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RESEARCH ARTICLE

Effects of the active amyloid beta immunotherapy CAD106 on PET measurements of amyloid plaque deposition in cognitively unimpaired *APOE ε4* homozygotes

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Abstract

INTRODUCTION: Alzheimer's Prevention Initiative Generation Study 1 evaluated amyloid beta (Aβ) active immunotherapy (vaccine) CAD106 and BACE-1 inhibitor umibecestat in cognitively unimpaired 60- to 75-year-old participants at genetic risk for Alzheimer's disease (AD). The study was reduced in size and terminated early. Results from the CAD106 cohort are presented.

METHODS: Sixty-five apolipoprotein E ε4 homozygotes with/without amyloid deposition received intramuscular CAD106 450 μg ($n = 42$) or placebo ($n = 23$) at baseline; Weeks 1, 7, 13; and quarterly; 51 of them had follow-up Aβ positron emission tomography (PET) scans at 18 to 24 months.

RESULTS: CAD106 induced measurable serum Aβ immunoglobulin G titers in 41/42 participants, slower rates of Aβ plaque accumulation (mean [standard deviation] annualized change from baseline in amyloid PET Centiloid: −0.91[5.65] for CAD106 versus 8.36 [6.68] for placebo; $P < 0.001$), and three amyloid-related imaging abnormality cases (one symptomatic).

DISCUSSION: Despite early termination, these findings support the potential value of conducting larger prevention trials of Aβ active immunotherapies in individuals at risk for AD.

KEYWORDS
active immunotherapy, Alzheimer's disease, amyloid, apolipoprotein E genotype, biomarkers, CAD106, cognitively unimpaired, positron emission tomography, preclinical, prevention, vaccine

Eric M. Reiman and Ana Graf have equal contribution.

Highlights

- This was the first amyloid-lowering prevention trial in persons at genetic risk of late-onset Alzheimer's disease (AD).
- Active immunotherapy targeting amyloid (CAD106) was tested in this prevention trial.
- CAD106 significantly slowed down amyloid plaque deposition in apolipoprotein E homozygotes.
- CAD106 was generally safe and well tolerated, with only three amyloid-related imaging abnormality cases (one symptomatic).
- Such an approach deserves further evaluation in larger AD prevention trials.

1 | BACKGROUND

Progress toward developing disease-modifying agents for Alzheimer's disease (AD) has been made by targeting one of the major neuropathological hallmarks of the disease: amyloid plaques.[1] Administering antibodies (passive immunotherapy) or inducing a humoral immune response (active immunotherapy, also referred to as vaccination) against the protein fragment amyloid beta (Aβ) are two promising strategies to decrease the level of Aβ plaques in the brain. Recent advances supporting the relationship between the plaque-reducing effects of a monoclonal antibody and slower clinical decline led to accelerated approval (in the United States) of the passive immunotherapies aducanumab and lecanemab.[2,3]

Active immunotherapy may offer a complementary approach to monoclonal antibodies in the preclinical phase of AD. Stimulating the immune system to produce antibodies against Aβ with the injection of short or fragmented Aβ peptides has the advantage of requiring less frequent administration via an intramuscular route, resulting in a more sustained polyclonal Aβ-specific response, at lower yet sufficient levels to slow down Aβ plaque deposition, thereby potentially associated with fewer amyloid-related imaging abnormalities (ARIA).[4]

CAD106 is an Aβ-based active immunotherapy comprising multiple copies of a short fragment of Aβ (Aβ_{1-6} peptide) coupled to a carrier that contains 180 copies of the bacteriophage Qβ coat protein.[5] Containing the N-terminus (B-cell epitopes) of the Aβ protein, CAD106 is a second-generation immunotherapy, which is designed to stimulate a strong B-cell response without activating an Aβ-specific T-cell response formerly implicated in serious adverse effects.[4–6]

In a randomized, double-blind, placebo-controlled, 90-week Phase 2 study of individuals with mild dementia due to AD, CAD106 demonstrated an acceptable safety and tolerability profile, while showing a significant correlation between the antibody response and change in brain amyloid (as measured by positron emission tomography [PET] in CAD106-treated patients; $P = 0.0004$).[7]

CAD106 treatment may be optimal if initiated in the preclinical phase of the disease (i.e., before symptoms develop), in the early stage of central nervous system (CNS) Aβ accumulation, and in some cases before the onset of detectable Aβ plaques.[1] This hypothesis is based on results from non-clinical studies and is also supported by the fact that slow accumulation of pathological Aβ species in the brain begins a decade or more before the onset of symptoms.[8]

The Alzheimer's Prevention Initiative (API) Generation Program included the first National Institutes of Health (NIH)- and industry-supported prevention trials of putative amyloid-modifying treatments in cognitively unimpaired persons at genetic risk for late-onset AD.[9] Under the API umbrella, Generation Study 1 was conducted in cognitively unimpaired adults at risk for the onset of clinical symptoms of AD, determined by the presence of two ε4 alleles of the apolipoprotein E (*APOE*) gene (*APOE* ε4 homozygotes).

APOE ε4 plays a crucial role in both the inflammatory and neurodegenerative processes associated with AD. Having two copies of the *APOE* ε4 allele substantially increases the risk of developing AD and lowers the average age of onset (from 84 to 68 years).[10–15] Evidence has shown that it is possible to detect and track biomarkers and cognitive decline in *APOE* ε4 homozygotes, who may therefore enrich AD prevention trials.[16–20]

Generation Study 1 (Cohort I) was designed to determine the effects of CAD106 on progression to clinical symptoms of AD in a cohort of cognitively unimpaired *APOE* ε4 homozygotes aged 60 to 75 years. Individuals with and without evidence of Aβ plaques, as measured by PET or cerebrospinal fluid (CSF), were recruited, with the objective to characterize treatment biomarkers as well as cognitive effects. The study was terminated early in light of negative results from other anti-amyloid therapies at that time. The aims were revised to determine the impact of CAD106 on Aβ immune response and PET measurements of brain amyloid.

2 | METHODS

2.1 | Planned study design and amendments

Generation Study 1 (ClinicalTrials.gov NCT02565511) was planned as a randomized, double-blind, placebo-controlled, parallel-group, event-driven trial with a variable treatment duration of a minimum of 5 years. Participants were enrolled into Cohort I (CAD106 vs.

placebo) from 28 sites in five countries from March 2016 to July 2018. Generation Study 1 also comprised a cohort (Cohort II) assessing another investigational drug (the beta-site-APP cleaving enzyme-1 [BACE-1] inhibitor umibecestat vs. placebo), the results of which will be reported separately. The study was terminated early (September 23, 2019 for Cohort I).

The study protocol was approved by the institutional review board at each study site. The study was designed, executed, and reported according to the International Council for Harmonisation guidelines for Good Clinical Practice and the ethical principles outlined in the Declaration of Helsinki.

2.1.1 | Participants

Participants were male or female individuals aged 60 to 75 years. They were eligible to participate if they were homozygous for the *APOE* ε4 genotype, regardless of their brain amyloid status; and cognitively unimpaired at screening, as defined by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS;[21] see the Supplementary File in supporting information for inclusion and exclusion criteria).

The study had multiple epochs (Figure 1A). After genotype disclosure, all participants, whether homozygotes or not, were assessed for the impact of disclosure and knowledge of their *APOE* ε4 status for up to 12 months (results to be reported separately). An analysis of baseline Aβ levels of the screened (homozygote) participants was performed and derived as elevated (A+) or not elevated (A−), as described in Table S1 in supporting information.

For both active and placebo arms, the clinical data and images from participants who consented to additional research were anonymized for upload to the Laboratory of NeuroImaging (LONI, https://loni.usc.edu/) server and most of the samples were provided to the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD; https://ncrad.iu.edu/). These data are now available upon request to these centers for external researchers to access and use for additional analysis.

2.1.2 | Randomization

Participants in Cohort I were randomized at a ratio of 5:3 to CAD106 450 μg + Alum 450 μg or placebo + Alum 450 μg; intramuscular injections were given at Weeks 1, 7, and 13, and quarterly (approximately every 13 weeks) thereafter. A sample size of 430 participants in the active treatment arm (CAD106) and 260 in the matching placebo arm was determined by a target power of 80% for the first primary endpoint (time to event), with an estimated event risk in 5 years of 30% to 40% based on the longitudinal cohort studies, a dropout rate of 30% over 5 years, and a type 1 error rate of 4%.[22]

The end of the treatment period was to be achieved when all ongoing participants for the respective cohort completed their Month 60 (5 years) assessment and the target number of 218 events had been reached in the respective cohort, whichever was later.

RESEARCH IN CONTEXT

1. **Systematic review**: A search of recent studies for Alzheimer's disease (AD) supports a relationship between amyloid beta (Aβ) plaque reduction and slower clinical decline achieved with amyloid-targeting monoclonal antibodies. It remains possible that anti-amyloid therapies may have a more profound benefit if initiated in the preclinical stage of the disease when amyloid plaques are still accumulating, that is, in cognitively unimpaired persons at genetic or biomarker risk for AD.
2. **Interpretation**: This prevention trial was the first to study Aβ-modifying therapies in persons at high genetic risk of late-onset AD before clinical symptoms appear. Despite its early termination due to negative results of some other anti-amyloid therapies, the study supports the potential for CAD106, an active immunotherapy (vaccine), to slow down amyloid plaque deposition as measured by positron emission tomography in cognitively unimpaired persons, with or without plaque burden.
3. **Future directions**: Our preliminary findings support the evaluation of anti-amyloid active immunotherapies such as CAD106 in larger and longer AD prevention trials.

2.1.3 | Study termination

In 2017, recruitment was halted in Cohort I following a protocol amendment after 65 participants were randomized. The aim was to determine whether CAD106 was associated with an immune response and a reduction in PET measurements of brain amyloid before exposing more participants to the drug (to be assessed by the data monitoring committee [DMC]). According to recent data on passive immunotherapies, robust CNS activity is required to maximize the chances that a clinical benefit might emerge after longer treatment.

Subsequently, after the decision to prematurely terminate treatment with umibecestat (Cohort II) on July 11, 2019[23] treatment with CAD106 in Cohort I was also terminated prematurely on September 23, 2019. The decision to terminate Cohort I early was not due to safety reasons but based on negative results from other anti-amyloid therapies. Therefore, an analysis of the unblinded data collected until the last assessment (≈ 18 months of treatment for the last participant) was performed to clarify the potential of CAD106 for future AD prevention trials. All participants on treatment at the time of study termination were to return for a final visit including PET scan and an end of study evaluation, as per the protocol.

2.2 | Endpoints

The original study design was based on a dual endpoints approach, with two separate primary endpoints including time to event (mild cognitive

impairment [MCI] or dementia due to AD) and measure of cognitive decline from baseline to Month 60.[22] Planned secondary endpoints included the effects of CAD106 versus placebo on Aβ-specific antibody titers and PET measurements of Aβ plaque deposition (using Centiloids to harmonize findings from the three [18] F-labeled Aβ PET tracers; see Supplementary File for more details on the original objectives and endpoints).

The primary analyses could not be performed as the planned treatment period of 5 to 8 years was not achieved and no data were collected at Month 60. However, an interim analysis for the assessment of CNS effects (i.e., change in Aβ plaque burden measured by PET) was preplanned at Year 2, and most of the 65 participants had a "Year 2" visit between 18 and 24 months from treatment initiation. This sample size and/or follow-up time was sufficient to assess antibody titers and brain amyloid PET measurements, which became the primary endpoints of the final analysis. Cognition was measured by both the RBANS[21] and the API preclinical composite cognitive (APCC) test score[24] as exploratory endpoints. ARIA and brain volumes, including whole brain, hippocampus, and lateral ventricles, were assessed in both groups by magnetic resonance imaging (MRI).

2.3 | Assessments and statistical analysis

Statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc.) and R version 3.4.3. Analyses of efficacy variables were based on observed cases only; that is, there was no imputation of missing data.

2.3.1 | Aβ-specific immune response

Aβ-antibody response was measured at scheduled visits by determination of Aβ-specific immunoglobulin G (IgG) titers in serum using enzyme-linked immunosorbent assay (ELISA) methods.[5] Titer levels were expressed in units relative to a reference polyclonal serum, as previously described,[7] rather than in titer units calculated based on serial dilutions of tested samples, as done with other active immunotherapies (such as AN1792).[25] T-cell lymphocyte responses to the $A\beta_{1\text{-}42}$ peptide, the $A\beta_{1\text{-}6}$ peptide, and the Qβ protein (positive control) were also assessed (Supplementary File). A Pearson correlation coefficient between the annualized change from baseline in Aβ PET Centiloid and the Cmax of the Aβ antibody titer was calculated.

2.3.2 | Aβ PET

All participants had a baseline Aβ PET scan and underwent a post-treatment Aβ PET scan if at least 18 months elapsed from the previous PET scan (Year 2 visit). The annualized change in Aβ PET Centiloids from baseline to this last assessment was compared across treatment groups using a two-sample *t* test. A linear regression adjusting for baseline Aβ level was also performed as a sensitivity analysis to improve the accuracy of the analysis of the treatment effect and to avoid potential conditional bias from baseline covariate imbalance. The technique used for serial Aβ image acquisition and unit transformation into standardized Centiloids is described in the Supplementary File.

In addition, subgroups of elevated and not elevated Aβ levels (A+/A−) at baseline were considered for most efficacy endpoints to illustrate the impact of baseline Aβ level on treatment effects. To identify the two subgroups of participants based on PET assessments, we used a Centiloid threshold of 24.33 at the time of treatment initiation (Supplementary File), which was defined based on florbetapir standardized uptake value ratio cut-off of 1.1. CSF measures were performed in parallel for four individuals (Tables S1 and S2 in supporting information).

2.3.3 | MRI

T1-weighted volumetric MRI scans were acquired on 3T systems using standardized protocols at screening (baseline) and at Weeks 26, 52, and 104. To assess rates of brain shrinkage, volumetric MRI scans from follow-up time points were compared to those from screening (see Supplementary File for technical details including the volumetric and ARIA-related MRI pulse sequences).

3 | RESULTS

3.1 | Participant characteristics

The disposition of participants is given in Figure 1B. Overall, these were cognitively unimpaired *APOE* ε4 homozygotes of mean (standard deviation [SD]) age of 65 (4) years, almost entirely from non-Hispanic White ethnic and racial groups, with comparable characteristics between the placebo ($N = 23$) and active treatment arms ($N = 42$; Table 1).

3.2 | Aβ-specific immune response

Most participants (92.9%) on CAD106 had an exposure of ≥18 months (median 24 months, representing an average of nine injections; Table S3 in supporting information). Aβ-specific IgG titers were measured in the active treatment group only (Figure 2), with 41/42 (97.6%) participants exposed to CAD106 having measurable Aβ-specific IgG titers in serum at ≥1 time point. The CAD106-induced mean maximum antibody titer concentration was 128.76 units/mL (95% confidence interval [CI]: 99.05, 167.37). For those participants who consented to (and had valid) quarterly titer measurements after study completion ($n = 17$), antibody titers were detectable (> lower limit of quantification) in 14 (82%) participants at the end of the follow-up period, which was approximately 2 years after the last dose (median 746 days; range: 233 to 1039 days). CAD106 did not activate Aβ-specific T cells (Supplementary File).

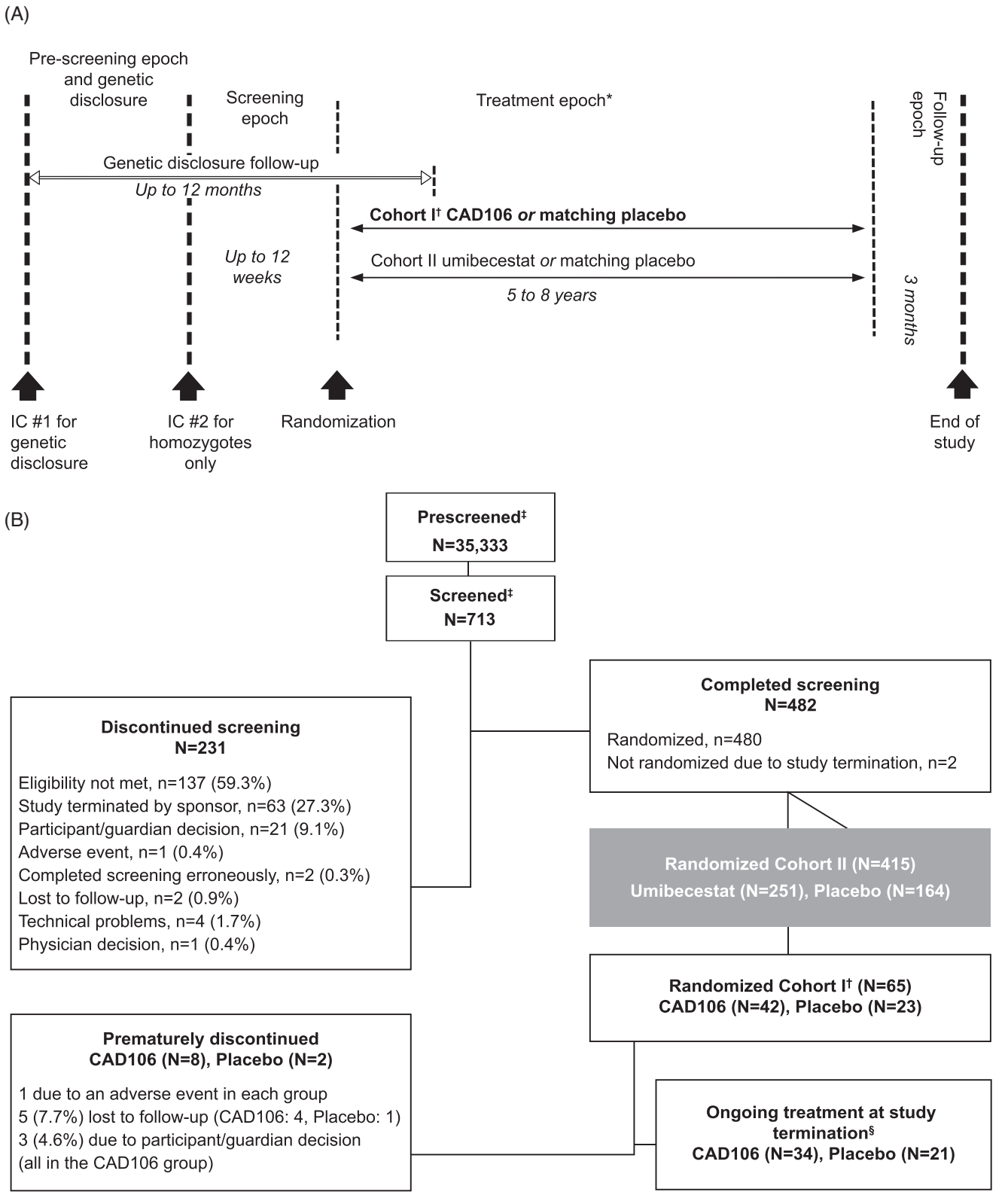

FIGURE 1 A, Study design and planned participant enrollment. B, Participant disposition and primary reasons for premature withdrawal, *Actual duration of treatment between 6 and 48 months (median: 24 months). † Planned $n = 690$ (CAD106: 430; placebo: 260) down to 65 (CAD106: 42; placebo: 23) after the halt of recruitment and subsequent trial termination. First three injections every 6 weeks, then quarterly until study termination. ‡Participants prescreened and screened for both Cohort I with CAD106 and Cohort II with umibecestat (note that another 8970 out of the 35,333 participants were screened for Generation Study 2); §Termination for CAD106 on September 23, 2019; last injection October, 19 2019. IC, informed consent; *N*, total number of participants in the cohort or in the treatment groups; *n*, number of participants in each sub-category

TABLE 1 Participant demographics and other baseline characteristics.

Baseline characteristic	CAD106 $N = 42$	Placebo $N = 23$	P value
Age (years)	64.9 (3.7)	66.1 (4.8)	0.275*
Sex—*n* (%)			0.581†
Female	27 (64.3)	17 (73.9)	
Race—*n* (%)			>0.990†
White	41 (97.6)	22 (95.7)	
Black	1 (2.4)	1 (4.3)	
Weight (kg)	80.9 (20.1)	71.7 (11.7)	0.022*
Years of education—*n* (%)			0.100†
≤12 years	8 (19.0)	1 (4.3)	
13–16 years	16 (38.1)	6 (26.1)	
≥17 years	18 (42.9)	16 (69.6)	
Family history of AD[a]—*n* (%)	36 (85.7)	18 (78.3)	0.702†
Brain characteristics			
Volumetric MRI-whole brain (cm^3)	1073.7 (117.3)	1063.9 (107.6)	0.735*
Hippocampus volume[b] (cm^3)	8.3 (0.9)	8.3 (1.0)	0.866*
Aβ PET Centiloid	49.7 (38.1)	49.1 (40.7)	0.955*
Aβ positive[c]—*n*/*N* (%)	29/42 (69.0)	15/22 (68.2)	>0.99†
Cognitive measures			
MMSE score	29.1 (1.1)	29.4 (0.8)	0.204*
APCC score	78.0 (5.5)	79.0 (6.8)	0.521*
RBANS total score	104.4 (12.0)	108.7 (12.8)	0.193*
RBANS—immediate memory index	108.0 (13.9)	110.0 (13.1)	0.568*
RBANS—delayed memory index	105.4 (12.7)	104.6 (11.4)	0.803*

Note: Values are means (SD) unless otherwise stated. Percentages are based on the numbers of participants with non-missing data. Age is defined as the age at randomization.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; APCC, Alzheimer's Prevention Initiative Composite Cognitive; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; *N*, total number of participants in each treatment group (safety analysis set); *n*, number of participants in the corresponding category; PET, positron emission tomography; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status, SD, standard deviation.

[a]Including siblings, parents, and grandparents.

[b]Sum of the left and right.

[c]PET positive (Centiloid ≥24.33) OR CSF positive (Elecsys ratio phospho-tau/$A\beta_{1-42}$ > 0.024 and Elecsys $A\beta_{1-42}$≤1700.0 pg/mL [upper limit of the measuring range]).

*P value derived using t test.

†P value derived using the chi-square test or Fisher exact test.

3.3 | Aβ deposition

3.3.1 | Annualized change in Centiloid

A total of 51 participants (CAD106: 35; placebo: 16) had a post-baseline PET scan after 18 to 24 months of treatment (Year 2 visit). As shown in Figure 3A and Table S4 in supporting information, the increase in amyloid plaques, measured by the mean (SD) annualized change from baseline in PET Centiloid, was significantly higher in the placebo group (8.4 [6.7]) compared to the CAD106 group (−0.9 [5.7] – no increase; $P < 0.001$). Analyses focusing on Aβ PET measurements based only on one tracer (florbetapir, which was used in 89% of the measurements) confirmed the results obtained in Centiloids across the three tracers.

In exploratory analyses, CAD106 treatment was associated with slower rates of Aβ plaque deposition than placebo in both the A+ subgroup ($N = 37$; $P = 0.001$, uncorrected for multiple comparisons) and the smaller A− subgroup ($N = 14$; $P = 0.071$, uncorrected for multiple comparisons).

Annualized change in Centiloid values plotted against baseline values for each individual are shown in Figure S1 in supporting information. The regression analysis adjusting for baseline Aβ level confirmed that treatment with CAD106 had a significant effect on Aβ clearance compared to placebo ($P < 0.001$).

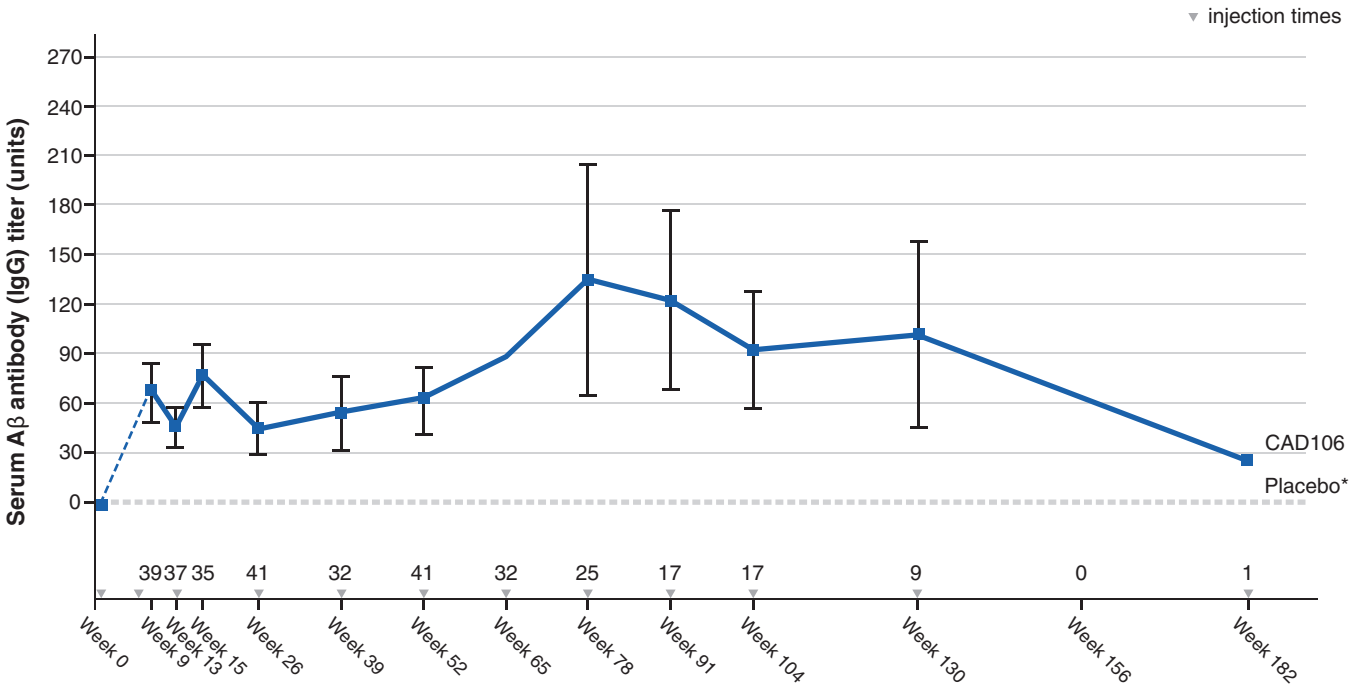

FIGURE 2 Mean and 95% CI of Aβ-specific IgG titer in serum over time. The numbers across the x axis represent the total number of participants in the CAD106-treated group that had a titer measurement at the corresponding visit (week) before study termination. All participants included in this graph were on treatment. *Aβ-IgG was not measured in this study for serum at baseline or in placebo-treated participants, but was assessed and not detected in a previous CAD106 study. Seven measurements at Weeks 9 and 15 were taken 2 weeks post-injection; all other measurements were taken before the injection. Aβ, amyloid beta; CI, confidence interval; IgG, immunoglobulin G

3.3.2 | Correlation with antibody titers

In the CAD106 arm, a weak linear relationship was observed between annualized change from baseline in Aβ PET and antibody titers, with a Pearson correlation $r = -0.20$ (Figure 3B). Correlations are not applicable in the placebo group as antibody titers are below detectable levels.[7]

3.4 | Brain volume

There was no significant difference between CAD106 and placebo at Weeks 26 and 52 on brain volume measures, as shown by the small mean percentage changes and relatively large SD, as well as no or minimal differences at the last assessment (Year 2 visit) (Table 2). Differences in brain volume in the A+ and A− subgroups at the last assessment are provided in Table S5 in supporting information.

3.5 | Cognition

There was no difference between CAD106 and placebo in the mean change from baseline in the RBANS total score up to the last assessment, as shown by the small mean changes and the large SD (−1.0 [9.27] for CAD106 and +0.4 [7.20] for placebo at the last assessment [Year 2 visit]). The baseline imbalance in RBANS scores remained at the same magnitude throughout the study as the cognition scores were stable over the 18–24 months of treatment in both groups. Likewise, the changes in the APCC score from baseline up to the last assessment were similar between CAD106 and placebo.

3.6 | Safety

Most participants in both groups reported at least one adverse event (AE; Table 3). There was a higher incidence of general disorders/injection site reactions with CAD106, affecting 64% (95% CI: 48.0, 78.0) of participants in this group, than with placebo (22% [8.3, 44.2]; Table 3; see also Table S6 in supporting information for details on preferred terms). All other AEs occurred in similar proportions between CAD106 and placebo, as shown by the overlapping 95% CIs (95%; Table 3).

CAD106 was discontinued in one participant due to an administration-related reaction and feeling abnormal, while there were no AEs leading to discontinuation in the placebo group.

MRI scans of three participants (7.1%) met criteria for ARIA in the CAD106 arm (one with symptomatic ARIA-edema [ARIA-E] and two with asymptomatic ARIA-hemorrhage [ARIA-H]; Table 3). The participant with ARIA-E had moderate sphenopalatine ganglioneuralgia-like symptoms that did not re-occur when treatment was restarted after missing two injections. The two participants who met the criteria for ARIA-H had subarachnoid hemorrhage with a new area of superficial

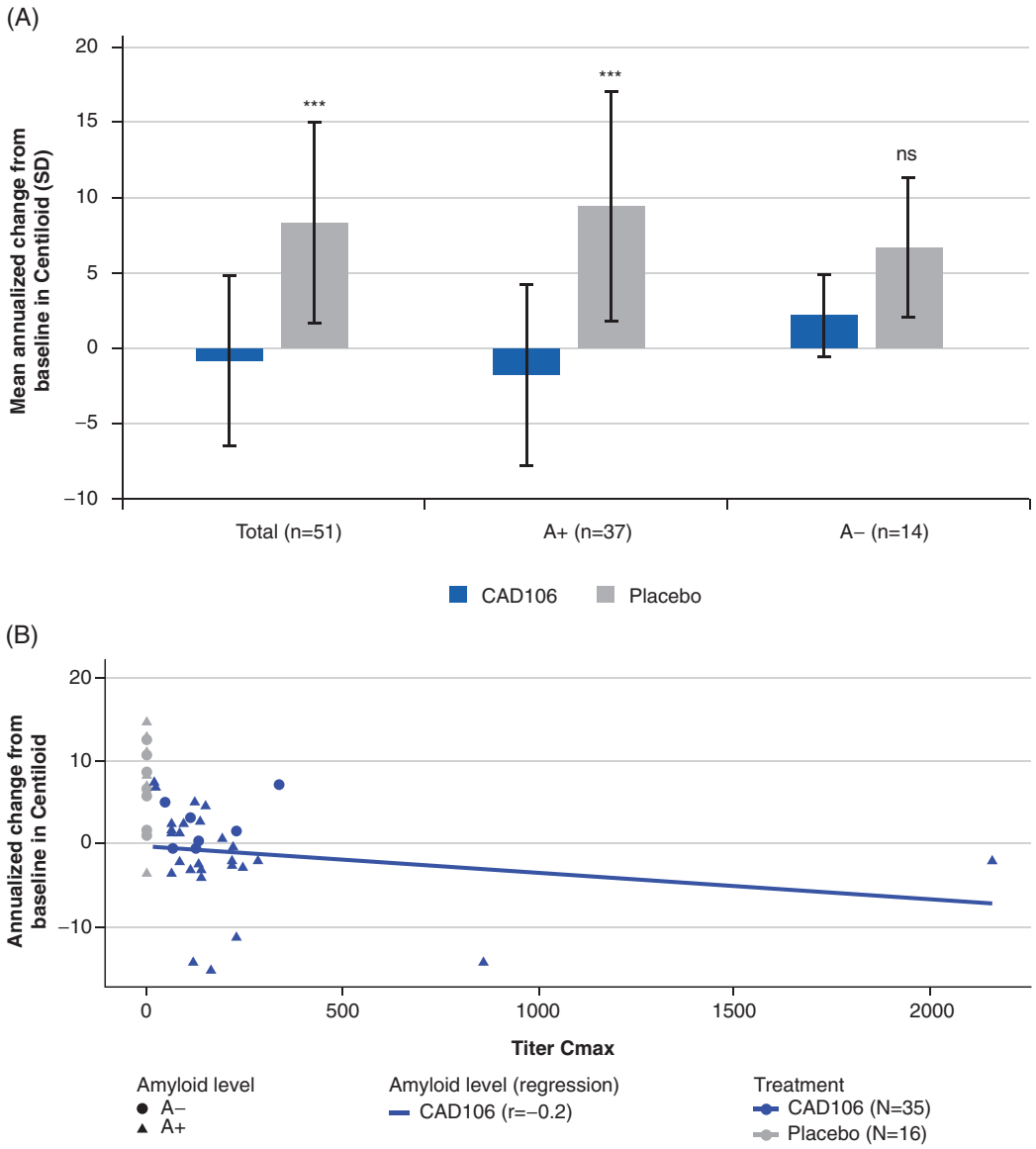

FIGURE 3 A, Annualized change in the PET measurement of Aβ plaques at the Year 2 visit. B, Correlation between annualized change in PET Aβ and Aβ-antibody response. The Year 2 visit occurred at Week 104 for participants assessed before study termination and between 18 and 24 months for other participants. Annualized change from baseline: change per participant/time interval (in days) × 365.25. The time interval was derived as (date of current assessment − date of baseline assessment + 1). Cmax is the maximum (or peak) serum concentration after dosing. Cmax for placebo is zero. A+ and A− are participants with and without elevated Aβ at the time of treatment initiation, respectively. *** $P \leq 0.001$; ns, $P > 0.05$. Aβ, amyloid beta; ns, non-significant; PET, positron emission tomography; r, Pearson correlation coefficient; SD, standard deviation

TABLE 2 Annualized percentage change in whole brain, hippocampus, and lateral ventricle volume.

Annualized percentage change Mean (SD)	Week 26		Week 52		Last assessment	
	CAD106 $n = 35$	Placebo $n = 23$	CAD106 $n = 37$	Placebo $n = 22$	CAD106 $n = 40$	Placebo $n = 23$
Whole brain (cm^3)	−0.76 (1.33)	−0.60 (1.29)	−0.51 (0.67)	−0.34 (0.76)	−0.46 (0.58)	−0.53 (0.47)
Hippocampus (cm^3)	−1.32 (2.35)	−0.92 (2.82)	−1.04 (1.44)	−0.78 (1.82)	−1.08 (1.38)	−1.05 (1.34)
Lateral ventricle (cm^3)	4.18 (5.77)	2.55 (7.54)	4.21 (3.93)	2.82 (5.04)	4.05 (3.75)	3.54 (3.54)

Note: The last assessment was performed between 18 and 24 months (Year 2 visit) when the PET scan was acquired and most individuals (> 92%) were still on treatment.
Abbreviations: PET, positron emission tomography; SD, standard deviation.

TABLE 3 Treatment-emergent AEs irrespective of relation to study treatment.

SOC with a difference of at least 5%	CAD106 *n* (%) 95% CI[a]	Placebo *n* (%) 95% CI[a]
Number of participants with at least one AE	38 (90.5) (76.5, 96.9)	21 (91.3) (70.5, 98.5)
General disorders and administration site conditions	27 (64.3) (48.0, 78.0)	5 (21.7) (8.3, 44.2)
Infections and infestations	21 (50.0) (34.4, 65.6)	8 (34.8) (17.2, 57.2)
Musculoskeletal and connective tissue disorders	19 (45.2) (30.2, 61.2)	9 (39.1) (20.5, 61.2)
Injury, poisoning, and procedural complications	18 (42.9) (28.1, 58.9)	4 (17.4) (5.7, 39.5)
Nervous system disorders	17 (40.5) (26.0, 56.7)	7 (30.4) (14.1, 53.0)
Psychiatric disorders	9 (21.4) (10.8, 37.2)	3 (13.0) (3.4, 34.7)
Skin and subcutaneous tissue disorders	9 (21.4) (10.8, 37.2)	2 (8.7) (1.5, 29.5)
Investigations	6 (14.3) (5.9, 29.2)	2 (8.7) (1.5, 29.5)
Metabolism and nutrition disorders	6 (14.3) (5.9, 29.2)	2 (8.7) (1.5, 29.5)
Respiratory, thoracic, and mediastinal disorders	6 (14.3) (5.9, 29.2)	2 (8.7) (1.5, 29.5)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	5 (11.9) (4.5, 26.4)	1 (4.3) (0.2, 24.0)
Ear and labyrinth disorders	4 (9.5) (3.1, 23.5)	0
ARIA[b]	**CAD106 *n* (%)**	**Placebo *n* (%)**
ARIA–E	1 (2.4%)	0
ARIA-H[c]	2 (4.8%)	0

Note: No new microhemorrhages were reported in either group. White matter disease worsening (score 1–3) was reported in two cases (8.7%) with placebo while there was no reported case with CAD106.

Abbreviations: AE, adverse event; ARIA, amyloid-related imaging abnormalities; ARIA-E, amyloid-related imaging abnormalities—edema; ARIA-H, amyloid-related imaging abnormalities—hemorrhage; CI, confidence interval; *n*, number of participants from the safety analysis set with an AE; MRI, magnetic resonance imaging; SOC, system organ class.

[a]95% CIs are estimated based on the binomial distribution.

[b]The ARIA values displayed are those confirmed by a safety MRI central reader.

[c]Criteria for ARIA-H: > 4 new microhemorrhages OR a large area of hemosiderin deposition (macrohemorrhage ≥10 mm) OR superficial siderosis.

siderosis after 24 and 45 months, respectively. No new microhemorrhages were identified in any participant in either group.

All the suspected drug-related AEs were mild or moderate in severity, except for one severe injection-related reaction in a participant receiving CAD106. No meningitis or encephalitis AEs were reported.

4 | DISCUSSION

This study was the first NIH- and industry-supported AD prevention trial of potential Aβ-modifying treatments in cognitively unimpaired persons at genetic risk for late-onset AD. Two different anti-amyloid drugs were tested and here we report results from the active immunotherapy CAD106. The original randomized trial was intended to evaluate the efficacy to slow cognitive decline and clinical progression, as well as the safety and tolerability, of CAD106 in cognitively unimpaired *APOE* ε4 homozygotes (i.e., persons at the highest known genetic risk for late-onset AD) close to the estimated age of clinical onset. We also aimed to explore the differential effects of treatment in those with or without evidence of brain amyloid burden at baseline (A+ and A–, roughly corresponding to the use of a secondary vs. primary prevention therapy) and clarify the relationships among biomarker effects and clinical outcomes.

We found that CAD106 was safe and well tolerated, with relatively high retention rates. Despite the low sample size ($N = 65$) and short duration (18–24 months) due to early study termination, we demonstrated that *APOE* ε4 homozygotes treated with CAD106 had consistent elevations in Aβ antibody titers and no increase in PET measurements of Aβ plaques, contrasting with the increase observed with placebo ($P < 0.001$). The trends were similar in both the A+ and A– subgroups. Because no deposition of Aβ was observed in these asymptomatic participants receiving CAD106, we did not expect to observe a significant correlation with antibody titers as per the prior study in mild AD,[7] although we did find a weak correlation. While the original primary analyses, including cognitive outcomes, could not be performed, cognition was assessed as an exploratory endpoint and we did not observe cognitive worsening in this small CAD106-treated cohort.

As reported previously,[7] the majority of participants had injection-related reactions, all mild to moderate in severity except for one that was severe and constituted the only severe AE reported as possibly related to the drug. Three cases of ARIA were reported: one (2.4%) ARIA-E and two (4.8%) ARIA-H, all in the active treatment arm. Among these, only the single case of ARIA-E was symptomatic (of moderate severity) without reoccurring when treatment resumed. This contrasts with the high incidence of ARIA reported in Phase 3 trials of monoclonal antibodies in early AD. The proportion of patients with ARIA-E was 65% with aducanumab (combined results for the 10 mg/kg dose)[26] and 32.6% with lecanemab[2] in homozygous *APOE* ε4 carriers who, for both therapies, represented the group with the highest rate of ARIA. Our finding is consistent with results of the previous trial of CAD106 in mild AD,[7] which reported six (5.7%) cases of ARIA in the CAD106 arm, suggesting that the lower incidence of ARIA we observed with CAD106 is more likely to be related to the mode of action (active vs. passive immunotherapy) than to the type of population (preclinical vs. symptomatic). Indeed, active immunization with CAD106 induces a gradual development of long-lasting polyclonal antibody response in lieu of the high peak of monoclonal antibodies with passive infusions. Also consistent with the previous CAD106 study,[7] no drug-induced meningoencephalitis was reported, and our results confirmed the absence of

Aβ-specific T cells potentially responsible for pathological autoreactive T-cell responses.

Based on evidence from other studies, some anti-Aβ therapies were associated with an early and possibly non-progressive and reversible reduction in brain volume related to a reduction in Aβ plaques.[26,27] In our study and in line with results from the previous study of CAD106 in mild AD,[7] no significant difference in brain volume loss was observed in either group. The slight brain volume loss that occurred in both groups at Week 26 (−0.76% with CAD106 vs. −0.60% with placebo) did not increase and even reversed over the almost 2-year duration of treatment. These findings suggest that CAD106-related reductions and/or slower increases in Aβ PET measurements are not likely to be attributable to the combined effects of brain shrinkage and partial-volume averaging.

Strengths of this study include the demonstrated ability to conduct a multicenter prevention trial of an AD-modifying treatment in cognitively unimpaired persons at risk for AD, the innovations used to recruit cognitively unimpaired *APOE* ε4 homozygotes and disclose their genetic results,[28] and all trial data and images as well as most samples being made available to external researchers in accordance with the Collaboration for Alzheimer's Prevention (CAP) principles.[29] However, the revised protocol resulting from the study's early termination does not allow us to draw any conclusion on potential treatment effects on cognitive or other clinical outcomes. In addition, the evidence for a treatment effect in *APOE* ε4 homozygotes without Aβ burden (A−) is limited by the small sample size of this subgroup. Another limitation may be the use of different tracers for Aβ PET measurements, with seven (11%) participants assessed with another tracer than florbetapir (although it should be noted that baseline and follow-up scans were performed using the same tracer and the results were not changed after removing these few participants from the analysis).

Despite these limitations, our findings suggest that the clinical, cognitive, and/or biomarker effects of CAD106 merit further investigation in larger, longer, and potentially license-enabling primary and/or secondary prevention trials, including in cognitively unimpaired individuals perhaps at genetic risk. Recruiting such asymptomatic participants at very early stage (e.g., *APOE* ε4 homozygotes including A–) is feasible and could have benefits. Recent studies in early and mild AD suggest that high antibody titers and a dramatic reduction in PET measurements of Aβ plaques may be necessary for a drug to have a clinically significant benefit in the early clinical stages.[2,26] We postulate that CAD106 and other anti-amyloid treatments could have a greater clinical benefit if started in cognitively unimpaired persons at risk for AD, before amyloid plaques are apparent or have virtually plateaued and downstream (e.g., tau and neurodegenerative) elements of the disease are more extensive. Our findings suggest that relatively stable polyclonal antibody titers can reduce the rate of amyloid plaque deposition and thus offer particular promise prior to the onset of extensive plaques and related AD pathology. If these evaluations are successful, an infrequently administered, safe, and well tolerated active immunotherapy would have great value in AD prevention.

In conclusion, in this multicenter trial of an active immunotherapy in cognitively unimpaired *APOE* ε4 homozygotes, CAD106 treatment was associated with elevated Aβ antibodies, slower increases in PET measurements of Aβ plaque deposition, including in those who did or did not yet have baseline biomarker evidence of significant Aβ plaques, and a favorable safety and tolerability profile. Despite the study's small size and early termination, it supports the potential value of conducting a larger prevention trial of an active Aβ immunotherapy such as CAD106 for the primary and/or secondary prevention of AD.

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CONFLICT OF INTEREST STATEMENT

M.E.R., Y.S., P.C., J.R., K.M., A.C. and A.G. are employees of and shareholders in Novartis. M.E.R. has a US patent application pending covering a pharmaceutical formulation containing CAD106 and an adjuvant and its use in Aβ immunotherapy. A.G. has a US patent covering CAD106 and its use in Ab immunotherapy. This research was sponsored by Novartis Pharma AG, Basel, Switzerland. R.S.T. benefited from research support to Georgetown University from Lilly, Biogen, Roche, Genentech, Novartis, Janssen, and Eisai. J.O.R. serves as a neurology consultant for Clinical Research Services Turku (CRST Oy). P.N.T., E.M.R. and J.B.L. are full-time employees of Banner Health. Banner Health received financial support from Novartis Pharma AG and Amgen for the conduct of the API Generation Program, from Eli Lilly for the conduct of another Alzheimer's prevention trial, and from Genentech/Roche for another Alzheimer's prevention trial. P.N.T. reports receiving grants from the National Institute on Aging (NIA) (UF1AG046150, RF1 AG041705, R01AG055444,

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CONSENT STATEMENT

Written informed consent was obtained from all participants. Participants consented to genotyping for *APOE* and to receive genetic counseling and disclosure, including their risk estimates of developing clinical symptoms of AD based on their *APOE* genotype.

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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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