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Original Article

A phase 2 randomized, placebo-controlled study on the efficacy and safety of AR1001, a phosphodiesterase-5 inhibitor, in patients with mild-to-moderate Alzheimer's disease

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ABSTRACT

Background: AR1001 is a phosphodiesterase-5 inhibitor that produces improved cognitive performance and reduces amyloid-β and phosphorylated tau burdens in preclinical models of Alzheimer's disease (AD).
Objectives: To evaluate the safety and efficacy of AR1001 in participants with mild-to-moderate Alzheimer's disease (AD).
Design: Randomized, double-blind, placebo-controlled phase 2 trial conducted at 21 sites in the United States.
Participants: Adults aged 55–80 years with mild-to-moderate dementia as determined by National Institutes of Aging-Alzheimer's Association (NIA-AA) stage 4 or 5 and Mini-mental State Exam (MMSE) score 16-26.
Intervention: Once daily oral administration of placebo, 10 mg AR1001, or 30 mg AR1001 for 26 weeks followed by 26 weeks optional extension.
Measurements: Co-primary efficacy endpoints were changes from baseline at Week 26 in Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog 13) and Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC). Secondary endpoints included measures of cognition, daily living, and depression. Levels of plasma biomarkers pTau-181, pTau-217, Aβ42/40 ratio, glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL) were also examined.
Results: A total of 210 participants were enrolled and 82% completed 26 weeks of treatment. AR1001 10 mg and 30 mg were well-tolerated with a similar safety profile compared to placebo. After 26 weeks, there were no differences in ADAS-Cog13, ADCS-CGIC, or in secondary efficacy endpoints between groups. Levels of plasma biomarkers pTau-181, pTau-217, and GFAP were improved in the 30 mg AR1001 group compared to placebo.
Conclusion: AR1001 was safe and well tolerated. Although primary efficacy endpoints were not met after 26 weeks of treatment, participants receiving 30 mg AR1001 showed favorable changes in AD-related plasma biomarkers compared to placebo.
Trial registration: clinicaltrials.gov; NCT03625622

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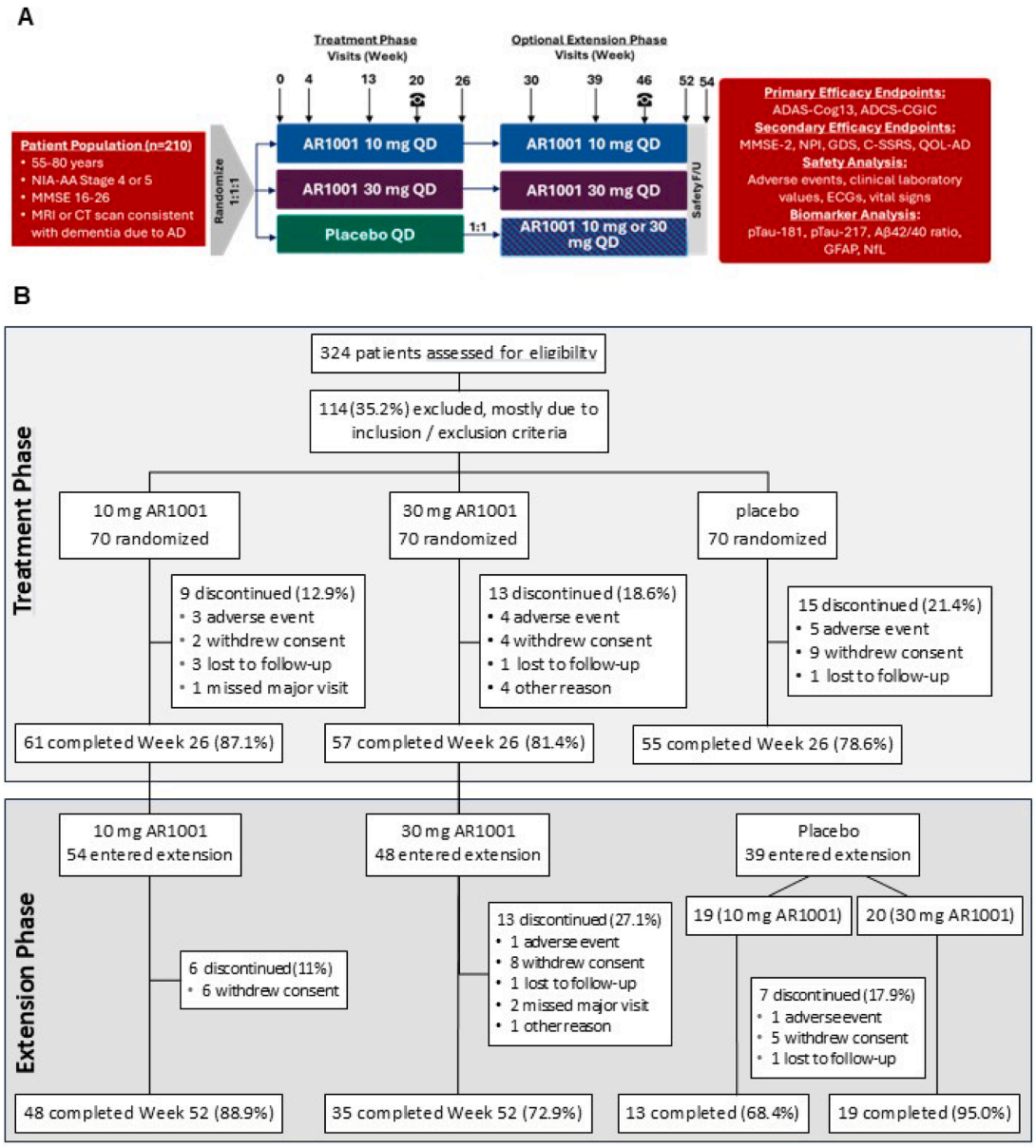

Fig. 1. Trial Design Summary and Patient Disposition. (A) Schematic of the 26-week randomized, placebo-controlled treatment phase and optional 26-week extension. Participants were randomized 1:1:1 to receive placebo, AR1001 10 mg, or AR1001 30 mg QD. Participants originally on placebo were re-randomized to 10 mg or 30 mg AR1001 in the extension phase. (B) CONSORT diagram showing participant flow, completion, and discontinuation rates across treatment and extension phases.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, accounting for 60–70% of cases [1]. According to the World Health Organization, more than 55 million people worldwide had dementia in 2021, with 10 million new cases per year, at a cost expected to reach $2.8 trillion USD per year globally by 2030 [1]. There are no medications or therapies that can arrest AD-related neurodegeneration and cognitive decline, creating a need for treatment options that at a minimum maintain quality of life for AD patients.

It is widely believed that the pathogenesis of AD is multifactorial, involving genetic factors [2], cholinergic dysfunction [3,4], amyloid plaque formation [5], tau aggregation [6,7], inflammation [8,9], and oxidative stress [10]. Monoclonal antibodies that target cerebral amyloid have recently received FDA approval for the treatment of early AD based on improvements in cognitive measures and reduction in amyloid burden compared to placebo, although targeting amyloid alone does not appear to be sufficient to prevent disease progression [11–13]. A polypharmacological approach that targets multiple pathogenic pathways may hold promise [14–17]. The development of safe and easily administered medicines would provide additional treatment options to patients, particularly those who are at risk for amyloid-related imaging abnormalities (ARIA) from monoclonal antibody therapy, such as ApoE4 homozygotes, patients with cerebral amyloid angiopathy, and patients taking concomitant antithrombotic or thrombolytic medication.

Phosphodiesterase-5 (PDE5) inhibitors, which include sildenafil (Viagra), vardenafil (Levitra), and tadalafil (Cialis), have been widely used for the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension (PAH), and have extensive clinical and safety data. PDE5 inhibitors increase cellular levels of cyclic GMP (cGMP), which activates protein kinase G (PKG) and its downstream effectors including cyclic adenosine monophosphate responsive element binding protein (CREB) and phosphatidylinositol 3-kinase (PI3K)/Akt. CREB has been linked to preservation of neuronal signaling and synaptic plasticity, and

Table 1
Baseline demographics and clinical characteristics of participants by treatment group.

Characteristic	Placebo (N = 70)	AR1001 10 mg (N = 70)	AR1001 30 mg (N = 70)
Age (years), mean (SD)	70.4 (5.5)	70.9 (6.5)	70.4 (6.8)
Gender, n (%)			
Female	48 (68.6)	43 (61.4)	47 (67.1)
Male	22 (31.4)	27 (38.6)	23 (32.9)
Race/Ethnicity, n (%)			
Black or African American	12 (17.1)	8 (11.4)	8 (11.4)
White	58 (82.9)	60 (85.7)	62 (88.6)
Other	0	2 (2.8)	0
Hispanic or Latino ethnicity, n (%)	13 (18.6)	13 (18.6)	16 (22.9)
BMI (kg/m^2), mean (SD)	28.1 (5.5)	29.6 (7.0)	28.7 (6.5)
Ongoing AD treatment*, n (%)	36 (51.4)	40 (57.1)	46 (65.7)
Baseline cognitive impairment, n (%)			
Mild (MMSE-2 $\geq$ 21)	52 (74.3)	44 (62.9)	37 (52.9)
Moderate (MMSE-2 of 16–20)	18 (25.7)	26 (37.1)	33 (47.1)
ApoE4 Status, n	62	59	60
Homozygous, n (%)	6 (9.7)	11 (18.6)	10 (16.7)
Heterozygous, n (%)	30 (48.4)	18 (30.5)	29 (48.3)

* Ongoing AD treatment indicates concomitant administration of an approved medication for Alzheimer's disease (donepezil, rivastigmine, galantamine, or memantine) at a stable dose.

Abbreviations: AD: Alzheimer's disease; ApoE4: apolipoprotein E4; BMI: body mass index; MMSE-2: Mini-mental Status Examination, 2nd Edition; SD: standard deviation

Akt activation protects neurons through inhibition of glycogen synthase kinase 3β and reduced phosphorylation of Tau [18].

Preclinical studies of PDE5 inhibitors in cell and animal models of neurodegenerative diseases support a role in neuroprotective and anti-inflammatory processes. In pluripotent stem cells derived from AD patients, sildenafil treatment reduced hyperphosphorylated Tau levels and enhanced neurite outgrowth [19]. Treatment with sildenafil and tadalafil reduced expression of pro-inflammatory cytokines, reduced apoptosis and autophagy, increased neurogenesis, and ameliorated cognitive and behavioral deficits in rodent models of neurodegenerative diseases including AD (reviewed in [18,20,21].

AR1001 (mirodenafil) is a phosphodiesterase 5 (PDE5) inhibitor approved in South Korea as Mvix® for treatment of erectile dysfunction. It is a highly potent inhibitor of PDE5, with an IC_{50} of 0.34 nM and 30-fold greater selectivity against PDE5 than PDE6 (IC_{50}= 10.2 nM) [22]. In neuroblastoma SH-SY5Y cells treated with the toxic amyloid form Aβ42, administration of mirodenafil reduced levels of Aβ and markers of apoptosis (cleaved caspase-3 and poly ADP-ribose polymerase), and increased levels of the autophagy marker p62 [23]. In the APP-C105 and ApoE4 knock-in transgenic mouse models of AD, mirodenafil-treated mice showed significant improvements in cognitive performance compared to control animals, had lower levels of Aβ42 and phosphorylated Tau, and had improved cerebrovascular perfusion and tight junction gene expression [24]. A recent study also showed that mirodenafil significantly improved sensorimotor and cognitive recovery and reduced the amount of degenerative and apoptotic cell neurons in experimental models of stroke [25]. Mirodenafil has been shown to penetrate the blood-brain barrier, as levels of ^{14}C[mirodenafil] were present in the brains of rats [26].

Due to the potent inhibition of PDE5 by AR1001, its blood-brain barrier penetration, and its amelioration of AD pathology and symptoms in preclinical models, AR1001 was evaluated in a Phase 2 study in patients with mild-to-moderate AD to determine its safety and preliminary efficacy.

Methods

Trial conduct and oversight

AR1001-ADP2-US01 (NCT03625622) was a 26-week, Phase 2, randomized, placebo-controlled, double-blinded study conducted across 21 sites in the United States from January 2019 to June 2021. All procedures were performed in compliance with the protocol, provisions in the Declaration of Helsinki, regulations of the United States Food and Drug Administration, and guidelines of Good Clinical Practice as outlined by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. WCG Institutional Review Board approved the study protocol and amendments. The privacy rights of human subjects were observed and informed consent was obtained from all participants and/or caregivers. The trial was sponsored by AriBio.

Study participants

Key eligibility criteria were 55-80 years of age, clinical staging 4 or 5 by 2011 NIA-AA criteria, and MMSE score between 16 and 26 (Fig. 1A). Study participants were required to have an MRI or CT scan after the onset of dementia symptoms that showed findings consistent with a diagnosis of AD at the time of the study, without clinically significant comorbid pathologies.

FDA-approved Alzheimer's disease medications (donepezil, rivastigmine, galantamine, memantine, or combinations of these [27]) were allowed if doses were stable for at least 3 months at the time of study enrollment.

ApoE4 proteotypic status was determined using Lumipulse *G* ApoE4 and Lumipulse *G* Pan-ApoE protein assays.

Randomization and intervention

Eligible patients were randomized 1:1:1 with a randomization block size of 6 to receive a daily oral dose of placebo, 10 mg AR1001, or 30 mg AR1001 for 26 weeks. The study was double blinded to participant and investigator. Upon completion of the 26-week treatment phase, participants were given the option to continue for an additional 26-week extension phase. Participants assigned to AR1001 for the treatment phase continued at the same dose in the extension phase, and participants assigned to placebo were rerandomized 1:1 with a block size of 2 to receive 10 mg or 30 mg AR1001 (Fig. 1A).

Endpoints and assessments

The co-primary efficacy endpoints were 1) change from baseline to Week 26 in the 13-item Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS-Cog 13), with scores ranging from 0 (no impairment)

Table 2
Summary of adverse events during the 26-week treatment and extension phases.

Treatment phase (Weeks 1-26)			
Event, n (%)	Placebo (N = 70) n (%)	AR1001 10 mg (N = 70) n (%)	AR1001 30 mg (N = 70) n (%)
Any adverse event	42 (60.0)	39 (55.7)	47 (67.1)
Adverse event related to study drug	13 (18.6)	4 (5.7)	7 (10.0)
Grade 1 (mild)	8 (11.4)	2 (2.9)	2 (2.9)
Grade 2 (moderate)	5 (7.1)	2 (2.9)	5 (7.1)
≥ Grade 3 (severe)	0	0	0
Any serious adverse event	6 (8.6)	6 (8.6)	8 (11.4)
Serious adverse event related to study treatment	0	0	1 (1.4)
Death	0	1 (1.4)	0
Death related to study treatment	0	0	0
Adverse event leading to treatment discontinuation	7 (10.0)	3 (4.3)	4 (5.7)
Adverse event leading to study discontinuation	6 (8.6)	3 (4.3)	4 (5.7)
Adverse events in clinical laboratory parameters	9 (12.9)	12 (17.1)	15 (21.4)
Visual disorders	2 (2.9)	0	0
Adverse events ≥5% in any group			
Fall	2 (2.9)	2 (2.9)	5 (7.1)
Nausea	2 (2.9)	1 (1.4)	5 (7.1)
Headache	3 (4.3)	2 (2.9)	4 (5.7)
Urinary tract infection	2 (2.9)	2 (2.9)	4 (5.7)
Dizziness	5 (7.1)	0	1 (1.4)
Arthralgia	5 (7.1)	0	0

Extension Phase (Weeks 26-52)		
Event, n (%)	10 mg AR1001 (N = 73)* n (%)	30 mg AR1001 (N = 68)* n (%)
Any adverse event	38 (52.1)	33 (48.5)
Adverse event related to study drug	4 (5.5)	6 (8.8)
Grade 1 (mild)	2 (2.7)	6 (8.8)
Grade 2 (moderate)	2 (2.7)	0
≥ Grade 3 (severe)	0	0
Any serious adverse event	2 (2.7)	2 (2.9)
Serious adverse event related to study treatment	0	0
Deaths	0	0
Adverse event leading to treatment discontinuation	3 (4.3)	4 (5.7)
Adverse event leading to study discontinuation	0	0
Adverse events in clinical laboratory parameters	10 (13.7)	9 (13.2)
Visual disorders	0	1 (1.5)
Adverse events for ≥5% of patients in any group		
Urinary tract infection	6 (8.2)	3 (4.4)

* Participants who received placebo during the treatment phase and opted to continue to the extension phase were randomized to receive 10 mg AR1001 (n=19) or 30 mg AR1001 (n=20). These participants were included in the extension phase safety analysis along with participants who continued their AR1001 treatment from the treatment phase.

Abbreviations: AE: adverse event; SAE: serious adverse event.

to 85; and 2) change from baseline to Week 26 in the Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC), a 7-point scale that evaluates improvement or worsening of AD symptoms. Secondary efficacy endpoints included changes from baseline to Week 26 in MMSE-2, Neuropsychiatric Inventory (NPI), Geriatric Depression Scale (GDS), and Quality of Life in Alzheimer's Disease (QOL-AD) (Fig. 1A).

Plasma samples for analysis of putative AD biomarkers pTau-181, pTau-217, Aβ42/40 ratio, glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL) were collected from consented participants in 10 ml EDTA tubes, centrifuged, and stored frozen in 1 ml aliquots. Plasma samples were diluted 4× and run in duplicate. The mean of duplicates for each sample was determined and included in the analysis if the coefficient of variation was < 25%. Plasma levels of pTau-181 were quantified using the Simoa Human pTau-181 Advantage V2 Kit (Quanterix). Plasma pTau-217 was quantified using Lumipulse® G pTau 217 kit (Fujirebio, Malvern, PA). Plasma levels of GFAP, Aβ42/40 ratio, and NfL were quantified using the Simoa Neurology 4-Plex E Advantage Kit (Quanterix). pTau-217 was quantified using Lumipulse® G pTau 217 kit (Fujirebio, Malvern, PA).

Investigators reported adverse events using MedDRA Version 24.0 based on assessments of patient symptoms, vital signs, physical examinations, electrocardiography (ECG) readings, clinical laboratory tests (hematology, blood chemistry, and urinalysis), and the Columbia Suicide Severity Rating Scale (C-SSRS) [28].

Cognitive and safety assessments were conducted at baseline (Week 0) and during the treatment phase at Weeks 4, 13, and 26, along with a safety phone call at Week 20. During the extension phase, cognitive and safety assessments were conducted at Weeks 30, 39, and 52, with a safety phone call at Week 46. A safety follow-up visit was conducted 2 weeks after the last dose of study drug (Fig. 1A).

Statistical methodology

Efficacy outcomes were analyzed in the Intent-to-Treat (ITT) population, which included participants who took at least one dose of study drug and had at least one post-dose efficacy measurement. Descriptive statistics were used to summarize continuous variables by treatment group. For the primary analysis, the change from baseline in ADAS-Cog 13 at Week 26 was analyzed with an ANCOVA model including the factor of treatment and the Baseline ADAS-Cog 13 result as a covariate. No repeated measures were included in the model. For the co-primary

endpoints, the overall Type I error of 0.05 was controlled using the Holm step-down procedure [29]. To reject the null hypothesis that AR1001 was not different from placebo with respect to changes from baseline in ADAS-Cog 13 and ADCS-CGIC at Week 26, both co-primary endpoints needed to show statistical significance. Similar ANCOVA models were used to analyze secondary outcomes at Week 26.

Biomarker analyses evaluated changes from baseline to Week 26 in pTau-181, pTau-217, Aβ 42/40 ratio, GFAP, and NfL, with independent samples tests for comparisons between treatment groups and placebo.

Safety outcomes were summarized overall and by the treatment group to which patients were randomized. Outcomes were further classified by severity, seriousness, and relation to the study drug. Descriptive statistics were used to describe the data.

Post-hoc analyses were performed in participants in the top tertile of baseline pTau-181 levels (corresponding to > 5.0 pg/mL) and corresponding lower two tertiles using Mann-Whitney U tests to compare between groups.

Sample size calculation

The population of 210 participants was selected based on an assumed 20% drop-out rate that would yield an evaluable sample size of 168 patients, providing the trial with 80% power to show that the AR1001 treatment groups altered disease progression compared to placebo by a 2-point difference at Week 26 in the group means of ADAS-Cog 13 scores, with an overall standard deviation of 6.7 points. For ADCS-CGIC, a 0.35 difference in group means can be detected as statistically significant with a sample size of 58 participants per group assuming the change from baseline in ADCS-CGIC has an overall standard deviation of 1.2 points [30].

Table 3
Summary of primary and secondary efficacy endpoints at week 26.

	Placebo	10 mg AR1001	30 mg AR1001
Primary Efficacy Endpoints			
ADAS-Cog-13			
No. participants*	51	59	57
Baseline value (SD)	24.2 (10.0)	25.5 (9.4)	26.8 (11.3)
LSM change from baseline (95% CI)#	-1.3 (-3.4, 0.7)	-1.7 (-3.6, 0.2)	-0.2 (-2.2, 1.7)
LSM difference vs. placebo		-0.4	1.1
P value vs. placebo		.81	.45
ADCS-CGIC			
No. participants	52	58	57
LSM (95% CI)	4.3 (4.0, 4.5)	4.2 (4.0, 4.4)	4.3 (4.1, 4.6)
LSM difference vs. placebo		-0.1	0.03
P value vs. placebo		.57	.88
Secondary Efficacy Endpoints			
MMSE-BV			
No. participants	52	59	56
Baseline value (SD)	11.8 (2.6)	11.2 (2.7)	10.4 (2.6)
LSM change from baseline (95% CI)	-0.4 (-1.0, 0.2)	-0.03 (-0.6, 0.5)	0.07 (-0.5, 0.7)
LSM difference vs. placebo		0.38	0.48
P value vs. placebo		.36	.27
MMSE-SV			
No. participants	52	59	56
Baseline value (SD)	22.5 (3.8)	21.8 (3.8)	20.6 (3.8)
LSM change from baseline (95% CI)	-0.2 (-1.1, 0.6)	-0.1 (-0.9, 0.7)	-0.2 (-1.0, 0.7)
LSM difference vs. placebo		0.12	0.10
P value vs. placebo		.83	.87
NPI			
No. participants	51	60	56
Baseline value (SD)	7.7 (10.2)	7.8 (9.0)	7.9 (9.9)
LSM change from baseline (95% CI)	-2.5 (-4.6, -0.4)	-1.7 (-3.6, 0.3)	-0.6 (-2.6, 1.4)
LSM difference vs. placebo		0.8	1.9
P value vs. placebo		.58	.21
GDS			
No. participants	51	60	57
Baseline value (SD)	2.7 (2.2)	2.3 (2.4)	2.7 (2.0)
LSM change from baseline (95% CI)	-0.58 (-1.1, 0)	-0.03 (-0.5, 0.5)	-0.36 (-0.9, 0.2)
LSM difference vs. placebo		0.55	0.22
P value vs. placebo		.16	.56
QOL-AD: FV			
No. participants	51	58	57
Baseline value (SD)	34.5 (5.3)	34.9 (6.8)	34.9 (6.2)
LSM change from baseline (95% CI)	1.1 (-0.2, 2.4)	0.9 (-0.2, 2.4)	0.5 (-0.7, 1.8)
LSM difference vs. placebo		-0.14	-0.55
P value vs. placebo		.88	.54
QOL-AD: PV			
No. participants	50	59	57
Baseline value (SD)	37.7 (6.0)	38.6 (6.6)	38.5 (6.2)
LSM change from baseline (95% CI)	0.17 (-1.1, 1.4)	0.23 (-0.9, 1.4)	0.36 (-0.8, 1.5)
LSM difference vs. placebo		0.07	0.19
P value vs. placebo		.94	.82

* Number of participants refers to participants evaluated at Week 26.

Abbreviations: ADAS-Cog13: Alzheimer's Disease Assessment Scale – Cognitive Subscale 13; ADCS-CGIC: Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; CI: confidence interval; GDS: Geriatric Depression Scale; LSM: least square mean; MMSE-2: BV: Mini-mental Status Examination, 2nd Edition: Brief Version; MMSE-2: SV: Mini-mental Status Examination, 2nd Edition: Standard Version; NPI: Neuropsychiatric Inventory; QOL-AD: FV: Quality of Life in Alzheimer's Disease: Family Version; QOL-AD: PV: Quality of Life in Alzheimer's Disease: participant version; SD: standard deviation.

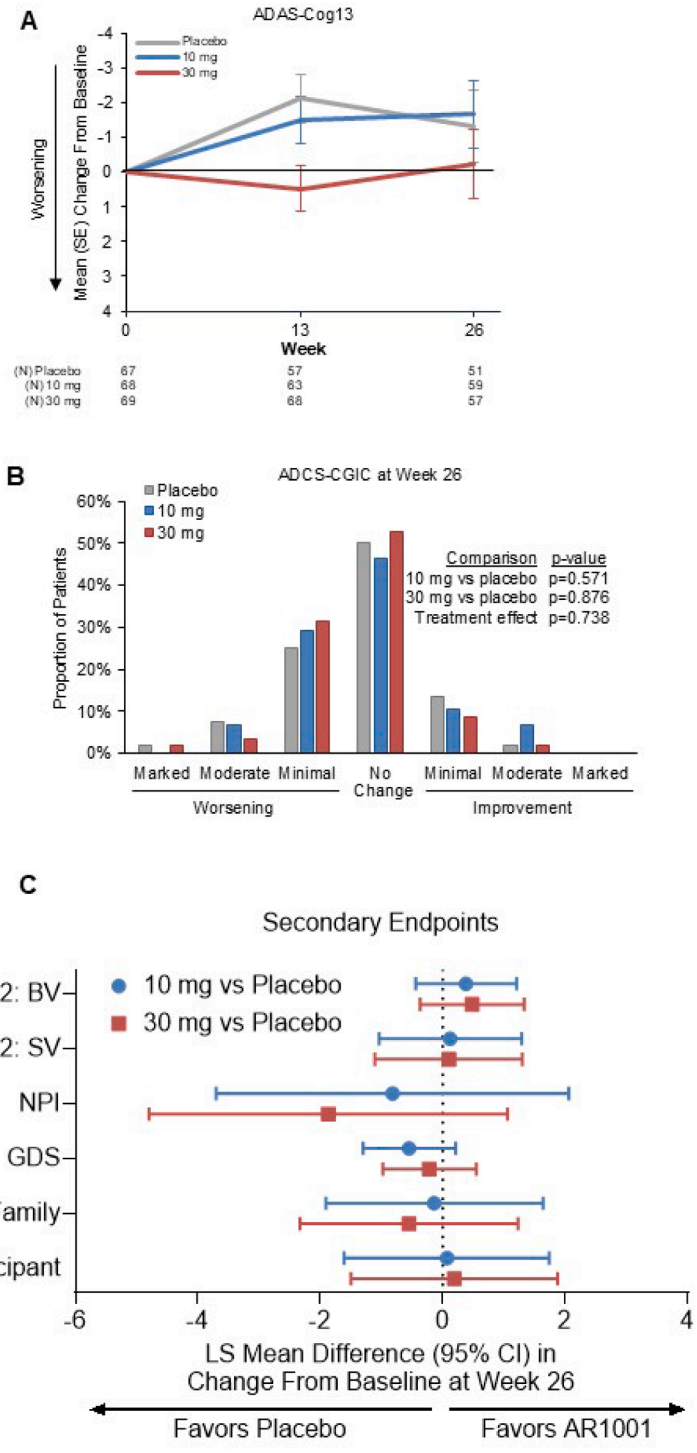

Fig. 2. Co-primary and secondary endpoints changes from baseline at Week 26 during the treatment phase. (A) Change from baseline in co-primary endpoint, ADAS-Cog13 total score at Weeks 13 and 26. Values on time axis are shifted slightly for ease of reading. (B) Distribution of co-primary endpoint, ADCS-CGIC scores at Week 26, reflecting clinician-rated change in global functioning. (C) Least squares (LS) mean difference (95% CI) from placebo for secondary endpoints at Week 26. Negative values are plotted for GDS and NPI to maintain directions.

Abbreviations: ADAS-Cog13: Alzheimer's Disease Assessment Scale – Cognitive Subscale 13; ADCS-CGIC: Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; CI: confidence interval; GDS: Geriatric Depression Scale; LS: least squares; MMSE-2: BV: Mini-mental Status Examination, 2nd Edition: Brief Version; MMSE-2: SV: Mini-mental Status Examination, 2nd Edition: Standard Version; NPI: Neuropsychiatric Inventory; QOL-AD: FV: Quality of Life in Alzheimer's Disease: Family Version; QOL-AD: PV: Quality of Life in Alzheimer's Disease: Participant Version; SE: standard error.

Table 4
Summary of biomarker results at week 26.

	Placebo	10 mg AR1001	30 mg AR1001
pTau-181 (pg/mL)			
Baseline value (SD)	4.24 (2.16)	4.65 (2.43)	4.65 (2.66)
No. participants evaluated*	41	51	45
Mean change from baseline (95% CI)	0.31 (-0.09, 0.70)	-0.29 (-0.85, 0.26)	-0.46 (-1.04, 0.12)
Mean difference vs. placebo		-0.60	-0.77
P value vs. placebo		0.093	0.034
pTau-217 (pg/mL)			
Baseline value (SD)	0.622 (0.609)	0.632 (0.531)	0.734 (0.744)
No. participants evaluated	40	51	42
Mean change from baseline (95% CI)	0.037 (-0.104, 0.162)	0.020 (-0.112, 0.123)	-0.118 (-0.223, -0.037)
Mean difference vs. placebo		-0.017	-0.155
P value vs. placebo		.530	.009
Aβ42/40 ratio			
Baseline value (SD)	0.084 (0.014)	0.086 (0.016)	0.089 (0.041)
No. participants evaluated	40	49	41
Mean change from baseline (95% CI)	0 (-0.004, 0.003)	0.001 (-0.001, 0.005)	0.001 (-0.003, 0.004)
Mean difference vs. placebo		0.001	0.001
P value vs. placebo		0.483	0.932
GFAP (pg/mL)			
Baseline value (SD)	255.7 (107.8)	259.2 (118.6)	256.6 (113.8)
No. participants evaluated	41	50	45
Mean change from baseline (95% CI)	23.9 (-3.8, 51.6)	11.9 (-14.0, 37.8)	-7.0 (-26.2, 12.2)
Mean difference vs. placebo		-12.0	-30.9
P value vs. placebo		0.393	0.042
NfL (pg/mL)			
Baseline value (SD)	29.4 (16.5)	28.0 (14.6)	33.2 (38.8)
No. participants evaluated	41	50	45
Mean change from baseline (95% CI)	1.06 (-2.69, 4.82)	0.95 (-2.69, 4.59)	-6.65 (-19.09, 5.79)
Mean difference vs. placebo		-0.25	-1.6
P value vs. placebo		.473	.052

* Number of participants refers to participants evaluated at Week 26.
Abbreviations: Aβ: amyloid beta; CI: confidence interval; GFAP: glial fibrillary acidic protein; NfL: neurofilament light chain; SD: standard deviation

Results

Trial population

The completion rate for the 26-week treatment phase was 78.6% in the placebo group, 87.1% in the 10 mg AR1001 group, and 81.4% in the 30 mg AR1001 group. Of the 173 participants who completed the treatment phase, 141 continued to the optional extension phase. The completion rate for participants entering the extension phase was 81.5% (115/141) (Fig. 1B).

The mean age of participants was 70.6 years, and the population was 65.7% female (Table 1). The racial and ethnic makeup of participants who were enrolled included 13.3% African-American and 20.0% Latino or Hispanic participants.

Of 181 participants tested for ApoE4 status, 27 (14.9%) were homozygous and 77 (42.5%) were heterozygous, an overall ApoE4 carrier rate of 57.4%. ApoE4 carrier status was broadly balanced across groups; however, the proportion of ApoE4 homozygotes was lower in the placebo group (9.7%) compared to the AR1001 10 mg (18.6%) and 30 mg (16.7%) groups (Table 1).

Safety assessments

During the 26-week placebo-controlled treatment phase, AR1001 at 10 and 30 mg had similar adverse event profiles compared to placebo (Table 2). There was one serious adverse event of syncope in the 30 mg AR1001 group that was considered related to study treatment. One participant in the 10 mg AR1001 group died due to a serious adverse event of COVID-19 that was not considered related to study treatment. Fourteen participants had adverse events that led to treatment discontinuation, with no notable differences in discontinuations between treatment groups. All adverse events considered related to treatment intervention were graded as mild or moderate (Grade 1 or 2) in severity. The most common adverse events during the treatment phase, occurring in 5% or more of participants in any treatment group, were fall, nausea, headache, urinary tract infection, dizziness, and arthralgia (Table 2).

Visual disturbances have been reported with sildenafil [35] and are attributed to its inhibition of PDE6 in the retina [23]. Mirodenafil has also been shown to inhibit PDE6 [22]. However, no clinically significant ocular safety signals were identified in this study. Two participants in the placebo group and no participants in AR1001 groups reported adverse events related to visual impairment during the treatment phase. In the extension phase, one participant in the 30 mg AR1001 group reported an adverse event of double vision (diplopia), which was assessed as mild by the investigator (Table 2) (data not shown).

During the extension phase, in which placebo participants were randomized to receive 10 or 30 mg AR1001, there were no Grade 3 or higher adverse events, serious adverse events, or study discontinuations related to study treatment. The only adverse event occurring in 5% or more of participants in either the 10 mg or 30 mg AR1001 groups during the extension phase was urinary tract infection (Table 2).

Efficacy assessments

For the co-primary efficacy endpoint of ADAS-Cog 13, least square mean change from baseline was not statistically different for 10 mg AR1001 vs placebo or 30 mg AR1001 vs placebo at Week 26 (Table 3). There was a moderate placebo response that resulted in an ADAS-Cog13 improvement of 2.2 points at Weeks 13 and 1.3 points at Week 26 (Fig. 2A).

Pairwise comparisons for the co-primary efficacy endpoint of ADCS-CGIC were not statistically different for 10 mg or 30 mg AR1001 vs placebo (Table 3). Nearly half of participants (49.7%) across groups were evaluated by investigators as having no change in cognitive performance after 26 weeks of treatment (Score = 4); 28.6% were evaluated as having minimal worsening (Score = 5), and 10.9% had minimal improvement (Score = 3). The remaining participants had moderate worsening/improvement or marked worsening (Fig. 2B).

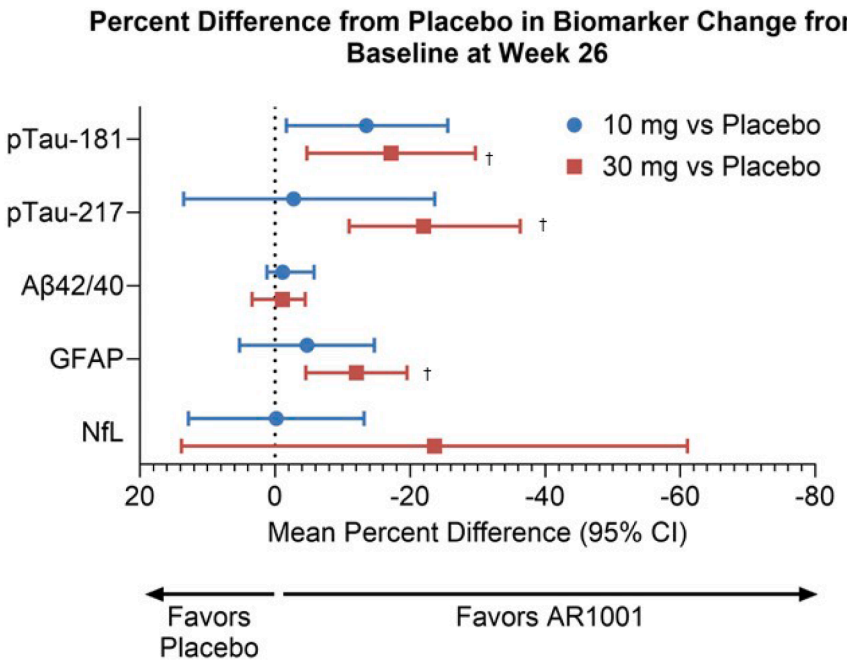

Fig. 3. Changes in Plasma Biomarkers at Week 26.
Forest plot of percent difference in change from baseline at Week 26 for plasma biomarkers comparing AR1001 treatment groups to placebo. †$p < .05$ for 30 mg vs placebo by Mann-Whitney U test. Negative values are plotted for Aβ42/40 ratio to reflect direction of improvement.
Abbreviations: Aβ: amyloid beta; GFAP: glial fibrillary acidic protein; NfL: neurofilament light chain.

Evaluation of secondary efficacy endpoints of MMSE-2, NPI, GDS, and QOL-AD found no statistically significant differences between placebo and AR1001 treatment groups 30 mg AR1001 (Table 3). The mean changes from baseline for secondary endpoints in the 10 mg and 30 mg AR1001 treatment groups are compared to placebo in Fig. 2C.

Post-hoc biomarker analysis

Plasma samples were collected for analysis of biomarkers pTau-181, pTau-217, Aβ42/40 ratio, GFAP, and NfL. Participants on placebo had worsening mean change from baseline at Week 26 for pTau-181, pTau-217, GFAP, and NfL. In contrast, participants on 30 mg AR1001 had improved mean change from baseline at Week 26 for the same biomarkers (Table 4). There were no appreciable changes in Aβ42/40 ratio observed in any group. Between-group comparisons showed statistically significant improvements ($p < .05$) in the 30 mg AR1001 group compared with placebo for pTau-181, pTau-217, and GFAP (Table 4). Although the difference in NfL did not reach statistical significance ($p = .052$), the direction of effect favored AR1001. A Forest plot of the mean percentage difference from placebo in biomarker levels in 10 mg and 30 mg AR1001 groups is shown in Fig. 3. Variability was high in the biomarker assays, nevertheless the difference in mean change from baseline between the 30 mg AR1001 group and placebo supports a potential biomarker effect of AR1001 on tau and glial markers.

Baseline pTau-181 post-hoc subgroup analyses

At the time of the trial, NIA-AA criteria did not require biomarker confirmation of cerebral amyloid in clinical trials. In basing eligibility on cognitive measures (e.g., MMSE), there was a possibility that the population had mixed AD and non-AD forms of dementia. To select for participants with a likelihood of having AD, we performed post-hoc analyses on participants in the top tertile of baseline pTau-181 (corresponding to ≥ 5.0 pg/mL), along with the corresponding lower two tertiles (pTau-181 < 5.0 pg/mL). pTau-181has been shown to correlate with cerebral amyloid PET [31]. A similar post-hoc tertile analysis was used to analyze donanemab and baseline tau PET [32].

Participants taking placebo in the high pTau-181 cohort showed worsening performance in ADAS-Cog-13 (Fig. 4A) and ADCS-CGIC (Fig. 4B) at Week 26, whereas participants in the lower pTau-181 cohort had improved cognitive performance. These data support a scenario in which higher pTau-181 correlates with more rapid AD progression [33].

Participants in the high pTau-181 cohort treated with AR1001 had slower cognitive decline in ADAS-Cog13 and ADCS-CGIC relative to participants treated with placebo (Fig. 4), though sample sizes were small and the study was not powered to detect a statistical difference.

Discussion

This Phase 2 study aimed to evaluate the safety and preliminary efficacy of AR1001 in patients with mild-to-moderate AD dementia. Oral AR1001 at 10 or 30 mg once daily was well-tolerated, with a safety profile similar to that of placebo and few serious or severe adverse events related to treatment over 52 weeks. Although visual disturbances are a known side effect of other PDE5 inhibitors [34], no meaningful ocular safety concerns were identified with AR1001 in the Phase 2 trial. This favorable ocular safety profile may be attributed to the lower dose of AR1001 used in this trial relative to doses for erectile dysfunction, and the 30-fold higher selectivity of AR1001 for PDE5 over PDE6 [22]. Although post-treatment MRI was not performed to assess ARIA, no ARIA-like symptoms or serious adverse events were observed. This suggests that AR1001 may represent a viable treatment option for genetically at-risk individuals who may not tolerate or qualify for anti-amyloid therapies, including ApoE4 homozygotes.

The completion rate in both the 26-week treatment phase and 26-week extension phase was 82%, suggesting the drug was well tolerated and low burden to use. The favorable safety profile of AR1001 and easy-to-use oral administration are strengths of this treatment.

The study did not meet the co-primary endpoints as measured by ADAS-Cog 13 and ADCS-CGIC or secondary endpoints through 26 weeks of treatment. The placebo group improved in ADAS-Cog13 and was mostly unchanged in ADCS-CGIC at Week 26. A positive placebo

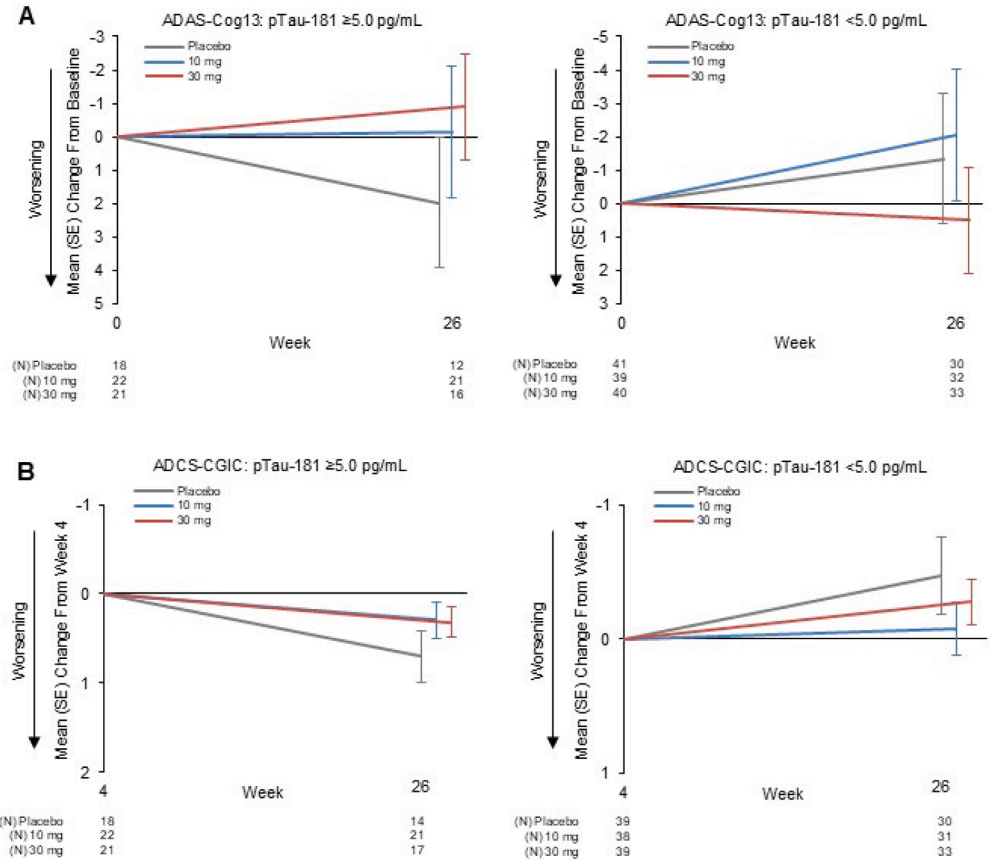

Fig. 4. Primary Efficacy Outcomes Stratified by Baseline Plasma pTau-181 Levels.
Change from baseline in (A) ADAS-Cog13 and (B) ADCS-CGIC at Week 26 in participants with the highest tertile of baseline pTau-181 (≥ 5.0 pg/ml) and in the corresponding lower two-thirds tertiles (< 5.0 pg/ml).
Abbreviations: ADAS-Cog13: Alzheimer's Disease Assessment Scale – Cognitive Subscale 13; ADCS-CGIC: Alzheimer's Disease Cooperative Study – Clinician Global Impression of Change; SE: standard error.

response was also found in a second AD clinical trial after 26 weeks treatment [35], suggesting that the placebo effect may be present in short-duration AD trials. As this was the first trial of AR1001 in AD patients, the duration was kept to 26 weeks by FDA to first determine the drug's safety. An extension phase was later approved and implemented to provide additional safety information.

In post-hoc analyses, differences were observed between 30 mg AR1001 and placebo in change from baseline in levels of plasma AD biomarkers pTau-181, pTau-217, and GFAP. The biomarker findings are consistent with effects observed in preclinical models of AD in which treatment with AR1001 and other PDE5 inhibitors resulted in lower levels of toxic Aβ and phosphorylated Tau[18,23].

A limitation of the study was the possible heterogeneity of the population, which may have included participants with both Alzheimer's and non-Alzheimer's dementias. The use of biomarkers to determine cerebral amyloid was not required by NIA-AA criteria at the time of the trial [36]. The possible inclusion of participants with other types of dementia is supported by the lower level of participants who were ApoE4 positive (57%) compared to other AD trials that required amyloid positivity, which had ApoE4 carriers in the range of 65-70% [11–13,37].

The 30 mg AR1001 dose is currently being examined compared to placebo in a 52-week Phase 3 trial, POLARIS-AD (NCT05531526) in participants with biomarker-confirmed AD, NIA-AA Stage 3 or 4, and MMSE ≥ 20. The sample size for the Phase 3 trial is planned to be at approximately 1500 participants, and biomarker confirmation of AD is required by cerebrospinal fluid or PET scan. The primary endpoint is change from baseline after 52 weeks in Clinical Dementia Rating Scale - Sum of Boxes (CDR-SB), which has been used in other Phase 3 AD registration trials [11–13,37] and is considered more sensitive to changes in cognition in early AD than the primary endpoints used in this study [38]. The POLARIS-AD Phase 3 trial will thus provide a more definitive evaluation of AR1001 effects in early AD patients.

Conclusions

This Phase 2 study was designed to evaluate the safety and preliminary efficacy of AR1001 for the treatment of mild to moderate AD. AR1001 treatment was well-tolerated and safe. The trial did not meet its primary or secondary endpoints for efficacy after 26 weeks of treatment. There were improvements in levels of pTau-181, pTau-217, and GFAP biomarkers in the 30 mg group compared to placebo.

CRediT authorship contribution statement

David Greeley: Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Marshall Nash:** Writing – review & editing, Project administration, Investigation. **Brad Herskowitz:** Writing – review & editing, Project administration, Methodology, Investigation. **Fred Kim:** Writing – review & editing, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **James Rock:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Neils Prins:** Project administration, Methodology, Investigation. **SangYun Kim:** Writing – review & editing, Visualization, Formal analysis. **Tianyang Xi:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Formal analysis. **Jonathan A. Busam:** Writing – review & editing,

Investigation. **Benoit Tete:** Methodology, Investigation. **Jai Jun Choung:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Sharon J. Sha:** Writing – review & editing, Project administration, Methodology, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

David Greeley reports financial support was provided by Aribio Co., Ltd. Marshall Nash reports financial support was provided by Aribio Co., Ltd. Brad Herskowitz reports financial support was provided by Aribio Co., Ltd. Sharon J. Sha reports financial support was provided by Aribio Co., Ltd. Neils Prins reports financial support was provided by Aribio Co., Ltd. Jonathan A. Busam reports financial support was provided by Aribio Co., Ltd. Benoit Tete reports financial support was provided by Aribio Co., Ltd. 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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Author disclosures

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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.2025.100337.

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