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Original Article

Phase 3 randomized clinical trials of simufilam in mild-to-moderate Alzheimer's disease

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ABSTRACT

Background: Soluble amyloid β_{1-42} ($A\beta_{42}$) signals via the α7 nicotinic acetylcholine receptor to hyperphosphorylate tau in Alzheimer's disease (AD). Simufilam disrupts this pathogenic signaling by binding filamin A and disrupts its linkages with inflammatory receptors to reduce neuroinflammation. We assessed simufilam in two Phase 3 clinical trials in mild-to-moderate AD.

Methods: Participants were age 50–87 with Stage 4 or 5 CE, a mini-mental state exam (MMSE) ≥16 and ≤27 and a Clinical Dementia Rating Global Score (CDR-GS) of 0.5, 1 or 2. The criterion supporting AD pathology was plasma phosphorylated (p)-tau181 or prior amyloid PET. RETHINK randomized participants to simufilam 100 mg or placebo for 52 weeks. REFOCUS evaluated simufilam 50 and 100 mg versus placebo for 76 weeks. Co-primary endpoints were change from baseline on ADAS-Cog12 and ADCS-ADL. Sub-studies assessed exploratory plasma biomarkers and, in REFOCUS only, CSF and imaging biomarkers.

Results: Both trials failed to meet co-primary, secondary or exploratory biomarker endpoints. REFOCUS was terminated early, with 22% of participants still active in the trial. In the predefined mild subgroup in REFOCUS, simufilam was associated with slower cognitive decline than placebo through Week 64 ($p = 0.019$). This finding disappeared at Week 76 with 45% missing data and did not replicate in RETHINK. Favorable nominal exploratory post-hoc findings amongst participants with the highest half of screening plasma p-tau181 levels occurred in RETHINK but not REFOCUS. The plasma p-tau181 entry criterion did not reliably exclude amyloid PET negativity in the sub-study.

Conclusions: Simufilam did not meet co-primary or secondary endpoints in these Phase 3 trials. Simufilam was safe and well tolerated. Trials registered at clinicaltrials.gov: NCT04994483 and NCT05026177

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in Western societies, and accounts for 60 %–70 % of all cases [1]. Worldwide, nearly 50 million people have AD or a related dementia, and 10 million new cases are diagnosed each year [2]. AD is characterized by neurodegeneration beginning in the hippocampus and progressively affecting the entire brain [3].

Although amyloid beta (Aβ) plaque in the brain is a hallmark of AD, the level of soluble Aβ is more highly correlated with the extent of

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synaptic loss and cognitive impairment than is amyloid plaque deposition [4–7]. Currently available anti-amyloid antibodies lecanemab and donanemab target Aβ plaques, which some view as a reservoir of diffusible amyloid oligomers that can be shed to cause further damage [8], though lecanemab also binds soluble protofibrils and oligomers. Anti-amyloid antibody therapies produce a modest slowing of cognitive decline and can be associated with severe adverse effects [9,10].

A primary pathogenic pathway of soluble $A\beta_{42}$ in Alzheimer's disease is its ultra-tight binding to the α7 nicotinic acetylcholine receptor (α7nAChR) to activate kinases that hyperphosphorylate tau [11–15]. In support, blocking α7nAChR with α-bungarotoxin dramatically reduces $A\beta_{42}$-induced tau hyperphosphorylation [15]. Hyperphosphorylated tau can no longer stabilize microtubules, impairing intraneuronal transport and resulting in degeneration of neurons [16–18]. Abnormally phosphorylated tau is found in neurofibrillary tangles [19], the second hallmark pathology of Alzheimer's disease.

Simufilam disrupts the pathogenic signaling of soluble $A\beta_{42}$ through α7nAChR by targeting the scaffolding protein filamin A. Filamin A aberrantly links to α7nAChR and multiple inflammatory receptors in AD, and simufilam disrupts these linkages [20–22]. Simufilam was also shown to improve insulin receptor signaling and suppress overactive mechanistic target of rapamycin (mTOR) [23]. In preclinical models, simufilam reduced tau hyperphosphorylation, amyloid deposits, neurofibrillary lesions and inflammatory cytokine release, while restoring function of three neuronal receptors and improving synaptic plasticity [20,22].

In 2021, financial short sellers alleged that Hoau-Yan Wang and Cassava Sciences had engaged in scientific misconduct regarding foundational data of simufilam. No journal editors found evidence of data manipulation in any of his papers. Following thorough investigations by both Dr. Wang's academic institution and the US Department of Justice, all charges were dropped in 2025.

After a Phase 1 trial in healthy volunteers, simufilam was investigated in a first-in-patient study that assessed pharmacokinetics and safety, and showed improvements in cerebrospinal fluid (CSF) and lymphocyte biomarkers [24]. A subsequent 28-day placebo-controlled Phase 2 study in mild-to-moderate AD showed CSF biomarker improvements versus placebo. Both a one-year open-label safety study and a 6-month randomized withdrawal produced encouraging cognitive results in participants with mild AD. The Food and Drug Administration (FDA) approved the design of two Phase 3 trials via Special Protocol Assessments. We now report results from these two Phase 3 trials of simufilam in mild-to-moderate AD.

2. Methods

The trials, managed by independent clinical research organization Premier Research, were conducted in accordance with the Declaration of Helsinki, national law(s) of participating countries, with the International Council for Harmonisation, and Good Clinical Practices. The study protocols, amendments, and informed consent forms were reviewed and approved by a human ethics committee or institutional review board. There was no overlap in clinical sites between the two trials, and sites with sub-study capabilities were assigned to REFOCUS. All participants provided written informed consent prior to any study procedures. The authors affirm the accuracy of the reported data and study conduct consistent with the protocols. An independent data and safety monitoring board (DSMB) reviewed interim safety data on three occasions and affirmed both studies could continue without modification. Following a chain of custody independent of Cassava Sciences, data were collected and verified by Signant Health using their electronic clinical outcome assessment (eCOA) platform and transferred to Premier Research who managed data before transferring to Pentara Corporation for analysis. Samples for biomarkers were collected at sites, sent to the central lab and transferred to Meso Scale Discovery for analysis. Safety data were analyzed by Premier Research, and efficacy and biomarker data were analyzed by Pentara Corporation.

2.1. Study design

RETHINK-ALZ (NCT04994483; referred to as RETHINK) was a Phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of participants with mild-to-moderate AD randomized (1:1) to oral placebo or simufilam 100 mg twice daily for 52 weeks. REFOCUS-ALZ (NCT05026177; referred to as REFOCUS) was a Phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of participants with mild-to-moderate AD randomized (1:1:1) to oral placebo or simufilam 50 mg or 100 mg twice daily for 76 weeks. Both studies had the same inclusion and exclusion criteria and endpoints. The primary objective of both studies was to assess the effect of simufilam on the co-primary endpoints of the 12-item Alzheimer's Disease Assessment Scale – Cognitive Subscale (ADAS-Cog12)[25] and the Alzheimer's Disease Cooperative Study – Activities of Daily Living (ADCS-ADL) [26]. Clinic visits occurred at screening, the baseline visit, the safety visit at Week 4, and then every 12 weeks.

After completion of either trial, participants had the option to immediately enroll in a separate 52-week, open-label extension trial (NCT05575076) evaluating the long-term safety and tolerability of simufilam. The extension study assessed only safety measures at week 4 and every 12 weeks thereafter.

Both RETHINK and REFOCUS included sub-studies for exploratory plasma biomarkers. REFOCUS also included sub-studies for CSF biomarkers, amyloid positron emission tomography (PET), tau PET and volumetric MRI. Planned treatment group sizes for these sub-studies were $n = 50$ for plasma in RETHINK and up to $n = 90$ in REFOCUS, $n = 30$ for CSF, $n = 40$ for amyloid PET, $n = 50$ for tau PET, and $n = 50$ for volumetric MRI. Meso Scale Discovery conducted the plasma and CSF biomarkers analyses. Clario conducted all imaging analyses.

REFOCUS was terminated early on November 25, 2024 when the negative topline RETHINK results were disclosed. At termination, 246 (22 %) participants were still active in the trial and were approaching Week 64 or Week 76 visits, while 591 (53 %) had completed the Week 76 visit. Participants were contacted as soon as possible and instructed to stop taking study drug and to complete an early termination visit in December.

2.2. Participant selection

Inclusion and exclusion criteria were the same for both trials. Participants age 50–87 years presented with a diagnosis of AD consistent with Stages 4 or 5 on the Alzheimer's continuum (National Institute on Aging –Alzheimer's Association) [27], a gradual and progressive change in memory for >6 months, a Mini Mental State Examination (MMSE) [26,28] ≥16 and ≤27 (stratified by low [16–20] or high [21- 27] MMSE), and a Clinical Dementia Rating Global Score (CDR-GS)[29,30] of 0.5, 1 or 2. AD pathophysiology was supported by an elevated plasma P-tau181 level at screening, or in 5.7 % of RETHINK and 5.3 % of REFOCUS participants, a prior amyloid PET scan or prior CSF biomarkers consistent with AD. If receiving background AD medications, the dosing regimen was stable for at least 12 weeks prior to randomization and was expected to remain stable during the study. All participants underwent MRI scanning to assess potential exclusion criteria: intracerebral hemorrhage, infarct >1 cm^3, >3 lacunar infarcts, >10 microhemorrhages, cortical superficial siderosis, diffuse confluent deep white matter hyperintense lesions (Fazekas scale 3), cerebral contusion, symptomatic subdural hematoma or aneurysm. Please see the **Supplemental Material** for complete inclusion/exclusion criteria.

2.3. Study endpoints

For both trials, the co-primary endpoints were the differences between treatments for LS mean change from baseline to Week 52 for

ADAS-Cog12 and ADCS-ADL. Secondary endpoints included LS mean change for the Integrated Alzheimer's Disease Rating Scale (iADRS) [31], 10-item Neuropsychiatric Inventory (NPI-10) [32], MMSE, CDR-Sum of Boxes (CDR-SB), and the Zaret Burden Interview (ZBI) [33]. CDR was collected only at screening and end of study.

Safety was assessed from adverse events, clinical laboratory testing (chemistry, hematology, urinalysis), vital signs (heart rate, blood pressure, temperature), body weight, physical and neurological examination, electrocardiogram (ECG) parameters, and the Columbia-Suicide Severity Rating Scale (C-SSRS) [34].

Exploratory biomarker endpoints included plasma biomarkers collected in sub-studies in both trials and, in sub-studies in REFOCUS only, cerebrospinal fluid (CSF) biomarkers and imaging assessments of volumetric MRI (whole brain, ventricular, hippocampal) and amyloid and tau PET.

2.4. Statistical analysis

In RETHINK, sample size was determined by a power analysis of ADAS-Cog using data from a similar population over 52 weeks. Approximately 300 participants per group provided 90 % power with a 2-sided significance level of 0.05 to show a 45 % mean difference from placebo at 52 weeks. This calculation assumed a true mean change from baseline for placebo of 5.0 points and a standard deviation (SD) of 8.5 points. Assuming a drop-out rate of 20 %, each treatment group was expected to enroll approximately 375 participants. In REFOCUS, a power calculation required group sizes of 289 to provide 90 % power to detect a 45 % difference from placebo at 76 weeks, based on a two-sided significance level of 0.05. This power calculation assumed a true mean change from baseline for placebo of 6.0 points and a SD of 10.0 points. Assuming a drop-out rate of 20 %, each treatment group planned to enroll 361 subjects. No interim analyses for futility or efficacy were conducted.

In RETHINK and REFOCUS, both co-primary endpoints were analyzed using a linear mixed model for repeated measurements (MMRM). The dependent variable was the change from baseline, and the model included fixed effects for treatment group (three levels in REFOCUS and two levels in RETHINK), week (five or seven levels, corresponding to Weeks 4, 16, 28, 40, and 52 and including Weeks 64 and 76 for REFOCUS), the treatment group-by-week interaction, and the randomization stratification variable. The baseline value of the corresponding endpoint was included as a covariate, and the unstructured covariance model was used. For both co-primary endpoints, the primary analysis compared simufilam 100 mg and placebo at Week 52 (RETHINK) and Week 76 (REFOCUS) using a two-sided test at the alpha = 0.05 level of significance. Study success required that both co-primary comparisons were statistically significant. For REFOCUS, if both primary comparisons were statistically significant, then the corresponding comparisons at Week 76 for simufilam 50 mg versus placebo would be tested at the alpha = 0.05 level of significance (two-sided). Secondary clinical endpoints (iADRS, NPI, MMSE, and ZBI) measured repeatedly were analyzed by linear MMRM, with a missing at random (MAR) approach to missing data.

Plasma and CSF biomarker sub-studies included p-tau217, neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), and total tau. All plasma biomarker and MRI data measured repeatedly were analyzed by linear MMRM. All CSF biomarker data, PET imaging data, and CDR-SB were analyzed using ANCOVA models with treatment group as a factor and the baseline value of the corresponding endpoint as a covariate.

Adverse events of interest were analyzed using a chi-square test.

The intent-to-treat (ITT) population included all randomized participants. The safety population included all participants who received at least one dose of study drug. The primary analysis population for RETHINK was the ITT population. For REFOCUS, the primary analysis population, prespecified and reflecting RETHINK post hoc analyses, was the modified ITT (mITT) population (81.7 % of participants), defined by an entry plasma p-tau181 $\geq$ 44 pg/mL or a prior, positive amyloid PET scan or CSF biomarkers consistent with AD. To assess potential bias, the REFOCUS prespecified censored ITT (cITT) analysis set excluded all data collected after termination of REFOCUS when participants had been instructed to stop taking study drug, which was 6.6 to 8.6 % of Week 76 data in the ITT analysis.

In RETHINK and REFOCUS, the co-primary endpoints were prespecified to be analyzed in the ITT analysis set by baseline severity, with subgroups defined as screening MMSE 21–27 (mild) versus MMSE 16–20 (moderate). The statistical plan also prespecified pooling of 52-week data from RETHINK and REFOCUS, with analyses including co-primary and multiplicity adjusted secondary endpoints, and by subgroups. Since the primary endpoints in both trials did not reach statistical significance, all other p-values <0.05 for both predefined subgroups and post hoc analyses are considered nominally significant and hypothesis-generating.

In exploratory post hoc analyses of RETHINK, the ITT population was divided into halves by screening plasma p-tau181 levels. Those who qualified for the trial with a prior amyloid PET scan or CSF biomarkers instead of a screening plasma p-tau181 assessment (5.7 % of the ITT population) were not included. Any timepoints with $p < 0.05$ for these post hoc analyses are considered nominally significant.

3. Results

In RETHINK, conducted between October 7, 2021 and October 8, 2024, 2271 participants were screened at 85 clinical trial sites in the US, Canada and Australia, and 804 participants were randomized to simufilam or placebo. A similar proportion in the simufilam (310 participants, 77.7 %) and placebo (325 participants, 81.7 %) groups completed the study (Fig. 1). Of 162 participants (20.3 %) who discontinued the trial, the most common reasons were withdrawal of consent (9.9 %) and adverse event (5.4 %). Baseline characteristics for RETHINK were evenly distributed between simufilam and placebo groups with a mean (SD) age of 74.0 (7.7) years, 436 participants (54.2 %) $\geq$ 75 years age, and 447 (55.6 %) female (Table 1). The majority of participants were white (742 participants, 92.3 %) and not Hispanic or Latino (675 participants, 84.0 %). Overall, 496 (62.2 %) participants reported use of a cognitive enhancer. APOE4 homozygotes in RETHINK were slightly imbalanced, with 13.9 % in the simufilam group and 8.2 % in placebo.

In REFOCUS, conducted between November 18, 2021 and December 30, 2024, 2671 participants were screened at 87 clinical trial sites in the U.S.A., Canada, Puerto Rico, and the Republic of Korea, and 1125 were randomized to simufilam 50 or 100 mg or placebo (Fig. 1). As noted above, the trial was terminated early on November 25, 2024 when the negative topline RETHINK results became available. Data from these early termination visits collected after trial termination are included in the ITT and mITT analyses. Of 502 (44.6 %) participants who discontinued the trial, the most common reasons were trial terminated (18.8 %), withdrawal by participant (14.1 %), and adverse event (7.4 %). Baseline characteristics for REFOCUS were evenly distributed between simufilam and placebo groups with a mean (SD) age of 73.9 (7.9) years, 604 participants (53.7 %) $\geq$ 75 years age, and 629 (55.9 %) female (Table 1). The majority of participants were white (965 participants, 85.8 %) and not Hispanic or Latino (1033 participants, 91.8 %). Cognitive enhancers were used by 607 (54.1 %) participants.

Although there was no correlation between screening p-tau181 levels and MMSE in these studies, the mean p-tau181 levels ranged from 66.9 to 74.8 pg/mL for mild treatment groups and 79.3 to 87.0 pg/mL for moderate treatment groups across studies (Table 1). It is also worth noting that of 1208 participants in the pooled mild subgroups, 393 had screening p-tau181 levels that fell in the top half.

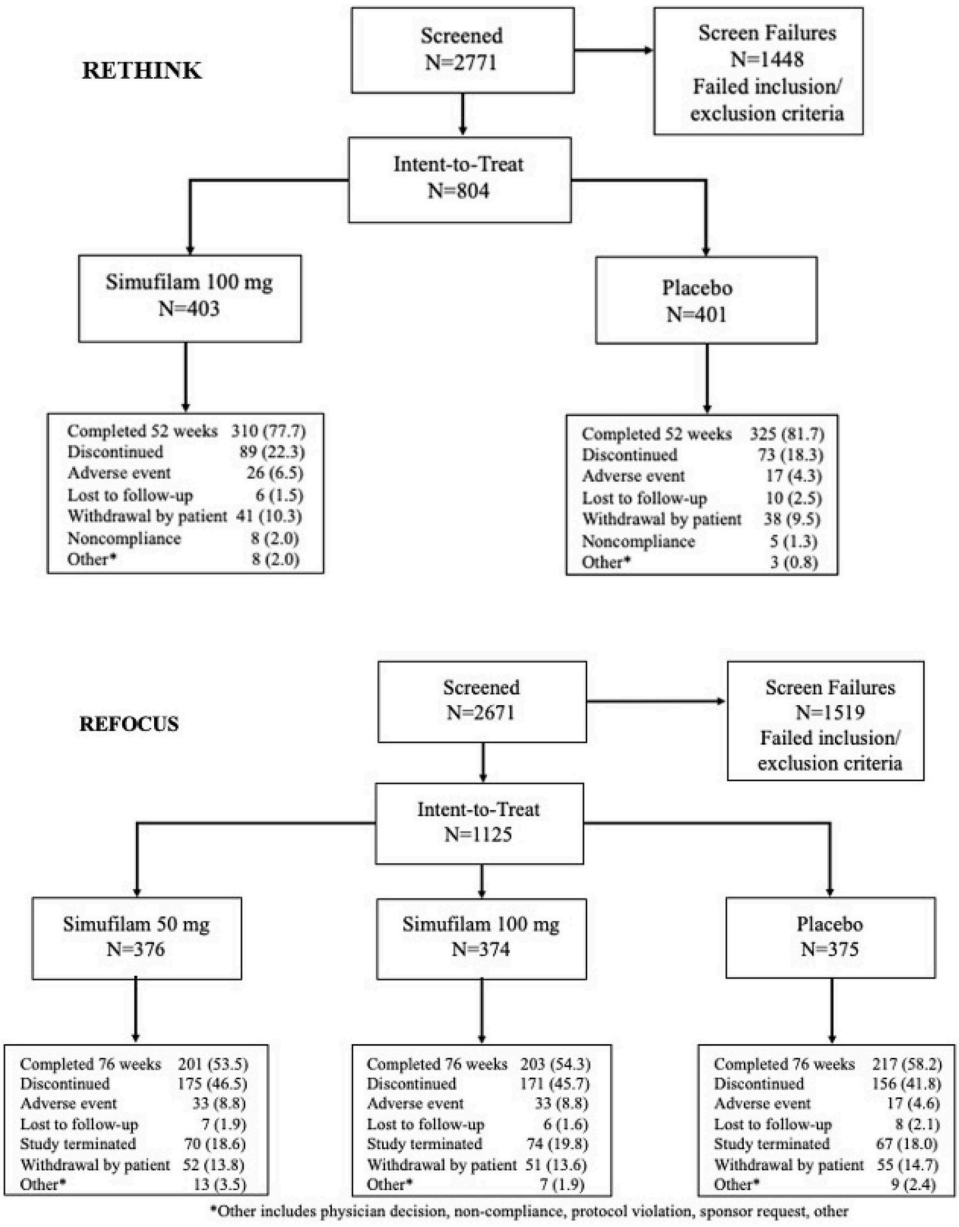

Fig. 1. CONSORT diagram for RETHINK and REFOCUS trials.

3.1. *Efficacy*

All primary, secondary and exploratory plasma biomarker endpoints are summarized in Table 2 and depicted in forest plots for RETHINK and REFOCUS (**Supplemental Figure 1**).

3.1.1. *Primary and secondary endpoints*

For the primary endpoint of ADAS-Cog12, in RETHINK, no significant differences were observed between simufilam and placebo for changes from baseline to Week 52 (Table 2) or at earlier timepoints (Fig. 2**).** There was also no significant difference in change from baseline to Week 52 for the other co-primary endpoint, ADCS-ADL (**Supplemental Figure 2**). No significant differences from baseline to Week 52 were observed for secondary endpoints iADRS, MMSE, NPI, CDR, and ZBI (Table 2), nor were there any significant differences for secondary endpoints over time (**Supplemental Figures 3 to 7**).

In REFOCUS, the primary analysis population was the modified ITT population, defined as participants with plasma p-tau181 $\geq$44 pg/mL or prior amyloid PET or CSF biomarkers consistent with AD. No significant differences between simufilam and placebo for changes from baseline to Week 76 (Table 2) or at other timepoints were observed for ADAS-Cog12 (Fig. 2) or for ADCS-ADL (**Supplemental Figure 8**). There were no significant differences from baseline for secondary endpoints iADRS, MMSE, NPI, CDR, and ZBI (Table 2 and **Supplemental Figures 9–13**).

3.1.2. *Subgroup analyses*

A prespecified pooled analysis of mild (MMSE 21–27) and moderate (MMSE 16–20) subgroups of RETHINK and REFOCUS for ADAS-Cog12 through Week 52 showed nominal treatment group differences in mild disease (n = 1208) at Weeks 4 and 28 ($p < 0.01$; **Supplemental Figure 14**). An exploratory post hoc analysis using a plasma p-tau181 cutoff of $\geq$ 67 (the highest half) for the pooled mild subgroups (n = 393) showed a nominal slowing of decline at Weeks 4, 28 and 40, with a trend at Week 52 (p = 0.03, 0.001, 0.006, and 0.066, respectively; **Supplemental Figure 14**).

In the predefined mild subgroup of the REFOCUS ITT population, simufilam 100 mg was associated with slower cognitive decline on ADAS-Cog12 than placebo. This finding did not replicate in RETHINK.

Table 1
Baseline characteristics (ITT population).

	RETHINK		REFOCUS		
	Simufilam 100 mg BID N = 403	Placebo N = 401	Simufilam 50 mg BID N = 376	Simufilam 100 mg BID N = 374	Placebo N = 375
Age, years [a]	73.7 ± 7.9	74.3 ± 7.6	74.5 ± 7.6	73.6 ± 8.2	73.7 ± 7.9
Age range, years	50 – 87	50 – 87	50 – 87	50 – 87	50 – 87
Age ≥75 years, n (%)	215 (53.3)	221 (54.8)	214 (56.9)	196 (52.4)	194 (51.7)
Female, n (%)	225 (55.8)	222 (55.4)	207 (55.1)	208 (55.6)	214 (57.1)
Race, n (%)					
Asian	8 (2.0)	2 (0.5)	21 (5.6)	28 (7.5)	32 (8.5)
Black or AfricaAmerican	20 (5.0)	18 (4.5)	23 (6.1)	17 (4.5)	21 (5.6)
White	366 (90.8)	376 (93.8)	326 (87.7)	326 (87.2)	313 (83.5)
Other	9 (2.2)	5 (1.2)	6 (1.6)	3 (0.8)	9 (2.4)
Not Hispanic or Latino, n (%)	334 (82.9)	341 (85.0)	345 (91.8)	338 (90.4)	350 (93.3)
Body weight, kg [a]	73.7 ± 14.7	72.8 ± 15.4	72.1 ± 14.9	72.7 ± 15.9	73.5 ± 15.6
Body mass index, kg/m^2 [a]	26.4 ± 4.2	26.1 ± 4.1	26.0 ± 4.0	26.2 ± 4.4	26.4 ± 4.4
Alzheimer's disease family history, n (%)	202 (50.1)	183 (45.6)	183 (48.7)	190 (50.8)	185 (49.3)
Mini Mental State Examination Score, n (%)					
21–27 (Mild)	244 (60.5)	250 (62.3)	242 (64.4)	240 (64.2)	235 (62.7)
16–20 (Moderate)	155 (38.5)	146 (36.4)	134 (35.6)	134 (35.8)	138 (36.8)
Clinical Dementia Rating-Global Score, n (%)					
0.5	200 (49.6)	218 (54.4)	213 (56.8)	212 (56.8)	219 (58.4)
1.0	185 (45.9)	163 (40.6)	147 (39.2)	146 (39.1)	143 (38.1)
2.0	18 (4.5)	20 (5.0)	15 (4.0)	15 (4.0)	13 (3.5)
Plasma p-tau181, pg/mL [a]					
Mild (MMSE 21–27)	72.4 ± 37.6	66.9 ± 30.6	71.5 ± 37.0	74.8 ± 40.1	71.2 ± 37.8
Moderate (MMSE 16–20)	80.8 ± 39.5	83.8 ± 36.9	87.0 ± 40.4	79.3 ± 27.6	87.0 ± 43.4
Apolipoprotein E genotype, n (%)					
E2/E2	1 (0.2)	0	1 (0.3)	1 (0.3)	1 (0.3)
E2/E3	23 (5.7)	14 (3.5)	24 (6.4)	23 (6.1)	13 (3.5)
E2/E4	12 (3.0)	13 (3.2)	9 (2.4)	13 (3.5)	16 (4.3)
E3/E3	149 (37.0)	137 (34.2)	138 (36.7)	146 (39.0)	131 (34.9)
E3/E4	159 (39.5)	201 (50.1)	161 (42.8)	154 (41.2)	177 (47.2)
E4/E4	56 (13.9)	33 (8.2)	43 (11.4)	37 (9.9)	36 (9.6)
Concomitant medications					
Memantine	112 (28.1)	127 (31.9)	78 (20.7)	76 (20.3)	62 (16.6)
Anticholinesterase inhibitors	191 (47.9)	209 (52.5)	176 (46.8)	179 (47.9)	185 (49.6)
Years of education [a]					
Mild (MMSE 21–27)	12.9 (4.8)	13.6 (4.0)	14.6 (2.8)	13.9 (3.8)	14.1 (3.4)
Moderate (MMSE 16–20)	12.8 (4.2)	13.2 (3.9)	14.0 (3.3)	13.2 (3.6)	13.2 (3.9)

[a] mean ± standard deviation
Other = American Indian or Alaska Native; not reported; other; unknown.

Nominal differences versus placebo occurred at Week 4 for both doses ($p = 0.01$ for 100 mg and $p = 0.05$ for 50 mg) and from Week 28 through Week 64 for the 100 mg dose ($p = 0.01$, $p = 0.02$, $p = 0.02$ and $p = 0.02$), with no treatment difference at Week 76 (Fig. 2). With earlier discontinuations and the trial termination, Week 76 data had 45 % missing data, which was modeled using an MAR approach for the MMRM statistical model. ADCS-ADL showed no significant differences in the mild subgroup of either trial. On iADRS, a nominal treatment difference versus placebo occurred in the 100 mg group at Week 64 ($p = 0.01$) in REFOCUS only. There were no significant differences in the moderate subgroup in either trial on these endpoints (Fig. 2).

3.1.3. Responder analyses

In a responder analysis of RETHINK participants who improved from baseline to Week 52, no significant differences between treatment groups were observed for co-primary endpoints ADAS-Cog12 and ADCS-ADL (**Supplemental Table 1**). However, in a responder analysis by mild versus moderate AD, a greater percentage of moderate participants in the simufilam group improved on the ADAS-Cog12 at Week 52 compared to placebo (31 % versus 21 %) ($p = 0.039$).

In a responder analysis of REFOCUS participants who improved from baseline to Week 76, there were no significant differences for co-primary endpoints ADAS-Cog12 and ADCS-ADL (**Supplemental Table 1**), nor were there any significant differences in responders in mild or moderate subgroups at Week 76.

3.1.4. Progression analyses

Progression analyses examined the number and percentage of participants who entered the studies with mild disease and had progressed to moderate or more severe disease (MMSE <16) at the end of the study, or those who entered with moderate disease and progressed to more severe disease. In RETHINK, mild participants with a Week 52 assessment did not differ between simufilam ($n = 193$) and placebo ($n = 210$) in rates of progression to moderate or more severe AD by Week 52 (33 % for simufilam versus 30 % for placebo). However, of RETHINK participants entering the study with moderate AD who had a Week 52 assessment (116 for simufilam and 114 for placebo), 50 (43 %) on simufilam progressed to more severe AD by Week 52 compared to 65 (57 %) on placebo (p value not determined).

REFOCUS showed no group differences in progression from mild to moderate AD or from moderate to more severe disease at Week 76.

3.1.5. RETHINK exploratory post hoc analyses

Post hoc analyses of RETHINK by screening plasma p-tau181 were conducted to potentially inform the REFOCUS statistical analytical plan. These exploratory post hoc analyses divided the ITT population (excluding the 5.7 % who qualified by prior amyloid PET or CSF biomarkers) at the median entry p-tau181 level. Nominal differences versus placebo occurred in the highest half of plasma p-tau181 on ADAS-Cog12 at Weeks 28, 40 and 52 ($p = 0.009$, $p = 0.048$ and $p = 0.036$) (**Supplemental Figure 14**).

Table 2
Change from baseline of primary and secondary endpoints and exploratory plasma biomarkers at Week 52 (RETHINK) and Week 76 (REFOCUS) (ITT populations).

	RETHINK		REFOCUS		
Endpoint	Simufilam 100 mg BID N = 403	Placebo N = 401	Simufilam 50 mg BID N = 376	Simufilam 100 mg BID N = 374	Placebo N = 375
ADAS-Cog12					
LS mean (SE)	2.79 (0.36)	3.19 (0.36)	5.26 (0.46)	4.97 (0.46)	4.70 (0.46)
LS mean difference (SE)	−0.39 (0.50)		0.56 (0.63)	0.27 (0.63)	
95 % CI	−1.37, 0.59		−0.68, 1.80	−0.97, 1.51	
P-value	0.431		0.375	0.666	
ADCS-ADL					
LS mean (SE)	−3.26 (0.44)	−3.76 (0.44)	−6.43 (0.57)	−6.27 (0.57)	−5.32 (0.57)
LS mean difference (SE)	0.51 (0.61)		−1.10 (0.79)	−0.95 (0.79)	
95 % CI	−0.68, 1.7		−2.65, 0.45	−2.50, 0.60	
P-value	0.403		0.164	0.232	
iADRS total					
LS mean (SE)	−5.53 (0.61)	−6.49 (0.60)	−10.50 (0.79)	−10.1 (0.79)	−9.53 (0.78)
LS mean difference (SE)	0.96 (0.83)		−0.93 (1.09)	−0.58 (1.09)	
95 % CI	−0.67, 2.59		−3.07, 1.21	−2.72, 1.55	
P-value	0.248		0.392	0.592	
NPI total score					
LS mean (SE)	0.54 (0.59)	0.90 (0.58)	2.08 (0.71)	1.74 (0.69)	0.45 (0.68)
LS mean difference (SE)	−0.36 (0.78)		1.63 (0.95)	1.28 (0.93)	
95 % CI	−1.89, 1.18		−0.23, 3.49	−0.54, 3.10	
P-value	0.647		0.086	0.167	
MMSE					
LS mean (SE)	−1.95 (0.22)	−2.14 (0.22)	−3.10 (0.26)	−3.06 (0.25)	−3.09 (0.25)
LS mean difference (SE)	0.19 (0.30)		−0.01 (0.34)	0.03 (0.34)	
95 % CI	−0.39, 0.77		−0.68, 0.65	−0.64, 0.69	
P-value	0.522		0.966	0.935	
CDR-SB					
LS mean (SE)	1.04 (0.14)	0.85 (0.14)	1.57 (0.17)	1.67 (0.17)	1.70 (0.17)
LS mean difference (SE)	0.19 (0.18)		−0.13 (0.20)	−0.03 (0.20)	
95 % CI	−0.16, 0.54		−0.53, 0.26	−0.41, 0.36	
P-value	0.281		0.510	0.895	
Zarit Burden Inventory					
LS mean (SE)	2.52 (0.56)	2.3 (0.55)	3.0 (0.65	4.75 (0.65)	4.2 (0.64)
LS mean difference (SE)	0.22 (0.76)		−1.18 (0.88)	0.58 (0.88)	
95 % CI	−1.27, 1.71		−2.90, 0.55	−1.15, 2.30	
P-value	0.773		0.181	0.512	
Exploratory Plasma Biomarker Sub-studies	Simufilam 100 mg BID N = 62	Placebo N = 57	Simufilam 50 mg BID N = 72	Simufilam 100 mg BID N = 55	Placebo N = 70
P-tau217					
LS mean (SE) CFB	−0.40 (1.15)	0.14 (1.26)	2.18 (0.91)	1.77 (0.95)	2.70 (0.89)
LS mean difference (SE)	−0.54 (1.65)		−0.53 (0.94)	−0.93 (1.04)	
95 % CI	−3.81, 2.74		−2.38, 1.32	−2.98, 1.12	
P-value	0.746		0.575	0.370	
Glial Fibrillary Acidic Protein					
LS mean (SE) CFB	0.89 (27.75)	55.03 (28.38)	19.70 (7.85)	10.14 (6.82)	29.69 (7.88)
LS mean difference (SE)	−54.14 (39.3)		−9.99 (9.71)	−19.55 (10.75)	
95 % CI	−132.2, 24.0		−29.19, 9.21	−40.79, 1.70	
P-value	0.172		0.305	0.071	
Neurofilament light chain					
LS mean (SE) CFB	159.2 (41.74)	86.6 (42.85)	25.50 (11.60)	−5.67 (12.35)	11.89 (11.40)
LS mean difference (SE)	72.57 (59.37)		13.60 (12.77)	−17.56 (14.22)	
95 % CI	−45.37, 190.5		−11.67, 38.88	−45.70, 10.58	
P-value	0.225		0.289	0.219	
Total Tau Protein					
LS mean (SE) CFB	4.88 (4.2)	0.33 (4.35)	1.88 (2.11)	−0.21 (2.32)	−0.83 (2.10)
LS mean difference (SE)	4.55 (5.97)		2.72 (2.54)	0.63 (2.81)	
95 % CI	−7.31, 16.41		−2.30, 7.73	−4.92, 6.17	
P-value	0.448		0.286	0.824	

ADAS-Cog12 = 12-item Alzheimer's Disease Assessment Scale – Cognitive Subscale; ADCS-ADL = Alzheimer's Disease Cooperative Study – Activities of Daily Living; CDR-SB = Clinical Dementia Rating Sum of Boxes; CFB = change from baseline; CI = confidence intervals; iADRS = Integrated Alzheimer's Disease Rating Scale; LS = least squares MMSE = Mini-Mental State Examination; NPI = Neuorpsychiatric Inventory; p-tau217 = plasma tau phosphorylated at threonine 217.

Notably, the higher plasma p-tau181 levels did not correspond to moderate participants, and there was no correlation with MMSE despite slightly different group means for mild and moderate treatment arms (Table 1). Similar post hoc analyses by plasma p-tau181 in REFOCUS did not show group differences.

3.1.5. *Biomarker sub-studies*

There were no significant differences between simufilam and placebo for changes from baseline to Week 52 or Week 76 on tertiary endpoints of sub-studies for plasma biomarkers in either trial ($n = 27–47$ per group; Table 2), although there was a favorable trend on plasma GFAP, a neuroinflammation marker, in the 100 mg group in REFOCUS ($p = 0.07$).

There were no findings in REFOCUS sub-studies of CSF biomarkers, amyloid or tau PET, or volumetric MRI (**Supplemental Table 2**), except for a trend toward reduced tau PET density in the 50 mg group ($p = 0.08$). The CSF and PET biomarker groups with Week 76 data were much smaller than expected due to withdrawal of sub-study consent and trial

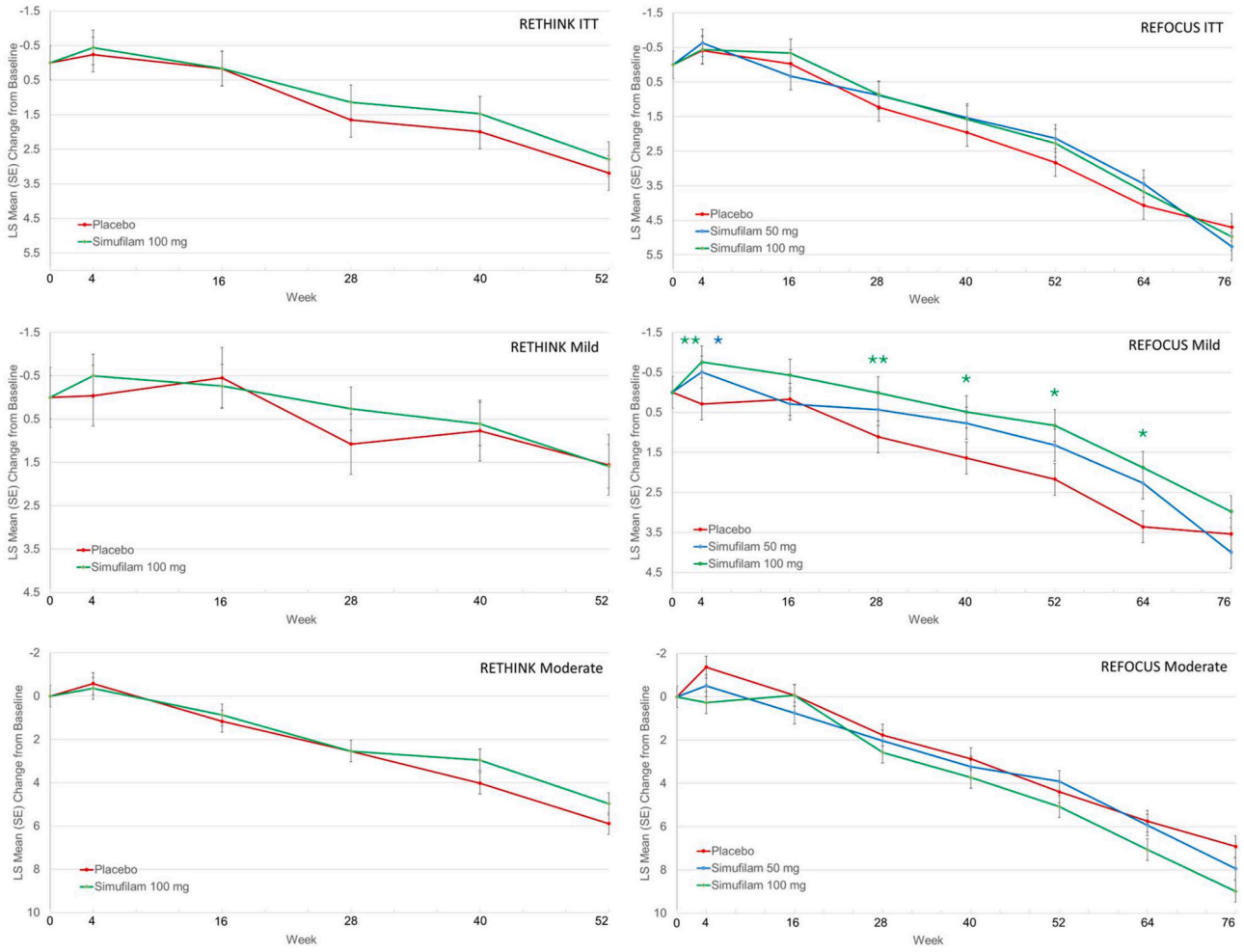

Fig. 2. Change from baseline for ADAS-Cog12 in RETHINK and REFOCUS in ITT populations and mild and moderate subgroups. *p ≤ 0.05, **p = 0.01 in the corresponding group's color. N = 714 and 494 for REFOCUS and RETHINK mild subgroups, respectively.

termination, resulting in group sizes of 7 to 14 for CSF, 21 to 40 for tau PET, and 23 to 35 for amyloid PET. In the volumetric MRI sub-study (48 to 52 per group), simufilam did not significantly impact hippocampal or ventricular volumes (**Supplemental Figure 15**).

Notably, of 160 baseline amyloid PET scans in that sub-study, 33 or 20.6 % were amyloid negative. This error rate indicates a low predictive power of the plasma p-tau181 screening criterion for amyloid pathology and resulted in an unknown number of non-AD participants enrolling in these studies.

3.1.7. REFOCUS early termination

Because REFOCUS was terminated early, some participants had a close-out visit and clinical assessments after being instructed to stop taking study drug (18, 14 and 18 for placebo, 50 and 100 mg). With a 25.6 % dropout rate at trial termination, 52.5 % of participants had completed Week 76, and close-out visit data increased this percentage to 55.2 %. To determine the impact of data collected after study termination, a censored ITT (cITT) population excluded observations on and after the early termination announcement. In the ITT population, the LS mean (SE) difference for simufilam 100 mg was 0.27 (0.631); in the cITT population, this difference changed to −0.04 (0.648). The 50 mg LS mean difference also changed slightly from 0.56 (0.633) in the ITT population to 0.34 (0.648) in the cITT population. A greater impact on the Week 76 timepoint was likely the 45 % missing data, requiring modeling.

Although the MMRM approach is the preferred and most conservative analysis of the data, in view of the 45 % missing data at Week 76, a completer analysis was also conducted. Week 76 completer analyses left group sizes of 106 to 120, including close-out visit data, and were not significant.

3.2. Safety and tolerability

In RETHINK, 553 (69.4 %) participants reported a treatment-emergent adverse event (TEAE), and treatment-related TEAEs occurred in 117 (14.7 %) participants (Table 3), mostly of mild or moderate severity. Few participants discontinued due to a TEAE: 26 (6.5 %) for simufilam and 17 (4.3 %) for placebo. The most commonly reported TEAE leading to discontinuation was diarrhea in three (0.8 %) participants in simufilam and in two (0.5 %) in placebo. One death occurred from cardiac arrest with simufilam but was not related to therapy. Three participants with placebo died from pancreatic mass, respiratory failure, and an unknown cause all unrelated to therapy. The most frequently reported serious AEs were hip fracture (8 participants, 1.0 %); syncope (6 participants, 0.8 %); urinary tract infection (5 participants, 0.6 %); and COVID-19, pneumonia, and pulmonary embolism (4 participants each, 0.5 %). The only notable serious AE difference between groups was the incidence of hip fracture: simufilam (7 participants, 1.8 %) and placebo (1 participant, 0.3 %). This hip fracture difference between treatment groups in RETHINK was neither significant ($p = 0.08$) nor observed in REFOCUS (see below). None of the 8 participants experiencing a hip fracture reported concomitant dizziness, but all had sustained a fall. If serious AE reports of hip fracture, femoral neck fracture, femur fracture, pelvic fracture, and ankle fracture were

Table 3
Treatment-emergent adverse events (safety populations).

	Number (%) of Participants				
	RETHINK		REFOCUS		
	Simufilam 100 mg BID N = 399	Placebo N = 398	Simufilam 50 mg BID N = 376	Simufilam 100 mg BID N = 374	Placebo N = 373
Any TEAE	284 (71.2)	269 (67.6)	288 (76.6)	286 (76.5)	282 (75.6)
TEAE by severity					
Mild	137 (34.3)	136 (34.2)	144 (38.3)	147 (39.3)	163 (43.7)
Moderate	113 (28.3)	109 (27.4)	111 (29.5)	114 (30.5)	92 (24.7)
Severe	34 (8.5)	24 (6.0)	33 (8.8)	25 (6.7)	27 (7.2)
Drug-related TEAEs	68 (17.0)	49 (12.3)	50 (13.3)	70 (16.7)	53 (14.2)
TEAEs leading to study discontinuation	26 (6.5)	17 (4.3)	34 (9.0)	32 (8.6)	17 (4.6)
Serious TEAEs	52 (13.0)	36 (9.0)	61 (16.2)	43 (11.5)	45 (12.1)
Discontinuation from study	10 (2.5)	5 (1.3)	11 (2.9)	6 (1.6)	6 (1.6)
Resulting in death	1 (0.3)	3 (0.8)	6 (1.6)	2 (0.5)	3 (0.8)
TEAEs occurring in at least 3 % in any group					
COVID-19	32 (8.0)	36 (9.0)	49 (13.0)	45 (12.0)	40 (10.7)
Urinary tract infection	31 (7.8)	29 (7.3)	41 (10.9)	32 (8.6)	34 (9.1)
Dizziness	21 (5.3)	1 (0.3)	11 (2.9)	26 (7.0)	23 (6.2)
Headache	18 (4.5)	11 (2.8)	16 (4.3)	17 (4.5)	13 (3.5)
Fall	30 (7.5)	30 (7.5)	8 (2.1)	6 (1.6)	5 (1.3)
Diarrhea	16 (4.0)	16 (4.0)	19 (5.1)	14 (3.7)	15 (4.0)
Nausea	10 (2.5)	5 (1.3)	9 (2.4)	13 (3.5)	15 (4.0)
Constipation	12 (3.0)	8 (2.0)	6 (1.6)	11 (2.9)	11 (2.9)
Weight decreased	6 (1.5)	10 (2.5)	6 (1.6)	17 (4.5)	8 (2.1)
Anxiety	14 (3.5)	9 (2.3)	15 (4.0)	13 (3.5)	12 (3.2)
Depression	10 (2.5)	11 (2.8)	12 (3.2)	9 (2.4)	16 (4.3)
Agitation	9 (2.3)	5 (1.3)	16 (4.3)	5 (1.3)	14 (3.8)
Athralgia	15 (3.8)	14 (3.5)	14 (3.7)	12 (3.2)	15 (4.0)
Fatigue	11 (2.8)	11 (2.8)	15 (4.0)	16 (4.3)	10 (2.7)
Back pain	7 (1.8)	7 (1.8)	16 (4.3)	13 (3.5)	9 (2.4)
Hypertension	8 (2.0)	5 (1.3)	11 (2.9)	5 (1.3)	14 (3.5)

Treatment-emergent AEs (TEAEs) were defined as events that occurred or worsened on or after the first administration of study drug. A patient who experienced multiple events was counted once.

combined to evaluate the overall frequency of lower extremity fractures requiring hospitalization, then the AE difference between groups lessens: simufilam (9 participants) and placebo (5 participants). The total number of all fracture-related AEs between treatment groups was similar: 14 simufilam participants (17 fracture events) and 18 placebo participants (22 fracture events). Fractures occurred most commonly after a fall, but there was no significant difference in the incidence of falls between groups (30 participants [7.5 %] in each group reported a fall).

In REFOCUS, the overall incidence of any TEAE was comparable between treatment groups (Table 3). The majority of TEAEs were mild or moderate (85 participants, 7.6 %). Drug-related TEAEs occurred in 15.4 %, serious TEAEs occurred in 13.3 %, and discontinuation for TEAEs occurred in 7.4 % of participants. Discontinuation for TEAEs occurred in 34 (9.0 %) participants on simufilam 50 mg (at least 2 patients: weight decreased, hepatic enzyme increased, AD dementia, headache, aggression), 32 (8.6 %) participants on simufilam 100 mg (weight decreased, ALT increased, diarrhea), and 17 (4.6 %) with placebo most commonly for weight decreased (1.2 %) and AD dementia (0.5 %). The most frequently reported serious AEs were fall (5 participants [1.3 %] 50 mg; 1 participant [0.3 %] 100 mg; 3 participants [0.8 %] placebo), hip fracture (3 participants [0.8 %] 50 mg; 1 participant [0.3 %] 100 mg; 2 participants [0.5 %] placebo [$p > 0.99$ for the pooled active treatment groups vs. placebo]), syncope (4 participants [1.1 %] 50 mg; 1 participant [0.3 %] 100 mg; 2 participants [0.5 %] placebo), and urinary tract infection (4 participants [1.1 %] 50 mg; 1 participant [0.3 %] 100 mg; 0 participants placebo). No deaths or serious TEAEs related to therapy were reported. The most common TEAEs were COVID-19, urinary tract infection and dizziness (Table 3).

For both trials, no remarkable changes from baseline were observed for serum chemistry, hematology or urinalysis parameters throughout the study, and no notable differences were observed between treatment groups. Estimated glomerular filtration rate remained stable relative to baseline during the study and showed little change over time, regardless of renal function category and treatment group. Results from ECG monitoring showed little change over the study. Baseline ECGs interpreted to be abnormal and clinically significant remained consistent over time, and results were similar between treatment groups. Simufilam showed no safety concerns on C-SSRS, with suicidal ideation rates between 0.5 and 1.3 % across groups.

The MRI sub-study in REFOCUS found no substantial increase in the incidence of MRI abnormalities or microhemorrhages for simufilam versus placebo from baseline to Week 76 (**Supplemental Table 3**). The incidence of MRI abnormalities and persistence of microhemorrhages or new occurrences were low at Week 40 and Week 76 with simufilam. One asymptomatic patient, an Apo E4 homozygote with baseline microhemorrhages randomized to simufilam 100 mg, showed an approximate 30 mm area of ARIA-E in the right parietal region at Week 76. This patient, who had already entered the open-label extension study by the time the MRI was read, remained asymptomatic on simufilam, and subsequent MRI scans showed resolution of the ARIA-E.

4. Discussion

Of these two Phase 3 clinical trials, RETHINK was completed, while REFOCUS was terminated early, with 22 % of participants still active in the trial and 53 % having completed the Week 76 visit. None of the co-primary or pre-specified secondary and exploratory endpoints in either trial showed a significant difference versus placebo. Hence, all subgroup or post hoc differences with $p < 0.05$ are considered nominal and hypothesis generating.

The primary analytical populations of mild-to-moderate AD for RETHINK (ITT) or REFOCUS (mITT) showed no significant effects of simufilam on ADAS-Cog12 in either trial. Although the predefined mild

and moderate subgroups in both trials also did not demonstrate significant results at the final timepoints, the predefined mild subgroup in REFOCUS showed nominal treatment group differences on ADAS-Cog12 at Week 4 and from Week 28 through Week 64, which disappeared at Week 76. This finding was not replicated in RETHINK. Week 76 data was impacted by close-out visit data collected after discontinuation of study drug, which may have been biased by participants being off study drug and knowing that RETHINK failed. A greater impact to Week 76 data was likely the 45 % missing data, of which 19 % was due to trial termination.

Missing data were modeled using a missing at random method, although a missing not at random (such as due to trial termination) could also be considered. More importantly, if more than 40 % of data are missing, the result should be considered exploratory [35]. In such cases, a completer analysis is recommended as well while considering the limitations of excluding participants with missing data [35]. A completer analysis, which included close-out visit data, left group sizes of 106 to 120 and was not significant. We consider the most conservative approach to be the current MMRM analysis with the understanding that the final timepoint with 45 % missing data is exploratory and not confirmatory. With the last reliable timepoint at Week 64, these data cannot predict longer-term outcomes.

This predefined mild subgroup population of 714 participants in REFOCUS represents the largest subgroup analysis conducted, but its failure to replicate in the mild subgroup in RETHINK undermines its interpretation. Nevertheless, when mild subgroup data to Week 52 from each trial were pooled ($n = 1208$), simufilam showed nominal treatment differences between simufilam and placebo at two timepoints and trends at two others.

The lack of replication in RETHINK of the mild subgroup finding in REFOCUS deserves scientific consideration. There was no overlap in clinical sites between the two studies, and sites with the capability to conduct CSF and imaging biomarker sub-studies were assigned to REFOCUS. The possibility that the plasma p-tau181 entry criterion with no confirmatory amyloid PET was more problematic in RETHINK may help explain the lack of replication of REFOCUS's predefined mild subgroup finding. The mild placebo group in RETHINK declined less on ADAS-Cog12 than the already low 2-point decline of the mild placebo group in REFOCUS at 52 weeks, and there were slight imbalances in mean plasma p-tau181 in mild treatment groups and in APOE4 homozygotes in RETHINK that did not favor simufilam. Finally, it is worth considering that REFOCUS participants had a 7 % higher level of university or higher education than RETHINK participants, which has been reported to correlate with more advanced disease or faster rate of decline[36,37] and may illustrate a potential difference in stage of disease between the two trials not evident in MMSE. Regardless of these considerations, the lack of replication between trials hampers interpretation of the predefined mild subgroup finding.

These clinical trials were the first Phase 3 studies to rely solely on a plasma biomarker for biological confirmation of AD, unless participants had a prior positive amyloid PET scan, which was 5–6 % of participants. Prior to the availability of amyloid PET scans, clinical trials in AD relied solely on clinical diagnosis, which has been estimated to have a false positive rate of 30 % [38]. The amyloid PET sub-study in REFOCUS showed that 21 % (33 of 160) were unexpectedly amyloid negative at baseline, suggesting that the plasma p-tau181 assay cut-off was insufficiently validated as an entry criterion to accurately predict brain amyloid deposition at the time these trials were initiated. Because amyloid deposition occurs up to two decades before cognitive symptoms appear [39,40], the negative amyloid PET scans observed in the sub-study likely reflect non-AD pathology. Although it is difficult to extrapolate a potential overall amyloid PET negative rate for REFOCUS or RETHINK, both trials used the same plasma p-tau181 assay and cutoff for inclusion and were likely impacted. Considering some false positives in enrolling AD participants, the nominal treatment group differences observed in the mild subgroup of REFOCUS may have been impacted by inclusion of cognitive impairment due to other causes. The mild subgroup finding might also include a clinical effect of simufilam's suppression of neuroinflammation on other cognitive impairments. Speculation is greatly limited by the lack of replication in RETHINK. Regardless, it is difficult to believe that this entry criterion did not negatively impact the evaluation of simufilam in these trials.

Although higher plasma p-tau181 levels have previously been reported to increase from cognitively unimpaired to MCI to AD [41,42], there was no correlation between screening p-tau181 and entry MMSE in these studies. Additionally, the moderate subgroup, which was 35 % of the ITT, did not neatly fit into the top half of the screening p-tau181 distribution from the RETHINK ITT population in the exploratory post hoc analysis. Because participants with higher plasma p-tau181 levels showed nominal treatment differences for simufilam versus placebo, these higher plasma p-tau181 may reflect better disease confirmation or a population more sensitive to the mechanism of simufilam rather than increased disease severity.

Suggesting inclusion of early disease, the placebo decline rate on ADAS-Cog12 at Week 52 in RETHINK was unexpectedly low, at 3.19 points, compared to 4.5 or 5.5 historically reported for mild-to-moderate AD [37,43], and the 18-month placebo decline rate of 5.26 on ADAS-Cog12 in REFOCUS was lower than the 7-point placebo decline on ADAS-Cog13 in a recent Phase 3 clinical trial in early AD (MCI to mild AD) [9]. Despite an entry criterion of diagnosed Stage 4 or 5 CE corresponding to mild or moderate stages, other entry criteria, including the plasma p-tau181 cutoff, MMSE up to 27 and CDR 0.5 global scores, may have allowed entry of participants with a stage of AD prior to dementia and perhaps also MCI due to other causes.

Sub-studies of plasma, CSF, PET and volumetric MRI biomarkers were hampered by small group sizes, particularly CSF ($n = 7$ to 14) and PET ($n = 21$ to 40), which included both mild and moderate participants. In the MRI sub-study ($n = 48$ to 52 per group), the nonsignificant changes for simufilam relative to placebo in hippocampal and ventricular volumes suggest adequate safety.

Oral administration of 50 or 100 mg of simufilam was generally safe and well tolerated in this elderly adult population. The proportion of participants reporting TEAEs was similar in simufilam and placebo groups, and most TEAEs were mild or moderate in severity. The incidence of drug-related TEAEs was markedly lower than the overall incidence of TEAEs. The incidence of serious TEAEs or TEAEs causing study discontinuation was modestly higher in the simufilam treatment groups in each study. A nonsignificant increase in hip fracture incidence requiring hospitalization for simufilam participants in RETHINK was not corroborated by any differences in the incidence of all lower extremity fractures, all fractures, or the equivalent data from REFOCUS. There was no suggestion of an increased incidence of falls in participants administered simufilam in either trial. No safety signals were observed in physical and neurological examinations, clinical laboratory testing, vital signs, ECG, renal function or suicide risk.

The MRI safety assessments at Weeks 40 and 76 showed that simufilam was not associated with ARIA-H. In AD patients not administered anti-amyloid antibodies, ARIA-E prevalence is < 0.1 % to 0.8 %, and ARIA-H prevalence ranges from 9.2 % to 33 % [44]. In REFOCUS, ARIA-H was 26 % to 28 % at screening, and its treatment emergence in participants without ARIA-H at baseline was low (1 % to 5 %). It is unlikely that the single isolated region of ARIA-E at Week 76 in an ApoE4 homozygote with pre-existing ARIA-H was due to simufilam.

The major limitations of these studies were the potential inclusion of MCI participants with or without amyloid pathology, the small group sizes of the biomarker sub-studies, and the early termination of REFOCUS, which mostly impacted Week 76 data. The use of a clinical diagnosis as a gating mechanism prior to MMSE and CDR cognitive assessments at screening, along with the novel plasma p-tau181 assay, may not have reliably excluded participants with non-AD pathology or MCI from these trials designed to assess mild-to-moderate AD. The plasma p-tau181 assay did not reliably exclude participants with

negative amyloid PET scans. The potential evidence of clinical benefit is in the predefined mild subgroup through the last reliable timepoint in REFOCUS, which did not replicate in RETHINK, although the predefined pooling of mild subgroups did show nominal treatment differences at two timepoints.

Although these two large Phase 3 trials did not demonstrate efficacy, the safety and tolerability profile of simufilam in this elderly adult population, together with treatment differences demonstrated in the predefined mild subgroup in one of the two trials, supports further development of simufilam in carefully designed studies for various indications. These include other disorders involving FLNA phosphorylation at serine 2152 such as cancer[45] and pituitary adenomas [46], inflammatory disorders involving inflammatory receptors that require FLNA recruitment [21], and disorders of FLNA overexpression such as tuberous sclerosis complex epilepsy [47].

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Consent statement

All participants and their caregivers provided written informed consent prior to any study procedures.

Role of funding source

The sponsor together with authors were involved in the design and conduct of the study as well as the collection, analysis, and interpretation of data, in the preparation of the manuscript. All authors reviewed and approved the manuscript for submission.

Data sharing statement

All data for this study are reported in the manuscript and Data Supplement. Additional data may be available upon written request to the Sponsor. The study protocol and statistical analysis plan are available as supplemental material.

CRediT authorship contribution statement

James W. Kupiec: Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Anton P. Porsteinsson:** Writing – review & editing, Investigation. **Raymond S. Turner:** Writing – review & editing, Investigation. **Suzanne Hendrix:** Writing – review & editing, Visualization, Validation, Formal analysis. **Craig Mallinckrodt:** Writing – review & editing, Visualization, Validation, Software, Formal analysis. **Arifulla Khan:** Writing – review & editing, Investigation. **Ian Cohen:** Writing – review & editing, Investigation. **Jonathan Liss:** Writing – review & editing, Investigation. **Roger Clarnette:** Writing – review & editing, Investigation. **Kee Hyung Park:** Writing – review & editing, Investigation. **Antonio M. Hernandez:** Writing – review & editing, Project administration. **Lindsay H. Burns:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: James Kupiec reports financial support was provided by Cassava Sciences Inc. James Kupiec reports a relationship with Cassava Sciences Inc that includes: employment and equity or stocks. Cassava Sciences, Inc., employee, JAN 2021 to MAY 2025, received salary and stock options; all stock in the company, purchased entirely with personal funds, was sold DEC 2024; all stock options cancelled 30 days after my retirement from the company. I currently own no stock in the company. Premier Research: Serving as a consultant to provide medical expertise as an Endpoint Committee member (adjudication) for a project that has nothing to do with this manuscript of its contents. Member independent review committee, Target ALS. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tjpad.2025.100469.

References

[1] Liang CS, Li DJ, Yang FC, et al. Mortality rates in Alzheimer's disease and non-Alzheimer's dementias: a systematic review and meta-analysis. Lancet Healthy Longev 2021;2(8):e479–88.
[2] Morovic S, Budincevic H, Govori V, Demarin V. Possibilities of dementia prevention - it is never too early to start. J Med Life 2019;12(4):332–7.
[3] Liu G, Liu C, Qiu A. Spatial correlation maps of the hippocampus with cerebrospinal fluid biomarkers and cognition in Alzheimer's disease: a longitudinal study. Hum Brain Mapp 2021;42(9):2931–40.
[4] Näslund J, Haroutunian V, Mohs R, et al. Correlation between elevated levels of amyolid beta-peptide in the brain and cognitive decline. JAMA 2000;283:1571–7.
[5] Lue LF, Kuo YM, Roher AE, et al. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol 1999;155(3):853–62.
[6] McLean CA, Cherny RA, Fraser FW, et al. Soluble pool of abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol 1999;46(6):860–6.
[7] Wang J, Dickson DW, Trojanowski JQ, Lee VM. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. Exp Neurol 1999;158(2):328–37.
[8] Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons learned from the Alzheimer's amyloid b-peptide. Nat Rev Mol Cell Biol 2007;8:101–12.
[9] Sims JR, Zimmer JA, Evans CD, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. Jama 2023;330(6):512–27.
[10] van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. N Engl J Med 2023;388(1):9–21.
[11] Burns LH, Pei Z, Wang HY. Targeting α7 nicotinic acetylcholine receptors and their protein interactions in Alzheimer's disease drug development. Drug Dev Res 2023.
[12] Dineley K, Bell K, Bui D, Sweatt J. β-amyloid peptide activates α7 nicotinic acetylcholine receptors expressed in xenopus oocytes. J Biol Chem 2002;227:25056–61.
[13] Wang HY, Lee D, D'Andrea M, Peterson P, Shank R, Reitz A. β-Amyloid1-42 binds to α7 nicotinic acetylcholine receptor with high affinity:implication for Alzheimer's disease pathology. J Biol Chem 2000;275:5626–32.
[14] Wang HY, Lee D, Davie C, Shank R. Amyloid peptide Aβ1-42 binds selectively and with picomolar affinity to α7 nicotinic acetylcholine receptors. J Neurochem 2000;75:1155–61.
[15] Wang HY, 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.
[16] Kolarova M, García-Sierra F, Bartos A, Ricny J, Ripova D. Structure and pathology of tau protein in Alzheimer disease. Int J Alzheimers Dis 2012;2012:731526.
[17] Hernández F, García-García E, Avila J. Microtubule depolymerization and tau phosphorylation. J Alzheimers Dis 2013;37(3):507–13.
[18] Tripathi T, Prakash J, Shav-Tal Y. Phospho-tau impairs nuclear-cytoplasmic transport. ACS Chem Neurosci 2019;10(1):36–8.
[19] Iqbal K, Grundke-Iqbal I. Discoveries of tau, abnormally hyperphosphorylated tau and others of neurofibrillary degeneration: a personal historical perspective. J Alzheimers Dis 2006;9(3):219–42. Suppl.
[20] Wang HY, 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.
[21] Wang HY, Cecon E, Dam J, Pei Z, Jockers R, Burns LH. Simufilam reverses aberrant receptor interactions of filamin A in Alzheimer's disease. Int J Mol Sci 2023;24(18).

[22] Wang HY, Lee KC, Pei Z, Khan A, Bakshi K, Burns LH. PTI-125 binds and reverses an altered conformation of filamin A to reduce Alzheimer's disease pathogenesis. Neurobiol Aging 2017;55:99–114.
[23] Wang HY, Pei Z, Lee KC, et al. Simufilam suppresses overactive mTOR and restores its sensitivity to insulin in Alzheimer's disease patient lymphocytes. Front Aging 2023. in press.
[24] Wang HY, Pei Z, Lee KC, et al. PTI-125 reduces biomarkers of Alzheimer's disease in patients. J Prev Alzheimer's Dis 2020;7:256–64.
[25] Mohs RC, Knopman D, Petersen RC, et al. Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's disease assessment scale that broaden its scope. The Alzheimer's disease cooperative study. Alzheimer Dis Assoc Disord 1997;11(Suppl 2):S13–21.
[26] Galasko D, Bennett D, Sano M, et al. An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's disease cooperative study. Alzheimer Dis Assoc Disord 1997;11(Suppl 2):S33–9.
[27] Jack Jr CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14(4):535–62.
[28] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189–98.
[29] Morris JC. The clinical dementia rating (CDR): current version and scoring rules. Neurology 1993;43(11):2412–4.
[30] O'Bryant SE, Lacritz LH, Hall J, et al. Validation of the new interpretive guidelines for the clinical dementia rating scale sum of boxes score in the national Alzheimer's coordinating center database. Arch Neurol 2010;67(6):746–9.
[31] Wessels AM, Siemers ER, Yu P, et al. A combined measure of cognition and function for clinical trials: the integrated Alzheimer's Disease rating scale (iADRS). J Prev Alzheimers Dis 2015;2(4):227–41.
[32] Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994;44(12):2308–14.
[33] Zarit S, Zarit J. The Memory And Behavior Problems Checklist And The Burden Interview. University Park, PA: Pennsylvania State University, Gerontology Center; 1990.
[34] Posner K, Brown GK, Stanley B, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry 2011;168(12):1266–77.
[35] Jakobsen JC, Gluud C, Wetterslev J, Winkel P. When and how should multiple imputation be used for handling missing data in randomised clinical trials - a practical guide with flowcharts. BMC Med Res Methodol 2017;17(1):162.
[36] Scarmeas N, Albert SM, Manly JJ, Stern Y. Education and rates of cognitive decline in incident Alzheimer's disease. J Neurol Neurosurg Psychiatry 2006;77(3):308–16.
[37] Thomas RG, Albert M, Petersen RC, Aisen PS. Longitudinal decline in mild-to-moderate Alzheimer's disease: analyses of placebo data from clinical trials. Alzheimers Dement 2016;12(5):598–603.
[38] Rabinovici GD, Gatsonis C, Apgar C, et al. Association of amyloid positron emission tomography with subsequent change in clinical management among Medicare beneficiaries with mild cognitive impairment or dementia. Jama 2019;321(13):1286–94.
[39] Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367(9):795–804.
[40] Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol 2013;12(4):357–67.
[41] Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. Alzheimers Dement 2018;14(8):989–97.
[42] Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. Nat Med 2020;26(3):379–86.
[43] Ito K, Ahadieh S, Corrigan B, et al. Disease progression meta-analysis model in Alzheimer's disease. Alzheimer's Dement 2010;6:39–53.
[44] Hampel H, Elhage A, Cho M, Apostolova LG, Nicoll JAR, Atri A. Amyloid-related imaging abnormalities (ARIA): radiological, biological and clinical characteristics. Brain 2023;146(11):4414–24.
[45] Wang HY, Burns Barbier L. Inventors; Pain Therapeutics, Inc., assignee. Method Inhib Growth Cancer Cells 2016.
[46] Marra G, Treppiedi D, Di Muro G, et al. A novel filamin A-binding molecule may significantly enhance somatostatin receptor type 2 antitumoral actions in growth hormone-secreting PitNET cells. In: Paper presented at: European Congress of Endocrinology; 2023.
[47] Zhang L, Huang T, Teaw S, et al. Filamin A inhibition reduces seizure activity in a mouse model of focal cortical malformations. Sci Transl Med 2020;12(531).