over 28 days in mild-to-moderate Alzheimer's disease patients, the biomarker changes that imply slowed disease progression may also reflect suppressed disease mechanisms and improved neuronal function. Certainly, any benefit to cognition over this trial's duration implies cognitive enhancement.

FDA Guidance requires clinical trials in Alzheimer's disease to show a clinical benefit on cognitive and functional co-primary endpoints. Meaningful benefits are unlikely to occur without concurrent improvements in a broad panel of disease biomarkers. There are few reports of drug effects on CSF biomarkers, and these effects on one to three markers have not always shown concurrent effects on cognition or function.[29] Drug effects on biomarkers that are compellingly related to the neurobiology of Alzheimer's disease in the pathway(s) affected by a drug candidate can support a regulatory claim for disease modification.[30]

Simufilam's potential to modify the disease and enhance cognition is supported by preclinical data. In a triple transgenic mouse model of Alzheimer's disease, simufilam improved cognitive behavior and reduced amyloid deposits, tau hyperphosphorylation, neurofibrillary lesions and inflammatory cytokine release.[17] Additionally, in brains of these transgenic mice, and in postmortem human brain tissue, simufilam restored function of α7nAChR, N-methyl-D-aspartate (NMDA) receptors and insulin receptors and improved synaptic plasticity (indicated by NMDA-induced activity-dependent expression of the master synaptic plasticity regulator Arc).[17] Improvements in receptor function and synaptic plasticity could underlie the apparent cognitive enhancement in this trial.

Limitations

There are several limitations to this study. The sample size is small. The directional changes and statistical significance are encouraging; however, the magnitude of observed biomarker changes is of uncertain significance. The relationships of changes in biomarkers to cognitive and functional measures have not been established, and multiple studies assessing a similar panel of biomarkers are required to determine these correlations and mechanistic relationships. Studies of simufilam large enough to detect treatment effects on clinical measures are warranted. Despite an interpretation of slowed disease processes, this study was not long enough to allow conclusions regarding disease modification. Longer studies are needed to measure effects on the trajectory of clinical decline.

Conclusions

Simufilam is the first of a new class of drug candidates to target altered filamin A, a proteopathy in Alzheimer's disease. This clinical dataset of CSF biomarker changes offers new insights into the pathophysiology of Alzheimer's disease and a potential new therapeutic strategy. Effect sizes on memory assessments indicate potential for cognitive enhancement. Simufilam's ability to slow disease progression in patients will need to be evaluated in large, definitive clinical trials.

Abbreviations

Amyloid-beta1-42 (Aβ42), phospho-tau181 (P-tau181), α7 nicotinic acetylcholine receptor (α7nAChR), toll-like receptor 4 (TLR4), cluster-of-differentiation14 (CD14), National Institute on Aging (NIA), Alzheimer's Association (AA), Mini-Mental State Exam (MMSE), soluble triggering receptor expressed on myeloid cells 2 (sTREM2), high mobility group box 1 (HMGB1), Cambridge Neuropsychological Test Automated Battery (CANTAB)

Declarations

Ethics Approval and Consent to Participate:

This study was reviewed and approved by Advarra, Inc., a central institutional review board. Written informed consent was obtained from all participants.

Consent for Publication:

As patient data is presented only in aggregate, no consent for publication was required.

Availability of Data:

Cassava Sciences has not established a data sharing repository for the data from this trial.

Competing Interests:

Simufilam is the chemical name for a compound owned by Cassava Sciences, Inc. CC, GBT, RB, NF and LHB are Cassava Sciences employees. H-YW and JC are consultants and scientific advisory board members for Cassava Sciences.

Funding:

This trial was supported by NIA grant AG050878. NIA personnel approved the clinical trial protocol. NIA personnel also approved the selection of Data and Safety Monitoring Board members and participate in these meetings.

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Author Contributions

RB, NF and LHB designed the clinical trial with guidance from JC. Biomarker analyses were conducted blind to treatment and time point by H-YW, ZP and K-CL. K-CL and H-YW conducted APOE genotyping. CC oversaw clinical operations and trial monitors. YGR, TAD, JP, BB, PS, ELB and BN were clinical investigators. GBT analyzed lymphocyte assays. LHB wrote the manuscript with help from HYW, RB and JC. All authors have access to the data via an electronic data capture system, except H-YW, ZP and K-CL who remain blinded to treatment.

References

1. Alzheimer's Association. 2020 Alzheimer's disease facts and figures. Alzheimers Dement 2020;16.
2. Cummings J, Feldman H, Scheltens P. The "rights" of precision drug development for Alzheimer's disease. Alzheimers Res Ther 2019;11.
3. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med 2018;284:643-63.
4. Hampel H, Toschi N, F B, et al. Alzheimer’s disease biomarker-guided diagnostic workflow using the added value of six combined cerebrospinal fluid candidates: Aβ1–42, total-tau, phosphorylated-tau, NFL, neurogranin, and YKL-40. Alzheimer's & Dementia 2018;14:492-501.
5. Bacioglu M, Maia L, Preische O, et al. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 2016;91:1-11.
6. Lista S, Hampel H. Synaptic degeneration and neurogranin in the pathophysiology of Alzheimer’s disease. Expert Rev Neurother 2017;17:47-57.
7. Wellington H, Paterson R, Portelius E, et al. Increased CSF neurogranin concentration is specific to Alzheimer disease. Neurology 2016;86:829-35.

8. Wang H-Y, Lee D, D'Andrea M, Peterson P, Shank R, Reitz A. b-Amyloid1-42 binds to a7 nicotinic acetylcholine receptor with high affinity:Implication for Alzheimer's disease pathology. J Biol Chem 2000;275:5626-32.
9. Wang H-Y, Lee D, Davie C, Shank R. Amyloid peptide Aβ1-42 binds selectively and with picomolar affinity to a7 nicotinic acetylcholine receptors. J Neurochem 2000;75:1155-61.
10. Wang H-Y, Li W, Benedetti N, Lee D. α7 nicotinic acetylcholine receptors mediate β-amyloid peptide-induced tau protein phosphorylation. J Biol Chem 2003;278:31547-53.
11. Dineley K, Bell K, Bui D, Sweatt J. b-Amyloid peptide activates a7 nicotinic acetylcholine receptors expressed in xenopus oocytes. J Biol Chem 2002;227:25056–61.
12. Hu M, Waring J, Gopalakrishnan M, Li J. Role of GSK-3beta activation and alpha7 nAChRs in Abeta(1-42)-induced tau phosphorylation in PC12 cells. J Neurochem 2008;106:1371-7.
13. Zhang L, Xie J, Yang J, Cao Y. Tyrosine phosphatase STEP61 negatively regulates amyloid b-mediated ERK/CREB signaling pathways via a7 nicotinic acetylcholine receptors. J Neurosci Res 2013;91:1581-90.
14. Johnson G, Stoothoff W. Tau phosphorylation in neuronal cell function and dysfunction. J Cell Sci 2004;117:5721-9.
15. Nakamura F, Stossel T, Hartwig J. The filamins: organizers of cell structure and function. Cell Adh Migr 2011;5:160-9.
16. Wang H-Y, Bakshi K, Frankfurt M, et al. Reducing amyloid-related Alzheimer's disease pathogenesis by a small molecule targeting filamin A. J Neurosci 2012;32:9773-84.
17. Wang H-Y, Lee K-C, Pei Z, Khan A, Bakshi K, Burns L. PTI-125 binds and reverses an altered conformation of filamin A to reduce Alzheimer's disease pathogenesis. Neurobiol Aging 2017;55:99-114.
18. Burns LH, Wang H-Y. Altered filamin A enables amyloid beta-induced tau hyperphosphorylation and neuroinflammation in Alzheimer's disease. Neuroimmunol Neuroinflammation 2017;4:263-71.
19. Gambuzza M, Sofo V, Salmeri F, Soraci L, Marino S, Bramanti P. Toll-like receptors in Alzheimer's disease: a therapeutic perspective. CNS Neurol Disord Drug Targets 2014;13:1542-58.
20. Wang H-Y, Pei Z, K.-C. Lee K-C, et al. PTI-125 reduces biomarkers of Alzheimer's disease in patients. J Prevent Alzheimer's Disease 2020;7:256-64.
21. Hansson O, Seibyl J, Stomrud E, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-β PETand predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. Alzheimer's Dement 2018;14:1470-81.
22. Wang HY, Pei Z, Lee KC, et al. PTI-125 Reduces Biomarkers of Alzheimer's Disease in Patients. The Journal of Prevention of Alzheimer's Disease 2020.
23. Yang J, Fu Z, Zhang X, Xiong M, Meng L, Zhang Z. TREM2 ectodomain and its soluble form in Alzheimer's disease. J Neuroinflammation 2020;17.

24. Paudel Y, Efthalia Angelopoulou E, Piperi C, Othman I, Aamir K, Shaikh M. Impact of HMGB1, RAGE, and TLR4 in Alzheimer’s disease (AD): From risk factors to therapeutic targeting. Cells 2020;9.
25. Bowman G, Dayon L, Kirkland R, et al. Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. Alzheimer's & Dementia 2018;14:1640-50.
26. Yang H, Liu H, Zeng Q, et al. Inhibition of HMGB1/RAGE-mediated endocytosis by HMGB1 antagonist box A, anti-HMGB1 antibodies, and cholinergic agonists suppresses inflammation. Mol Med 2019;25:13.
27. Krafft P, Caner B, Klebe D, Rolland W, Tang J, Zhang J. PHA-543613 preserves blood–brain Barrier integrity after intracerebral hemorrhage in mice. Stroke 2013;44:1743-7.
28. Zou D, Luo M, Han Z, et al. Activation of Alpha-7 Nicotinic Acetylcholine Receptor Reduces Brain Edema in Mice with Ischemic Stroke and Bone Fracture. Mol Neurobiol 2017;54:8278–86.
29. Tolar M, Abushakra S, Hey J, Porsteinsson A, Sabbagh M. Aducanumab, gantenerumab, BAN2401, and ALZ-801—the first wave of amyloidtargeting drugs for Alzheimer’s disease with potential for near term approval. Alzheimer's Res Ther 2020;12.
30. Cummings J. Challenges to demonstrating disease-modifying effects in Alzheimer’s disease clinical trials. Alzheimers Dement 2006;2:263-71.

Figures

Figure 1

Patient Flow Diagram

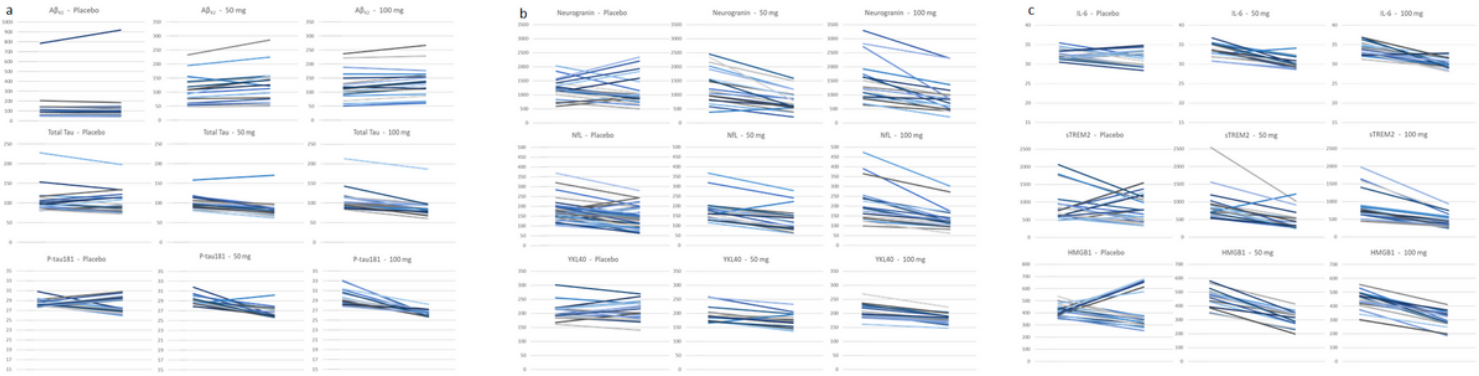

Figure 2

Simufilam improved biomarkers of AD pathology, neurodegeneration, neuroinflammation and BBB integrity. Percent change from baseline of CSF biomarkers (A) and lymphocyte target engagement markers (B). Reductions in filamin A linkages to α7nAChR or TLR4 in lymphocytes indicate target engagement. These secondary analyses of percent change from baseline on all biomarkers excluded the 3 patients with no detectable simufilam in plasma at return visits. Data are means ± SEM. *p ≤ 0.0001, #p < 0.001, †p < 0.01 and +p < 0.05 versus placebo. N=22, 20, 19 for placebo, 50 and 100 mg, respectively.

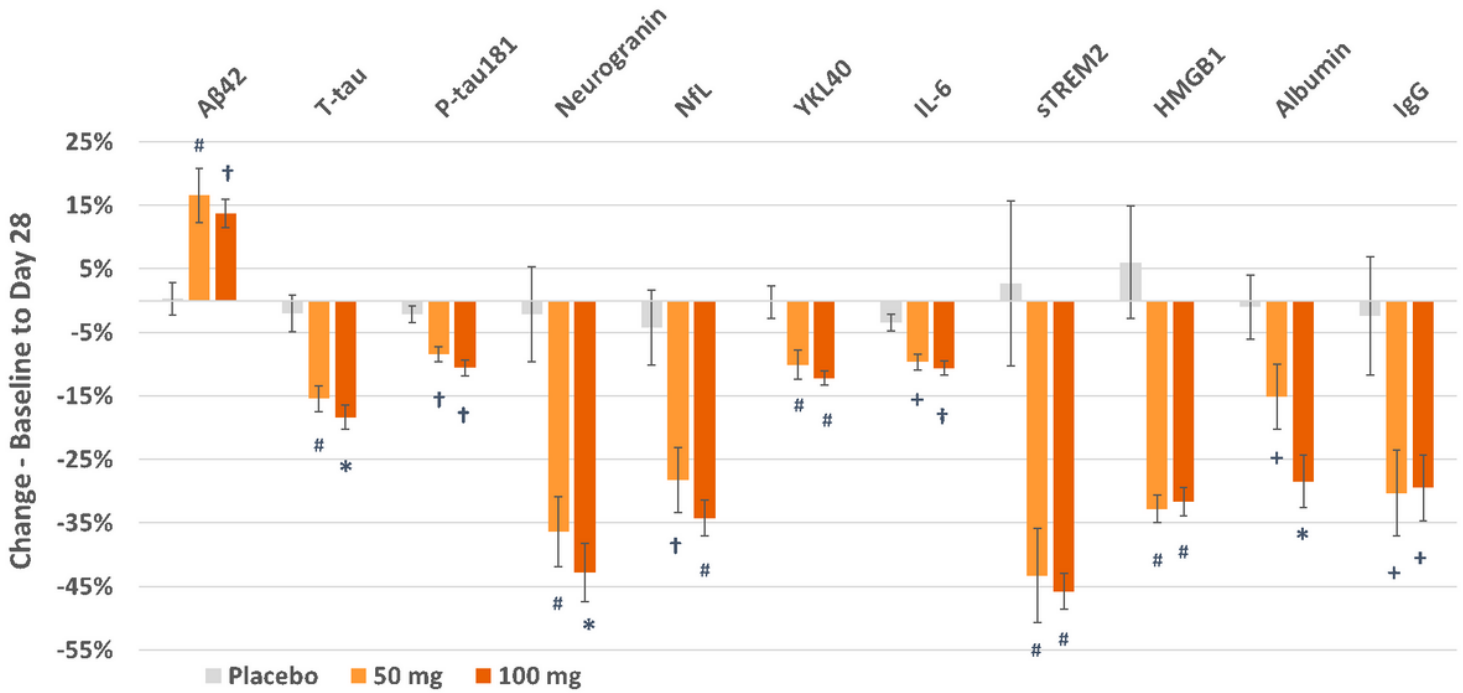

Figure 3

Spaghetti plots by group for biomarkers measured by ELISA. Plots show individual patient levels (pg/mL) at screening (left) and at Day 28 (right). All patients in simufilam groups show decreases in all biomarkers except one individual in the 50 mg group. By contrast, placebo patients show movement in

both directions for each biomarker. A: Core AD pathology biomarkers. B: Neurogranin, neurofilament light chain (NfL), and YKL-40. C: Secondary biomarkers IL-6, sTREM2 and HMGB1.

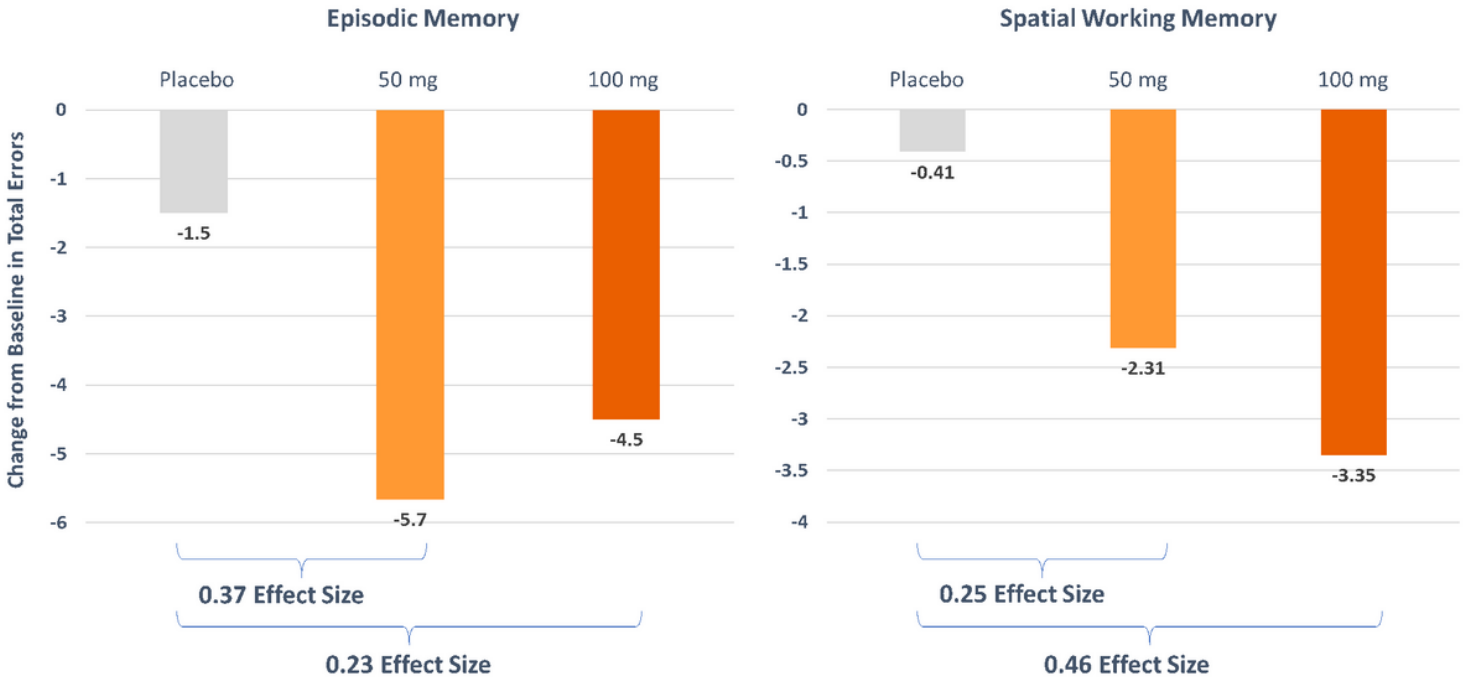

Figure 4

Simufilam appeared to improve both episodic memory and spatial working memory. Effect sizes were calculated by Hedge's g. For the episodic memory test (Paired Associates Learning), the least impaired patients (11 or fewer errors, representing a ceiling effect) and patients with 54 or more errors (very poor performance suggesting not understanding the task) were removed from the analysis. Both datasets removed the 3 patients with no detectable drug in plasma, 2 patients with ≥25% non-compliance by pill counts, one patient with no baseline test and one who did not understand instructions per rater notes. N=14, 13, 10 for PAL, and N=22, 17, 18 for spatial working memory for placebo, 50 and 100 mg, respectively.