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RESEARCH ARTICLE

Safety, tolerability, immunogenicity, and efficacy of ABvac40 active immunotherapy against Aβ40 in patients with mild cognitive impairment or very mild Alzheimer's disease: A randomized, double-blind, placebo-controlled phase 2 study

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Abstract

INTRODUCTION: ABvac40 is an investigational active immunotherapy (vaccine) targeting Aβ40. This study assessed the safety and immunogenicity of ABvac40 in patients with amnestic mild cognitive impairment or very mild Alzheimer's disease.

METHODS: AB1601 was a multicenter, randomized, double-blind, placebo-controlled phase 2 study. Patients (n = 124) received five monthly injections plus a 10-month booster of ABvac40 or placebo, with 18–24 months of follow-up. Primary endpoints included safety, tolerability, and immunogenicity. Secondary endpoints assessed immune response, neuropsychological changes, and disease biomarkers.

RESULTS: Treatment-emergent adverse events (TEAEs) and serious TEAEs were comparable between ABvac40 (90.6% and 26.6%) and placebo (93.3% and 26.7%). Amyloid-related imaging abnormalities-hemorrhage (ARIA-H) were similar (12.5% ABvac40; 15.0% placebo), with no ARIA-edema (ARIA-E) or meningoencephalomyelitis. ABvac40 induced a specific, sustained immune response in plasma, with detectable antibodies in CSF.

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DISCUSSION: These findings support further investigation of ABvac40 as a potential disease-modifying therapy.
Clinical Trial Registration Number: NCT03461276 (ClinicalTrials.gov)

KEYWORDS
ABvac40, active immunotherapy, Alzheimer's disease, amyloid-β 40, Aβ40, cerebral amyloid angiopathy, clinical trial, disease-modifying therapy, phase 2, randomized trial, vaccine

Highlights

- ABvac40 was safe and well-tolerated in early-stage Alzheimer's disease patients.
- No amyloid-related imaging abnormalities-edema (ARIA-E) or encephalitis observed; ARIA-hemorrhage (ARIA-H) rates were similar across groups.
- Specific, sustained immune response to ABvac40 in plasma, with cerebrospinal fluid (CSF) antibody penetration.
- Cognitive scales and magnetic resonance imaging (MRI) volumetric data favored ABvac40 over placebo.
- Results support further development of ABvac40 as a disease-modifying therapy.

1 | INTRODUCTION

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder involving diverse pathological mechanisms, including amyloid-beta (Aβ) deposition, phosphorylated tau protein aggregation, neuroinflammation, and synaptic dysfunction. Aβ has been a primary therapeutic target in recent decades,[1] with drug development efforts focusing on parenchymal aggregates of Aβ42. Recently, two anti-Aβ monoclonal antibodies—lecanemab and donanemab—have been approved in several countries. Lecanemab targets large soluble Aβ protofibrils, whereas donanemab specifically binds to insoluble, N-terminal truncated forms of Aβ found exclusively in brain amyloid plaques. These therapies have demonstrated significant efficacy in clearing amyloid plaques and a reduction in disease progression by approximately 25%–35% in early-stage AD patients.[2–4] However, these treatments have substantial limitations, including a risk of serious adverse events such as amyloid-related imaging abnormalities (ARIA) and limited clinical efficacy. Therefore, there is an urgent need for novel and safe therapies targeting alternative pathways involved in AD pathogenesis.

Compelling evidence suggests that Aβ40 also plays a critical role in AD pathogenesis.[5–8] Unlike Aβ42, which primarily aggregates in brain parenchyma, Aβ40 is predominantly associated with cerebral amyloid angiopathy (CAA), an age-related small vessel disease characterized by progressive accumulation of Aβ40 in the walls of cortical and leptomeningeal blood vessels.[9] This vascular deposition damages the vessel wall, leading to blood–brain barrier disruption, vessel occlusion or rupture, and hemorrhages, ultimately reducing cerebral blood flow and impairing cognitive function. Approximately 80% of AD patients exhibit mild to severe forms of CAA.[10] Moreover, the severity of CAA is strongly associated with AD pathology,[11] and its presence correlates with earlier dementia onset[12] and faster cognitive decline in AD patients.[13–15] Importantly, CAA contributes to AD dementia independent of senile plaques and neurofibrillary tangles,[14,15] highlighting its distinct role in disease progression. Furthermore, recent evidence also indicates that underlying CAA is closely linked to the occurrence of ARIA[16,17] which has been associated with passive anti-amyloid therapies. Aβ40-targeted therapies, which have shown efficacy in reducing Aβ40 deposition in cerebral vessels and restoring vascular reactivity in animal models of CAA,[18] could offer a promising approach for addressing CAA-related cognitive impairment in the early stages of AD.

ABvac40 is a peptide-based active immunotherapy (vaccine) targeting Aβ40. It is composed of multiple copies of a short fragment of Aβ40 (Aβ33-40 peptide, B-cell epitope) conjugated to a helper T-cell carrier protein (keyhole limpet hemocyanin [KLH]), and formulated in a Th2-biased adjuvant designed to minimize T-cell–mediated inflammatory responses. By incorporating the C-terminus of the Aβ40 peptide, ABvac40 was designed to elicit a robust B-cell response while avoiding the activation of Aβ-specific T-cells, which has previously been associated with severe adverse events such as meningoencephalitis.[19]

ABvac40 represents a novel approach with a different mechanism of action. In contrast to therapies targeting Aβ42 aggregated in the brain parenchyma, ABvac40 specifically targets Aβ40, focusing on Aβ deposited in the walls of cerebral blood vessels. In vitro studies have demonstrated that ABvac40-elicited antibodies are highly specific for Aβ40 peptides, recognizing different aggregation states.[20] In addition, ABvac40 overcomes several limitations of passive immunotherapies, such as the need for frequent infusions, regular magnetic resonance imaging (MRI) monitoring, and the high cost of administration.

A randomized, double-blind, placebo-controlled, phase 1 study (ClinicalTrials.gov NCT03113812)[20] conducted in patients with mild-to-moderate AD, showed that ABvac40 exhibited a favorable safety and tolerability profile, with no cases of meningoencephalitis or ARIA.

Furthermore, the study demonstrated a sustained and specific antibody response to Aβ40.

The present phase 2 clinical trial was designed as a confirmatory study to assess the safety, tolerability, and immunogenicity of ABvac40 in patients with amnestic mild cognitive impairment (a-MCI) and very mild AD (vm-AD). Additionally, the trial aimed to provide a deeper characterization of the immune response induced by ABvac40, including the exploration of its effects on clinical outcomes and disease biomarkers.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors conducted a literature search using PubMed and clinicaltrials.gov for clinical studies on active immunotherapies targeting amyloid-β (Aβ) for Alzheimer's disease (AD). To our knowledge, ABvac40 is the first active immunotherapy specifically targeting Aβ40, the main component of vascular deposits in cerebral amyloid angiopathy (CAA), a common neuropathological hallmark in AD that contributes to disease progression.
2. **Interpretation**: This phase 2 trial confirmed the safety and tolerability of ABvac40 in patients with amnestic mild cognitive impairment or very mild AD. The vaccine elicited a sustained and specific antibody response in plasma, with detectable antibodies in cerebrospinal fluid. Cognitive assessments and volumetric measures of brain atrophy indicated favorable trends in the ABvac40-treated group.
3. **Future directions**: These findings support further development of ABvac40 as a potential long-term disease-modifying therapy for early AD. Further studies are warranted to investigate the underlying relationship to CAA.

2 | METHODS

2.1 | Overview

This study (AB1601: EudraCT#: 2016-004352-30; ClinicalTrials.gov NCT03461276) was a multicenter, randomized, double-blind, placebo-controlled phase 2 clinical trial that enrolled patients with a-MCI or vm-AD, at 23 sites in four European countries (France, Italy, Spain, and Sweden).

The study was conducted in full conformance with standards for Good Clinical Practices and the Declaration of Helsinki. The protocol was prepared in accordance with the International Council for Harmonization (ICH) guidelines, and was approved by Institutional Review Boards/Ethics Committees (IRBs/ECs) from the sites and the health authorities from all countries. All enrolled participants and their caregivers provided written informed consent.

2.2 | Study design

The study consisted of two parts: a confirmatory phase 2 clinical trial with two parallel treatment groups (ABvac40 and placebo, 1:1, see supplementary methods in supporting Information for further details on randomization), which lasted up to 24 months (Part A), followed by an 18-month extension with cross-over treatment (Part B). Here, only the results of Part A are reported.

In Part A, participants in the ABvac40 group received six subcutaneous administrations of ABvac40 vaccine (1 mL, corresponding to 0.2 mg of immunogenic peptide). The first five doses were administered monthly, and the sixth dose, a delayed booster dose, was given at month 10, 6 months after the fifth dose. The placebo group followed the same administration schedule but received 1 mL of the ABvac40 vehicle. Rationale for ABvac40 dose selection is described in supplementary methods (Supporting Information).

2.3 | Participants

The study population consisted of a representative group of male and female patients aged 55 to 80 years with a-MCI, as defined by the National Institute on Aging and Alzheimer's Association (NIA-AA);[21] or vm-AD, as defined by the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). Patients were enrolled regardless of amyloid positron emission tomography (PET) status, which was not used as an inclusion criterion. Patients had a Mini-Mental State Examination (MMSE) score between 24 and 30 points, a Clinical Dementia Rating Scale (CDR) global score of 0.5, and a Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) score of 85 or lower. Key exclusion criteria included: presence or history of immunodeficiency, significant kidney and/or liver disease, a major uncontrolled systemic condition, history or signs of cerebrovascular disease (including vascular dementia), presence on MRI of a relevant pattern of microvascular disease or > 1 lacunar or territorial infarcts (presence of up to 3 microhemorrhages was acceptable), treatment with anticoagulants or antiaggregant therapy, or suicidal behavior or ideation. Additional inclusion and exclusion criteria are listed in the Supporting Information.

2.4 | Objectives

The primary safety objective was to evaluate the safety and tolerability of multiple doses of ABvac40 in individuals with a-MCI or vm-AD. The primary efficacy objective was to assess the immune response elicited by ABvac40 in the study population. Secondary (exploratory) efficacy objectives included characterizing the immune response triggered by

ABvac40, evaluating changes in cognition and function, and assessing changes in disease biomarkers throughout the study.

2.5 | Assessments

2.5.1 | Safety and tolerability

Safety and tolerability were assessed at regular intervals by monitoring and recording adverse events, physical and neurological examinations, laboratory assessments (hematology, immunology, toxicology, biochemistry, coagulation, serology, and urine test), electrocardiograms (ECG), vital signs (blood pressure, heart rate, respiratory rate, body temperature), and brain MRIs.

The primary safety endpoint was the rate of adverse events (AEs). AEs were coded using version 20.0 of the Medical Dictionary for Regulatory Activities (MedDRA) and classified as: treatment-emergent AEs (TEAEs), treatment-emergent serious AEs (TESAEs), and TESAEs of special interest (TESAESIs). TESAESIs were defined as ARIA, either ARIA-hemorrhage (ARIA-H), or ARIA-vasogenic edema and/or sulcal effusion (ARIA-E), and aseptic meningoencephalomyelitis.

Secondary safety variables included: withdrawal criteria, number of patients withdrawn due to AEs, and cause of withdrawal. Additionally, the frequency of clinically significant changes in physical and neurological examinations, laboratory tests, electrocardiograms, vital signs, and brain MRI was also assessed.

2.5.2 | Immune response

Immune response to ABvac40 was evaluated in blood samples collected at baseline and at 0.5, 1.5, 2.5, 3.5, 4.5, 6, 9.5, 10.5, 12, 18, and 24 months. Anti-Aβ40 antibodies in plasma were assessed by enzyme-linked immunosorbent assays (ELISAs). 96-well plates coated with the Aβ1–40 peptide were incubated with plasma samples diluted 1:10, and bound antibodies were detected using horseradish peroxidase-conjugated anti-human immunoglobulin G (IgG) secondary antibodies. Further details have been published elsewhere.[20]

The primary efficacy endpoint was the maximal increment (MΔ) in anti-Aβ40 antibody signal (optical density, [OD]) from baseline. MΔ for each participant was defined as the maximum change from baseline in anti-Aβ40 antibody signal across all post-baseline visits. Antibody specificity was confirmed by pre-adsorbing plasma samples with Aβ33-40 peptide before ELISA analysis. Participants in the ABvac40 group were classified as positive responders according to predefined criteria.[20] To further assess ABvac40 biological activity, antibody levels were quantified throughout the study in both plasma and CSF using ELISA, with plasma samples diluted 1:810 and CSF samples diluted 1:3. Quantification was performed with a monoclonal chimeric mouse (antigen-binding domains)/human (constant domains) antibody specific to Aβ40 (Araclon Biotech, Zaragoza, Spain) as an internal standard.

The frequency of B-lymphocytes secreting anti-Aβ40 antibodies was determined using an in-house Fluorescent Enzyme-Linked ImmunoSpot (FluoroSpot) assay (see further details in supplementary methods in supporting Information).

T-cell responses to ABvac40 drug substance (Aβ33-40 conjugated to KLH) were evaluated by measuring the frequency of IFN-γ and IL-4-secreting T cells, representing Th1 and Th2 cytokines, respectively, at baseline and after five immunizations using a dual IFN-γ/IL-4 FluoroSpot kit (Mabtech, Nacka, Sweden).

2.5.3 | Clinical assessments

Neuropsychological assessments were conducted at baseline and at 6, 12, 18, and 24 months. These included the MMSE and RBANS as cognitive scales; the Trail Making Test Part A (TMT-A) as an executive function scale; the Alzheimer's Disease Cooperative Study–Activities of Daily Living for use in MCI (ADCS-ADL MCI) as a functional scale; and the CDR Sum of Boxes (CDR-SB) as a global scale. Details of the neuropsychological tests are provided in the supplementary methods in the Supporting Information.

2.5.4 | Imaging

MRI scans were performed to assess safety and efficacy throughout the study. For safety evaluation, scans were acquired at baseline and at 2.5, 6, 9, 12, and 24 months, with the exception of French sites, where scans were taken at baseline and at 1.5, 3, 6, 9, 12, and 24 months. Volumetric MRI was performed at baseline and at 6, 12, and 24 months. Whole brain and hippocampal volumes were analyzed to assess longitudinal brain atrophy changes. All MRI scans were reviewed through a centralized radiology assessment. MRI scans were acquired using scanners with a magnetic field strength of 1.5T or 3.0T. Further details, including MRI sequences, are provided in the supplementary methods in the Supporting Information.

Cortical fibrillary amyloid deposition was assessed at baseline and at 12 and 24 months by ^{18}F-Flutemetamol PET scans. Details on amyloid-PET acquisition are described in the supplementary methods in the Supporting Information. At baseline, patients were stratified as amyloid-positive or -negative based on a visual read by a central reader. To monitor changes throughout the study, the standardized uptake value ratio (SUVR) was calculated for each timepoint on a global cortical region, using the pons as the reference region. SUVR values were then converted to the centiloid scale.[22]

2.5.5 | Plasma and CSF biomarkers

Levels of Aβ peptides in plasma were measured at baseline and at 0.5, 1.5, 2.5, 3.5, 4.5, 6, 9.5, 10.5, 12, 18, and 24 months, using an Aβ ELISA kit (ABtest-IA, Araclon Biotech, Zaragoza, Spain) and a mass

spectrometry-based method (ABtest-MS, Araclon Biotech, Zaragoza, Spain).

CSF samples were collected via lumbar puncture at baseline and at 12 and 24 months. CSF levels of Aβ40 and Aβ42 peptides, neurofilament light chain (NfL), and neurogranin were measured by ELISA (ABtest-IA, Araclon Biotech, Zaragoza, Spain; UmanDiagnostics, Umeå, Sweden; Euroimmun, Lübeck, Germany, respectively). Phospho-tau181 (p-tau181) and total tau (t-tau) were quantified by chemiluminescence enzyme immunoassay on the Lumipulse platform (Fujirebio Europe, Ghent, Belgium).

2.6 | Additional mechanistic studies: Immunohistochemical analyses

To further investigate the mechanism of action of ABvac40, immunohistochemical (IHC) analyses were performed. Paraffin-embedded occipital lobe brain sections from individuals diagnosed with AD and concomitant CAA, as well as from healthy controls, were obtained from the Biobank Banco de Tejidos CIEN (Madrid, Spain) and processed following standard operating procedures, with the appropriate approval of the Ethics and Scientific Committees. Briefly, after dewaxing and rehydration, sections underwent formic acid antigen retrieval and endogenous peroxidase inhibition. Slides were then incubated with post-immune plasma (diluted 1:50) or CSF (undiluted) samples, obtained after six ABvac40 doses. Next, sections were treated with a biotin-conjugated goat anti-human Fcγ-specific antibody, followed by the avidin-biotin complex. Finally, immunoreactivity was visualized using 3,3′-diaminobenzidine as the chromogen. Pre-immune CSF samples from the same individuals were used as negative controls, and post-immune CSF pre-adsorbed with Aβ33-40 peptide served as a specificity control.

2.7 | Statistical analysis

The sample size was calculated for the primary safety endpoint to ensure > 95% probability of detecting an AE occurring at a rate of at least 5% in the ABvac40-treated group. Using Hanley's simple approximation,[23] a minimum of 60 patients per group was required, that is, a total study sample size of 120 subjects. For the primary efficacy endpoint, assessing no difference in the mean maximum change from baseline in anti-Aβ40 antibody signal, a one-sided *t*-test ($\alpha = 0.025$) was used. Assuming a 40% dropout rate and a final sample of 70 patients, the study was powered at > 85% to detect a difference of 1.778 OD between the active and placebo groups, with standard deviations (SDs) of 2.0 and 1.0, respectively.[20]

All statistical analyses and tabulations were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Five analysis sets were used for analysis: (1) Safety population, which comprised all randomized patients who received any study treatment, analyzed according to the treatment received, regardless of the treatment assigned; (2) intent-to-treat (ITT) population, which comprised all randomized patients who received any study treatment, analyzed according to the treatment assigned, regardless of the treatment received; (3) modified intent-to-treat (mITT) population, which comprised all ITT patients who had a baseline and at least one post-baseline anti-Aβ40 antibody assessment; (4) per-protocol (PP) population, which comprised all ITT patients who received all doses of study medication, attended the safety visit after the sixth-dose booster and had no major protocol deviations that could affect the efficacy analyses; and (5) per-protocol cognition (PPc) population, which comprised all PP patients who had no major protocol deviations classified as "use of disallowed concomitant medication" relating to use of AD medication.

Safety endpoints were analyzed descriptively in the safety population, and results were presented as counts and percentages of patients with at least one AE within each system organ class and preferred term, as applicable.

The primary efficacy endpoint, defined as the MΔ in anti-Aβ40 antibody signal, was analyzed in the mITT population. As the assumption of normality of the distribution of MΔ in anti-Aβ40 antibody signal appeared to be violated, a Mann–Whitney *U* test was used to compare the treatment groups. The trial was considered confirmatory for efficacy if the average MΔ in the ABvac40 group was significantly greater than in the placebo group. Sensitivity analyses of the primary outcome are described in supplementary methods in the Supporting Information.

Secondary (exploratory) efficacy endpoints related to the characterization of the immune response were summarized by treatment and visit in the ITT population. Other secondary (exploratory) endpoints, including neuropsychological tests and biomarkers, were analyzed using Mixed-Model Repeated Measures (MMRM) in the PPc and PP population, respectively, to assess the potential efficacy of ABvac40 under optimal adherence conditions and to explore hypotheses that may inform future research. MMRM included change from baseline in the efficacy parameter as the dependent variable; treatment, protocol-specified visits, treatment-by-visit interaction, and amyloid positivity as the fixed effects; baseline efficacy parameter and baseline age as covariates; and measures within-patient at each visit as a repeated measure. The following factors were also included in the model: apolipoprotein E (*APOE*) ε4 carrier status, baseline use of AD symptomatic medication, and clinical subgroup, if found to be significantly associated with the response measure ($p < 0.15$). No imputation of missing data was performed. The MMRM approach implicitly handles missing data via the model, and data are assumed missing at random. No adjustments were made for multiple testing of study parameters (interpretation of the secondary analyses results should be considered descriptive in nature).

3 | RESULTS

3.1 | Treatment disposition and participant characteristics

A total of 238 patients were screened, of whom 134 were randomized to ABvac40 ($N = 66$) or placebo ($N = 68$) (Figure 1). Of these, 62 patients in each group received at least one dose of study treatment.

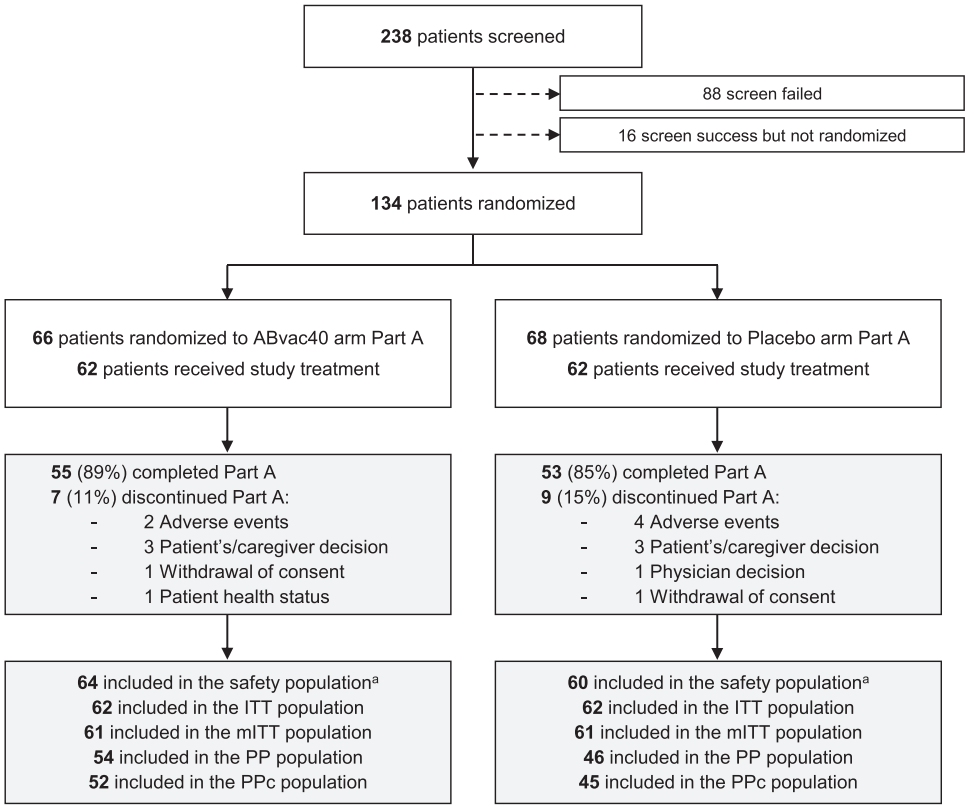

FIGURE 1 Patient disposition. [a]Two patients were randomized to placebo but inadvertently received one dose of ABvac40; therefore, these two patients were summarized for the safety population in the ABvac40 arm. ITT, intent-to-treat; mITT, modified intent-to-treat; PP, per-protocol; PPc, per-protocol cognition.

The percentage of patients who completed Part A of the study was 89% in the ABvac40 arm (55 out of 62) and 85% in the placebo arm (53 out of 62). Reasons for discontinuation were similar in both arms and were mostly due to AEs and patient/caregiver decisions. Details are shown in Figure 1. Part A of the AB1601 study was conducted between December 1, 2017, and July 1, 2021. It is important to note that the introduction of the crossover extension (Part B) shortened Part A from 24 to 18 months, leading some patients to transition directly to the crossover phase upon completing 18 months of follow-up in Part A. This modification led to a lower number of patients remaining in Part A at the 24-month time point.

Baseline demographic characteristics are summarized in Table 1. The mean (SD) age of all participants was 70.4 (5.7) years, 59.7% were female, and 95.2% were Caucasian. Baseline disease characteristics were comparable between the placebo and active treatment arms. The overall mean (SD) MMSE score at baseline was 25.8 (1.8). Additionally, 61.3% of patients were *APOE* ε4 carriers, and 64.5% were diagnosed with a-MCI. Amyloid-PET positivity, assessed by visual read, was observed in 74.2% of participants.

3.2 | Safety

Table 2 summarizes the incidence of TEAEs. Two patients were randomized to a placebo but inadvertently received one dose of ABvac40; therefore, these two patients were summarized for the Safety population in the ABvac40 arm. Most patients in the ABvac40 (90.6%) and placebo (93.3%) groups presented at least one TEAE, but only about half of them had treatment-related TEAEs (45.3% in the ABvac40 group and 43.3% in the placebo group). Common TEAEs in both groups were urinary tract infections, administration site and skin reactions, fall, and headache (Table S1 in Supporting Information). Treatment discontinuation due to TEAEs occurred in 6.3% of ABvac40 patients and 11.7% of placebo patients.

TESAEs were observed at similar rates in both arms (26.6% in ABvac40 and 26.7% in placebo), although treatment-related TESAEs were less frequent in the ABvac40 group (4.7%) compared to the placebo group (13.3%). There were two TESAEs leading to death, one in the ABvac40 group (general physical health deterioration) and one in the placebo group (pancreatic neoplasm), both deemed unrelated to treatment. TESAEs classified by system organ class, MedDRA Version 20.0, are shown in Table S2 in the Supporting Information. Three cases of pulmonary thromboembolism were reported in the ABvac40 group, all classified under respiratory, thoracic, and mediastinal disorders. All three cases presented with hypertension, and two were associated with low mobility. According to the investigator's assessment, none of the events were considered related to the treatment.

No events of ARIA-E and aseptic meningoencephalomyelitis were reported. Thus, ARIA-H was the only TESAESI reported, with similar incidences in the ABvac40 (12.5%) and placebo (15.0%)

TABLE 1 Baseline demographic and clinical characteristics of participants: ITT population.

Characteristic	ABvac40 (*N* = 62)	Placebo (*N* = 62)	Overall (*N* = 124)
Age (years)	70.6 (6.0)	70.1 (5.5)	70.4 (5.7)
Female sex, *n* (%)	38 (61.3)	36 (58.1)	74 (59.7)
Race/ethnicity, *n* (%)			
Caucasian	58 (93.5)	60 (96.8)	118 (95.2)
Other	1 (1.6)	1 (1.6)	2 (1.6)
Missing	3 (4.8)	1 (1.6)	4 (3.2)
Highest level of education, *n* (%)			
University degree	18 (29.0)	17 (27.4)	35 (28.2)
College graduate	4 (6.5)	7 (11.3)	11 (8.9)
High school graduate	15 (24.2)	22 (35.5)	37 (29.8)
Some school	25 (40.3)	16 (25.8)	41 (33.1)
BMI (kg/m^2)	25.8 (4.3)	25.8 (4.5)	25.8 (4.4)
MMSE score	25.7 (1.6)	25.9 (2.0)	25.8 (1.8)
Time from diagnosis (months)	14.7 (13.7)	14.5 (15.8)	14.6 (14.7)
Study disease, *n* (%)			
a-MCI	38 (61.3)	42 (67.7)	80 (64.5)
vm-AD	24 (38.7)	20 (32.3)	44 (33.5)
APOE ε4 status, *n* (%)			
Non-carriers	24 (38.7)	24 (38.7)	48 (38.7)
Carriers: Heterozygous	29 (46.8)	33 (53.2)	62 (50.0)
Carriers: Homozygous	9 (14.5)	5 (8.1)	14 (11.3)
Amyloid-PET status[a], *n* (%)			
Positive	47 (75.8)	45 (72.6)	92 (74.2)
Negative	15 (24.2)	17 (27.4)	32 (25.8)
Patients on anti-dementia drugs, *n* (%)	35 (56.5)	36 (58.1)	71 (57.3)

Note: Data are expressed as mean (standard deviation), except for categorical variables, which are presented as counts (%).
Abbreviations: a-MCI, amnestic mild cognitive impairment; APOE, apolipoprotein E; BMI, body mass index; ITT, intent-to-treat; MMSE, Mini-Mental State Examination; *N/n*, number of participants; PET, positron emission tomography; vm-AD, very mild Alzheimer's disease.
[a]Amyloid-PET status was assessed by visual read.

arms. The distribution of ARIA-H by *APOE* genotype showed no association with the ε4 allele in either treatment group. Two events led to treatment discontinuation, both in the placebo group.

The laboratory analyses, vital signs, and physical and neurological examination rarely revealed clinically significant abnormalities across groups (data not shown).

3.3 | Immunogenicity

The median MΔ of anti-Aβ40 antibody signal in the ABvac40 group (3.5390 OD; interquartile range [IQR] 3.2950–3.6595) was significantly higher than the average MΔ observed in the placebo group (0.0523 OD; IQR: 0.0128–0.1525) (primary efficacy endpoint; $p < 0.0001$; Figure 2A). In addition, all sensitivity analyses yielded confirmatory results, confirming the robustness of the primary efficacy endpoint (Table S3 in Supporting Information).

Increasing concentrations of anti-Aβ40 plasma antibodies from baseline (0.00 µg/mL) were observed during the ABvac40 administration schedule, reaching maximum mean values at month 4.5 (66.93 µg/mL; 95% confidence interval [CI] 47.06, 86.81), after the fifth dose. The concentration decreased at month 6 and month 9.5, but following booster administration at month 10, a marked concentration increase was observed (54.99 µg/mL; 95% CI 40.59, 69.38). In the subsequent visits, the concentration steadily decreased, being 4.87 µg/mL (95% CI 1.85, 7.89) at month 24. No increased concentrations were observed in patients receiving placebo. Details are shown in Figure 2C. Consistent with these findings, more than 85% of patients in the ABvac40 group were positive responders from month 1.5 to month 12, reaching a positive response rate of over 95% between months 2.5 and 12 (Table S4 in Supporting Information).

In CSF, anti-Aβ40 antibodies were detected in the ABvac40 group with a CSF-to-plasma ratio of 0.1%. The median concentration increased from 0.00 ng/mL at baseline to 21.90 ng/mL (IQR 8.25, 38.68) at month 12, and decreased thereafter to undetectable levels

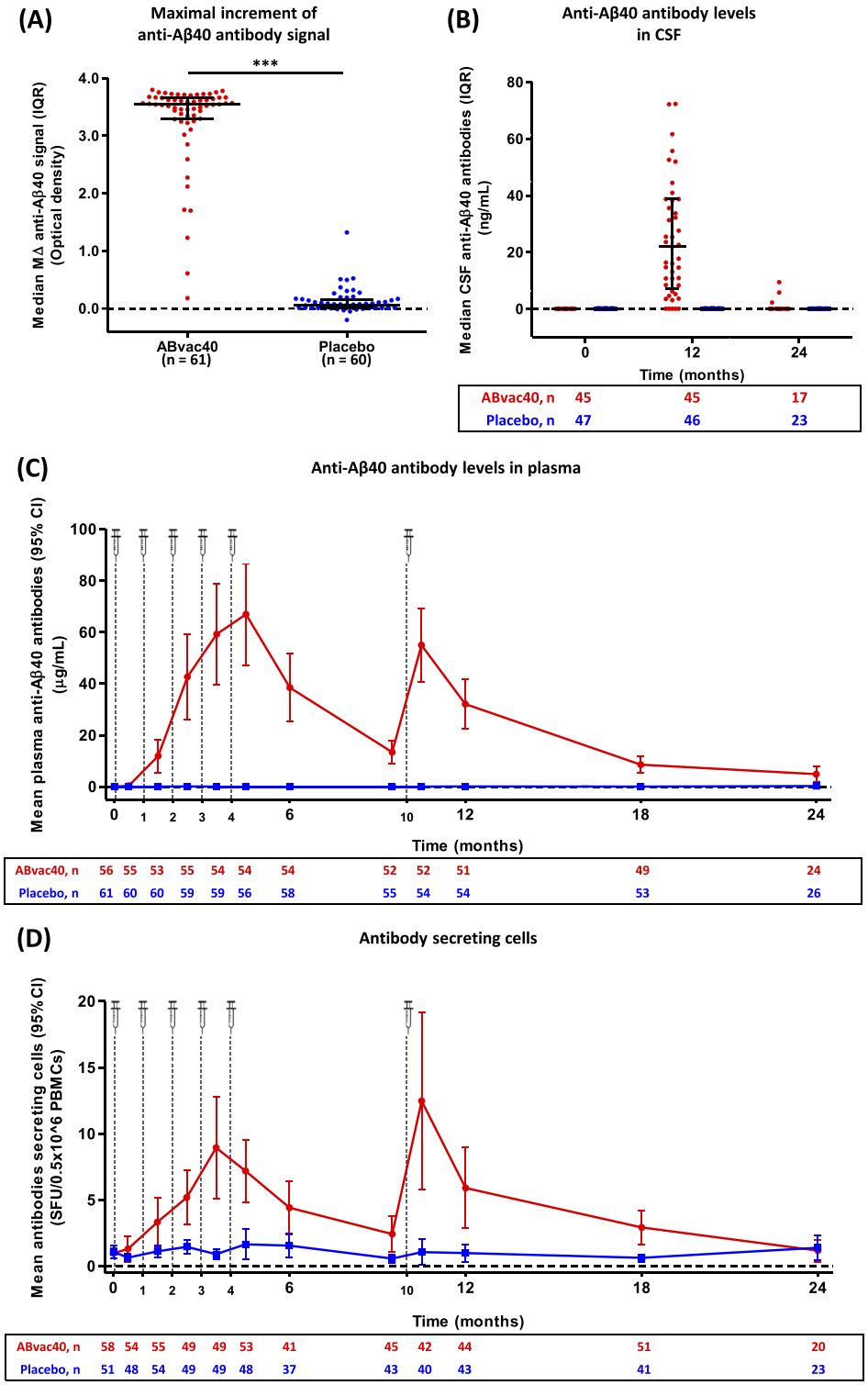

FIGURE 2 Immunogenicity of ABvac40. (A) Average MΔ of anti-Aβ40 antibody signal in plasma (optical density in ELISA) from baseline (mITT population). MΔ for each participant was defined as the maximum change from baseline in anti-Aβ40 antibody signal across all post-baseline visits. The line represents the median (horizontal line), and the error bars indicate the IQR. Individual values are also shown. Group differences were assessed using the Mann–Whitney *U* test. ***$p < 0.001$. (B) (C) Anti-Aβ40 antibody concentrations in CSF (B) and plasma (C) for ABvac40 and placebo groups (ITT population). Plasma values are presented as mean ± 95% CI, while CSF values are shown as median and IQR. In the CSF graph, two outliers from the ABvac40 group at 12 months (219.28 ng/mL and 119.10 ng/mL) are excluded for visualization but included in median and IQR calculations. (D) Frequency of memory B cells in blood (ITT population). Values are presented as mean ± 95% CI. Aβ, amyloid-beta; CI, confidence interval; CSF, cerebrospinal fluid; IQR, interquartile range; ITT, intent-to-treat; MΔ, maximal increment; mITT, modified intent-to-treat; *n*, number of participants; PBMC, peripheral blood mononuclear cells; SFU, spot-forming units. Syringe symbol: time points of product administration.

TABLE 2 Summary of TEAEs: Safety population.

TEAEs, *n* (%)	ABvac40 (*N* = 64)	Placebo (*N* = 60)
Any TEAE	58 (90.6)	56 (93.3)
Any treatment-related TEAE	29 (45.3)	26 (43.3)
Any TEAEs leading to treatment discontinuation	4 (6.3)	7 (11.7)
Any TEAE leading to death[a]	1 (1.6)	1 (1.7)
Any TESAE	17 (26.6)	16 (26.7)
Any treatment-related TESAE	3 (4.7)	8 (13.3)
Any TESAE leading to treatment discontinuation	2 (3.1)	4 (6.7)
Any TESAE leading to death[a]	1 (1.6)	1 (1.7)
Any TESAESI	8 (12.5)	9 (15.0)
Aseptic meningoencephalomyelitis	0 (0.0)	0 (0.0)
ARIA-E	0 (0.0)	0 (0.0)
ARIA-H	8 (12.5)	9 (15.0)
ARIA-H leading to treatment discontinuation	0 (0.0)	2 (3.3)
ARIA-H by *APOE* ε4 carrier status		
Non-carrier	4/25 (16.0)	4/23 (17.4)
Heterozygous carrier	3/30 (10.0)	5/32 (15.6)
Homozygous carrier	1/9 (11.1)	0/5 (0.0)

Note: Two patients were randomized to placebo but inadvertently received one dose of ABvac40; therefore, these two patients were summarized for the safety population in the ABvac40 arm.

Abbreviations: *APOE*, apolipoprotein E; ARIA, amyloid-related imaging abnormalities; ARIA-E, ARIA-edema; ARIA-H, ARIA-hemorrhage; *N/n*, number of participants; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event; TESAESI, treatment-emergent serious adverse event of special interest.

[a]TEAE/TESAE leading to death: General physical health deterioration (ABvac40), not related to treatment; Pancreatic neoplasm (placebo), not related to treatment.

at month 24 (Figure 2B). Anti-Aβ40 antibody levels significantly correlated between plasma and CSF (Spearman rho: 0.749; $p < 0.0001$; Figure S1 in supporting Information).

The frequency of anti-Aβ40-specific antibody-secreting memory B cells mirrored the trajectory of anti-Aβ40 antibody levels in the ABvac40 arm. Mean levels initially increased from baseline to month 3.5 (8.94 spot forming units [SFU]/0.5 × 10^6 peripheral blood mononuclear cells [PBMCs] [95% CI 5.10, 12.78]), peaked at month 10.5 following ABvac40-booster administration (12.47 SFU/0.5 × 10^6 PBMCs [95% CI 5.76, 19.18]), and subsequently declined steadily to baseline levels by month 24 (Figure 2D).

ABvac40 drug substance (Aβ33-40 conjugated to KLH carrier protein) elicited a predominant Th2 immune response in 86.5% (32/37) of the studied participants, while 8.1% (3/37) did not exhibit a polarized immune response, and 5.4% (2/37) showed a predominant Th1 response. In contrast, no response to ABvac40 drug substance was observed in the placebo group (Figure S2 in Supporting Information).

3.4 | Clinical assessment

The ABvac40 group demonstrated a maximum reduction in disease progression of 39% relative to placebo, as assessed by MMSE scores. Differences between groups became apparent at month 12 (least squares [LS]-mean change difference: 1.54 points, 95% CI 0.26, 2.82; $p < 0.05$; Figure 3A), with numerical improvements observed from month 6 onward. RBANS total scores also favored the ABvac40 group starting at month 12, with the largest difference at month 18 (Figure 3B). Performance in the TMT-A indicated improvements in processing speed and executive function in the ABvac40 group, with the greatest group difference observed at month 24 (LS-mean change difference: −12.51 s, 95% CI −24.72, −0.30; $p < 0.05$; Figure 3C). No differences were found between groups in the CDR-SB or ADCS-ADL MCI scores throughout the study period (Figures 3D and 3E).

3.5 | Brain imaging

Volumetric MRI showed lesser progression in whole brain atrophy at months 12 and 24 in the ABvac40 group versus placebo (LS-mean change difference at month 24: 1.51%, 95% CI 0.19, 2.82; $p < 0.05$; see Figure 4A). Hippocampal volumes decreased 6%–8% at 24 months, without observing differences between treatment arms (Figures 4B and 4C). Regarding amyloid-PET, rates of amyloid deposition were minimal and similar between groups, with LS-mean changes at month 24

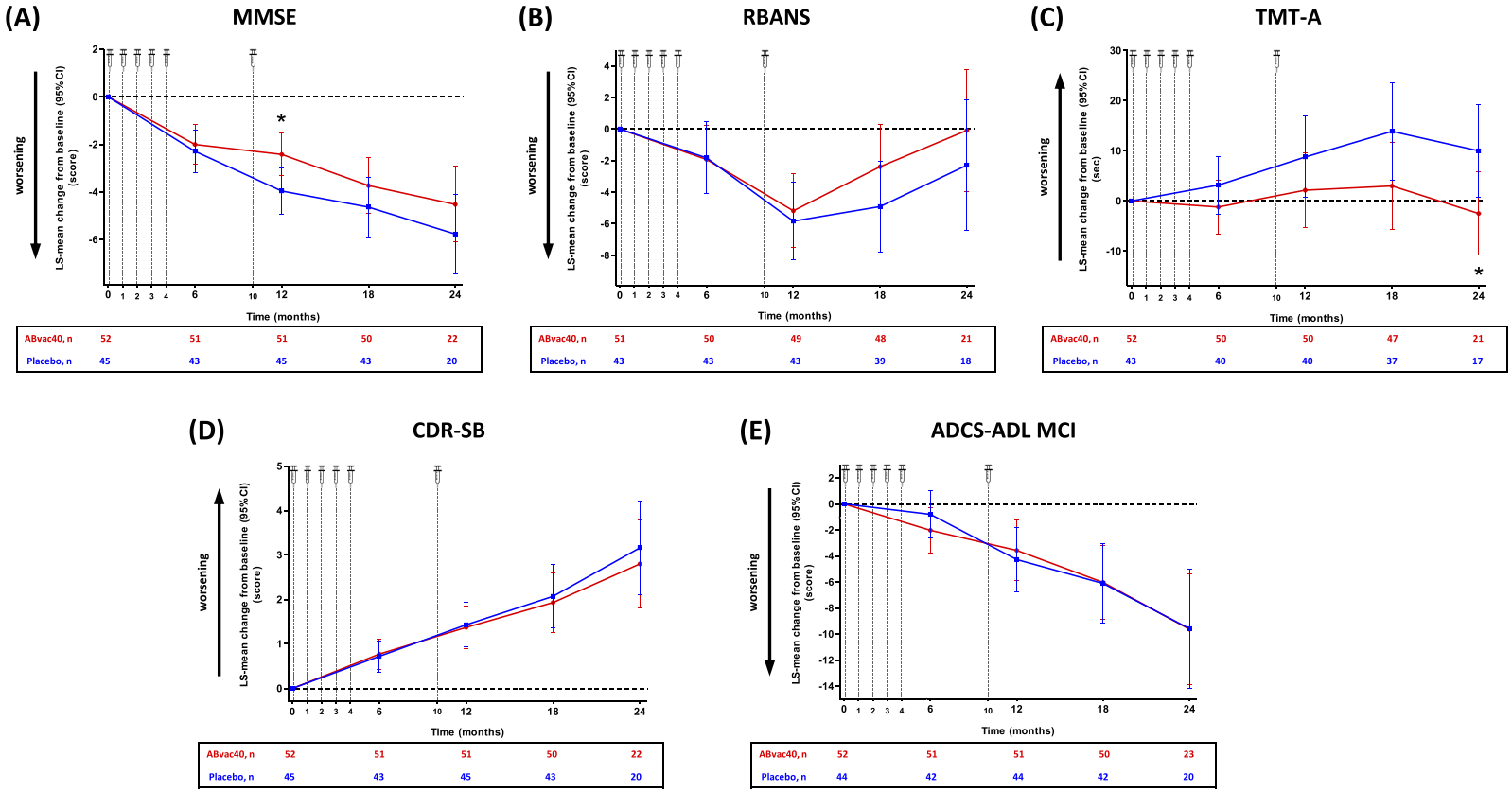

FIGURE 3 Performance in neuropsychological tests (PPc population). (A) MMSE; (B) RBANS; (C) TMT-A; (D) CDR-SB; (E) ADCS-ADL MCI. Plots show the LS-mean change from baseline and 95% CI based on an MMRM analysis. *$p < 0.05$. ADCS-ADL MCI, Alzheimer's Disease Cooperative Study–Activities of Daily Living, Mild Cognitive Impairment; CDR-SB, Clinical Dementia Rating Sum of Boxes; CI, confidence interval; LS, least squares; MMRM, mixed model for repeated measures; MMSE, Mini-Mental State Examination; *n*, number of participants; PPc, per-protocol cognition; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; TMT-A, Trail Making Test A. Syringe symbol: time points of product administration.

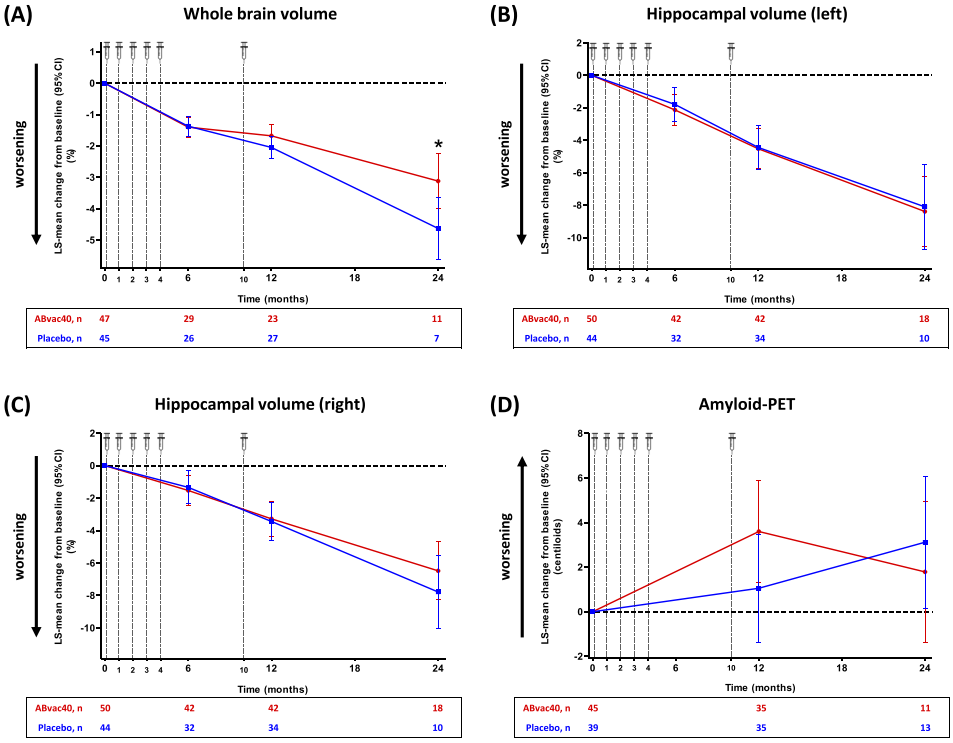

FIGURE 4 Brain imaging assessments (PP population). Volumetric MRI measures of whole brain (A) and hippocampal atrophy (B) (left hippocampus) and (C) (right hippocampus). (D) Rate of amyloid deposition as measured by amyloid-PET. Plots show the LS-mean change from baseline and 95% CI based on an MMRM analysis. $^{*}p < 0.05$. CI, confidence interval; LS, least squares; MMRM, mixed model for repeated measures; MRI, magnetic resonance imaging; *n*, number of participants; PET, positron emission tomography; PP, per-protocol. Syringe symbol: time points of product administration.

of 1.78 centiloids (standard error [SE] 1.58) for ABvac40 and 3.12 Centiloids (SE 1.48) for placebo (Figure 4D).

3.6 | Biomarkers

Total plasma levels of Aβ40 peptide, as quantified by a mass spectrometry-based assay, paralleled the increase observed in anti-Aβ40 antibodies, also observing a marked booster effect at month 10.5 (Figure 5A). In contrast, free levels of Aβ40, as measured by immunoassay, decreased as antibody levels increased (Figure 5B). The levels of plasma Aβ42 remained stable in both arms throughout the study (Figure S3 in Supporting Information).

In CSF, no differences were observed between ABvac40 and placebo groups for the levels of tested biomarkers (Aβ40, Aβ42, Aβ42/Aβ40, p-tau181, t-tau, NfL, and neurogranin) across the study. Details are shown in Figure S4 in the Supporting Information.

3.7 | Labeling of vascular amyloid by ABvac40-induced antibodies

Post-immune plasma and CSF samples from ABvac40-treated participants showed strong immunoreactivity against vascular amyloid deposits in brain sections from individuals with AD and concomitant CAA (Figure 6). Labeling was primarily observed in leptomeningeal, penetrating, and cortical arteries (Figure 6A and 6D), as well as arterioles (Figure 6B and 6D) and capillaries (Figure 6C and 6E). While occasional Aβ40-containing neuritic plaques were detected in the parenchyma, most of the antibody binding was restricted to vascular structures. In contrast, neither pre-immune samples nor post-immune samples pre-adsorbed with Aβ33-40 peptide showed any detectable labeling (Figure S5 in Supporting Information), confirming the specificity of ABvac40-elicited antibodies for Aβ40 peptides. Additionally, brain sections from healthy control cases showed no signal (data not shown).

4 | DISCUSSION

In this randomized, double-blind, placebo-controlled phase 2 clinical trial, ABvac40 active immunotherapy demonstrated a favorable safety and tolerability profile with no unexpected safety concerns. Common TEAEs included urinary tract infections, falls, and headaches, which are typical in this elderly population, as well as administration site and skin reactions, which are commonly reported with vaccines and injectable drugs. SAEs occurred at similar rates in both groups, and those SAEs leading to death were deemed unrelated to the treatment.

Importantly, no cases of ARIA-E were reported in either group throughout the study, and the incidence of ARIA-H was similarly dis-

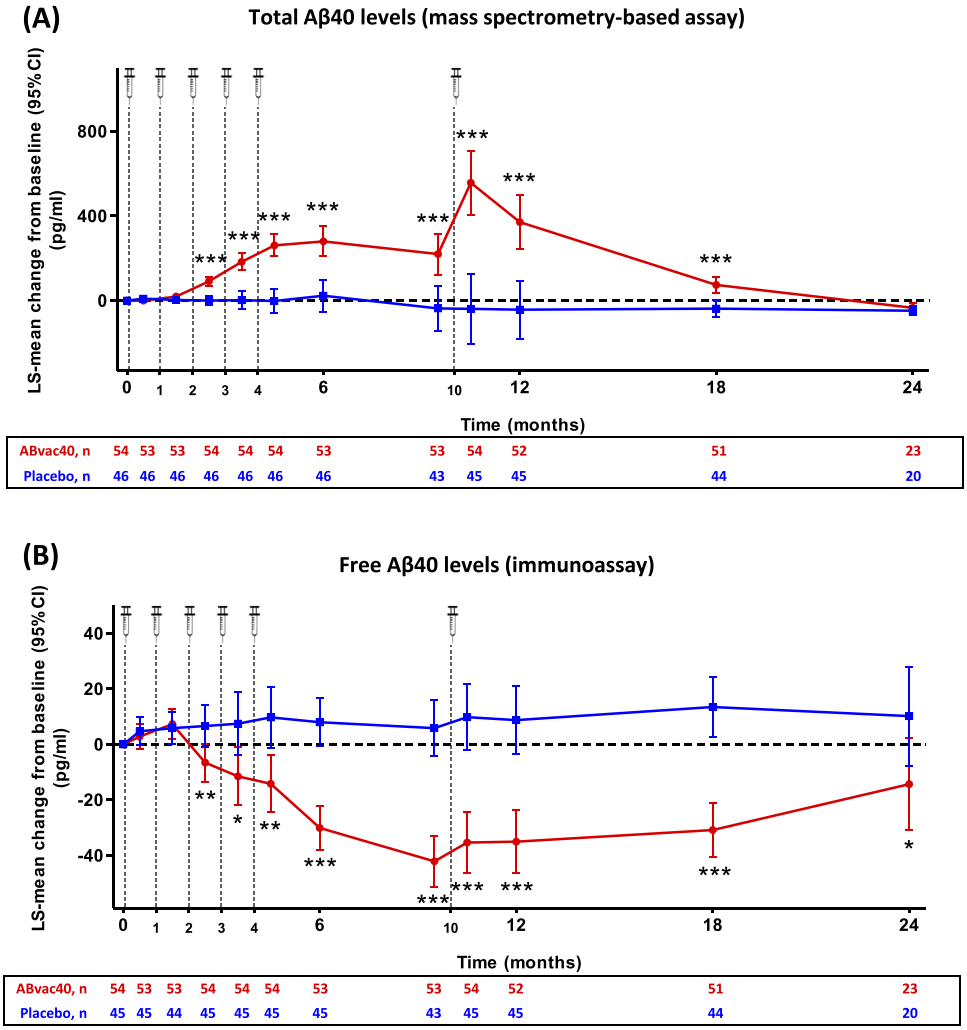

FIGURE 5 Plasma levels of Aβ40 peptide measured by (A) mass spectrometry-based assay (total levels) or (B) immunoassay (free levels) (PP population). Plots shows LS-mean change from baseline and 95% CI based on a MMRM analysis. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Aβ, amyloid-beta; CI, confidence interval; LS, least squares; MMRM, mixed model for repeated measures; *n*, number of participants; PP, per-protocol. Syringe symbol: time points of product administration.

tributed between ABvac40 (12.5%) and placebo (15.0%). This contrasts with the elevated ARIA rates reported in trials of passive immunotherapies for early AD.[3,24–27] The mechanisms underlying ARIA are not fully understood, but evidence suggests that antibody-mediated breakdown of neuritic plaques mobilizes amyloid from the brain parenchyma to the vasculature, exacerbating pre-existing CAA, and increasing perivascular inflammation and/or impaired perivascular clearance.[28,29] These changes compromise vascular integrity, causing extravasation and leakage of blood components through damaged vessel walls.[9,16] A key factor that may explain the lower ARIA incidence observed with ABvac40 is its specific targeting of Aβ40 species, which represents a fundamental difference in mechanism, by focusing on vascular amyloid rather than mobilizing parenchymal amyloid. Additionally, the gradual and sustained polyclonal immune response induced by ABvac40, as opposed to the rapid and high peak seen with intravenous monoclonal antibodies, may also contribute to its safety profile. It is important to note, however, that exclusion of participants with signs of cerebrovascular disease likely reduced CAA burden, a known risk factor for ARIA, potentially influencing this outcome. Nevertheless, other trials with passive immunotherapies using similar exclusion criteria have reported higher ARIA rates,[3,4] suggesting that additional factors may contribute to the safety profile of ABvac40.

Furthermore, no cases of aseptic meningoencephalomyelitis were observed, possibly due to the absence of Aβ-specific T-cell-mediated responses. Moreover, a predominant Th2 immune response was detected in PBMCs stimulated in vitro. These findings are consistent with the design of ABvac40, which lacks T-cell–activating epitopes and is formulated with a Th2-biased adjuvant.

Taken together, these safety findings are consistent with the results from the phase 1 trial[20] and support the favorable safety profile of ABvac40.

A key finding in this study was successful confirmation of the immunogenicity of ABvac40. The primary hypothesis, that ABvac40 would increase the specific anti-Aβ40 antibody signal, was met, and all

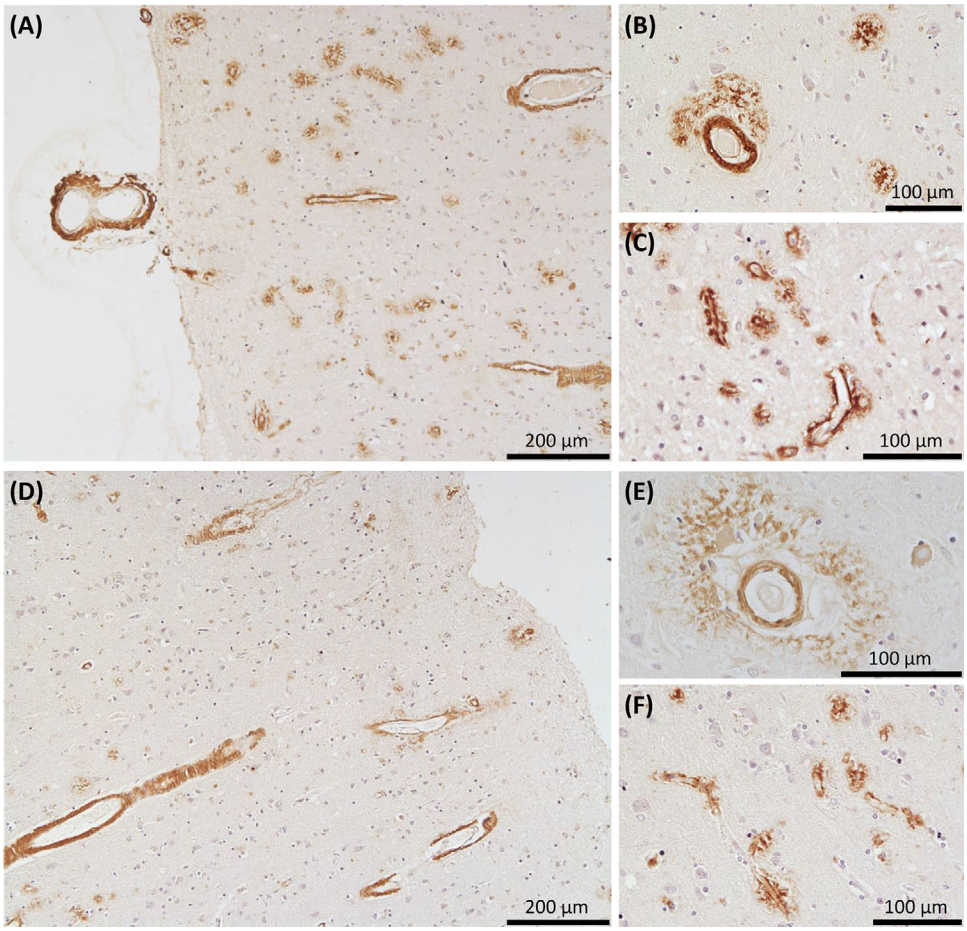

FIGURE 6 Reactivity of ABvac40-induced antibodies on paraffin-embedded occipital brain sections from patients with AD and concomitant CAA. IHC was performed using plasma (A–C) and CSF (D–F) samples from ABvac40-treated patients as primary antibodies. The images show specific labeling of vascular amyloid deposits in the walls of blood vessels throughout the brain, including leptomeningeal and penetrating arteries (A, D), arterioles (some of which also exhibit amyloid aggregates in the surrounding neuropil) (B, E), and capillaries (C, F). AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; IHC, immunohistochemistry.

sensitivity analyses also yielded confirmatory results. Compared to the phase 1 study,[20] the immune response was further enhanced, likely due to the inclusion of five administrations with a booster dose. Notably, phase 1 data have already shown an enhanced response when increasing from two to three doses. Future treatment strategies should further optimize the administration schedule to sustain high antibody levels over time, potentially through periodic booster doses.

To assess the immune response more precisely, antibody concentrations were quantified using a chimeric anti-Aβ40 antibody as an internal standard, rather than serial dilution titers.[19,30–32] This approach provides a more precise and consistent quantification of antibody levels, minimizing variability and enabling direct comparisons across multiple samples and time points, ultimately leading to a more accurate assessment of the immune response. Results confirmed that ABvac40 induced a strong, specific, boostable, and sustained immune response, highlighting its potential as a cost-effective, long-term therapeutic strategy for the primary and secondary prevention of AD. Notably, evidence of peripheral target engagement was observed, as indicated by increased total plasma levels of Aβ40 and decreased levels of free Aβ40. Anti-Aβ40 antibodies were also detected in CSF, with a CSF-to-plasma ratio of 0.1%, comparable to other immunotherapies.[33–36]

Neuropsychological assessments showed a modest but consistent trend favoring ABvac40 across multiple cognitive outcomes. Specifically, a between-group difference was observed in MMSE at 12 months. While the relatively steep decline in MMSE scores seen in the placebo group may seem unusual, it can be attributed to both limited sample size and heterogeneity in disease progression.[37–39] Parallel trends observed in RBANS and TMT-A further support the consistency of cognitive effects across different domains[40,41]. Although no differences were found in CDR-SB or ADCS-ADL, these instruments may be less sensitive to subtle cognitive/functional changes over short periods or in very early stages of disease.[42]

Importantly, these clinical findings are in line with imaging data showing slower progression of whole-brain atrophy in the ABvac40 group. This contrasts with reports from passive immunotherapies targeting aggregated parenchymal amyloid, where accelerated brain volume loss was observed.[43] Although it remains unclear whether

this is due to accelerated neuronal damage or pseudo-atrophy,[44] such changes are characteristic of immunotherapies that reduce amyloid plaques. However, ABvac40, by targeting vascular amyloid, was associated with a reduction in atrophy progression, suggesting potential neuroprotective effects. Given the exploratory nature of these outcomes and the limited sample size, the results should be interpreted with caution. Nonetheless, the alignment across cognitive and imaging outcomes provides biological plausibility to the modest cognitive findings[45] and supports the rationale for further investigation in adequately powered trials.

Consistent with its proposed mechanism of action, ABvac40 treatment did not impact fibrillary cortical amyloid deposition as measured by amyloid-PET. Current amyloid-PET radiotracers selectively bind to fibrillary Aβ, primarily reflecting parenchymal plaques, with minimal contribution from CAA to the amyloid-PET signal.[46] However, IHC analyses using plasma and CSF from ABvac40-treated participants demonstrated strong labeling of vascular amyloid deposits in brain tissue from AD patients with CAA, consistent with the intended mechanism of targeting Aβ40. While these ex vivo findings provide clear evidence of antibody binding to cerebrovascular amyloid, the absence of validated in vivo biomarkers for CAA in this study prevented direct assessment of ABvac40's impact on vascular amyloid burden.

No differences were observed in CSF biomarkers between groups. This could be explained by several factors, including a considerable degree of variability in the change from baseline observed among patients, which could reflect differences in disease progression. Additionally, the time points selected for assessment might not have been optimal for detecting meaningful treatment effects, or conventional AD CSF biomarkers may not adequately capture vascular-related effects of the treatment.

Limitations of this study include the relatively small sample size, which challenges the reliability of the clinical and biomarker efficacy endpoints. As a result, robust conclusions on efficacy cannot be drawn from these data, and larger clinical trials are needed to confirm these findings in a broader population. However, despite the sample size, the consistency of the results supports the validity of the findings. Additionally, the lack of demographic diversity in the study population, as most participants were Caucasian, may reduce the generalizability of the results to other ethnic groups. Another limitation is the absence of specific CAA-related outcomes, which might have provided more insights into the vascular mechanism of ABvac40.

While this study was primarily designed to evaluate safety and immunogenicity, it excluded individuals with clinical or radiological signs of cerebrovascular disease, likely resulting in a cohort with relatively limited CAA pathology. Given the high prevalence of CAA in AD, greater clinical benefits might be expected in broader AD populations. Notably, future trials including AD patients enriched for vascular amyloid pathology could better capture the full therapeutic potential of this approach. This potential is particularly relevant considering the multifactorial nature of AD, where therapies addressing different pathological components may provide complementary benefits. In this context, ABvac40's favorable safety profile, durable immune response, and low treatment burden, position it as a promising candidate not only for early intervention and prevention but also as part of combination strategies.

To date, a limited number of therapeutic strategies targeting vascular amyloid have been explored. Ponezumab, an anti-Aβ40 monoclonal antibody with Fc mutations to reduce immune effector functions, aimed to sequester Aβ40 in the bloodstream and clear brain deposits via a peripheral sink effect.[47] However, it was discontinued due to lack of efficacy,[47,48] which may be related to its limited ability to trigger Fc-mediated responses. In contrast, milvesiran, an RNA interference therapy targeting amyloid precursor protein, is currently in clinical trials for both AD[49] and CAA,[50] representing a promising alternative. Despite these efforts, no therapeutic approach has yet proven effective in preventing or reversing vascular amyloid deposition in AD and/or CAA, highlighting the urgent need for novel approaches in this field.

In conclusion, this phase 2 study met both primary objectives. ABvac40 showed a favorable safety and tolerability profile, with no increase in ARIA-H, no cases of ARIA-E, and no signs of inflammatory reactions. ABvac40 induced a robust immune response, supporting its potential as a cost-effective, long-term treatment for AD. Although not powered for efficacy analysis, positive findings in cognitive performance and brain atrophy suggest that ABvac40 could offer a potential benefit for AD and/or CAA, being a novel approach that leverages the advantages of active immunotherapy with a differential mechanism of action, primarily targeting vascular amyloid. Further large-scale studies are needed to confirm these findings and elucidate their underlying biological processes.

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CONFLICT OF INTEREST STATEMENT

M.P.L., A.M.L., M.M., J.C., J.L., I.M., J.A.A., L.S., N.F., and J.R. are full-time employees of Araclon Biotech-Grifols. MS was a full-time employee of Araclon Biotech–Grifols, held several patents related to Alzheimer's disease diagnosis and treatment, and was the founder and a shareholder of Araclon Biotech–Grifols. M.T., D.W., and J.T. are full-time employees of Grifols. G.P.R. has received consultancy fees, honoraria for lectures, and/or participated in advisory boards from the following companies: Grifols, Araclon Biotech, Lilly, Almirall, Nutricia, Schwabe Pharma, and Esteve. M.B. has received consultancy fees, honoraria for lectures, and/or participated in advisory boards from the following companies: Grifols, Araclon Biotech, Roche, Biogen, Lilly, Merck, Novo Nordisk, Bioiberica, Eisai, Servier, Schwabe Pharma, Nutricia, and Terumo. M.B. has also obtained research funding from Life Molecular Imaging, Bioiberica, Grifols, Araclon Biotech, Lilly, Roche, Janssen, Alzehon, Cortyzime, Novo Nordisk, and Schwabe Pharma. Author disclosures are available in the supporting Information.

DATA AVAILABILITY STATEMENT

Data and supporting documents, including the study protocol and statistical analysis plan, are available from the corresponding author upon reasonable request.

CONSENT STATEMENT

All participants provided written informed consent prior to any study-related procedures.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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