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Adeno-Associated Viral Vector (Serotype 2)–Nerve Growth Factor for Patients With Alzheimer Disease
A Randomized Clinical Trial

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IMPORTANCE Nerve growth factor (NGF) is an endogenous neurotrophic factor that prevents the death and augments the functional state of cholinergic neurons of the basal forebrain, a cell population that undergoes extensive degeneration in Alzheimer disease (AD).

OBJECTIVE To determine whether stereotactically guided intracerebral injections of adeno-associated viral vector (serotype 2)–nerve growth factor (AAV2-NGF) are well tolerated and exhibit preliminary evidence of impact on cognitive decline in mild to moderate AD-associated dementia.

DESIGN, SETTING, AND PARTICIPANTS In a multicenter phase 2 trial, 49 participants with mild to moderate AD were randomly assigned in a 1:1 ratio to receive stereotactically guided intracerebral injections of AAV2-NGF or sham surgery. Participants were enrolled between November 2009 and December 2012. Analyses began in February 2015. The study was conducted at 10 US academic medical centers. Eligibility required a diagnosis of mild to moderate dementia due to AD and individuals aged 55 to 80 years. A total of 39 participants did not pass screening; the most common reason was Mini-Mental State Examination scores below cutoff. Analyses were intention-to-treat.

INTERVENTIONS Stereotactically guided intracerebral injections of AAV2-NGF into the nucleus basalis of Meynert of each hemisphere or sham surgery.

MAIN OUTCOMES AND MEASURES Change from baseline on the Alzheimer Disease Assessment Scale–cognitive subscale at month 24.

RESULTS Among 49 participants, 21 (43%) were women, 42 (86%) self-identified as white, and the mean (SD) age was 68 (6.4) years. AAV2-NGF was safe and well-tolerated through 24 months. No significant difference was noted between the treatment group and placebo on the primary outcome measure, the Alzheimer Disease Assessment Scale–cognitive subscale (mean [SD] score, 14.52 [4.66] vs 9.11 [4.65], $P = .17$).

CONCLUSIONS AND RELEVANCE This multicenter randomized clinical trial demonstrated the feasibility of sham-surgery–controlled stereotactic gene delivery studies in patients with AD. AAV2-NGF delivery was well-tolerated but did not affect clinical outcomes or selected AD biomarkers. Pathological confirmation of accurate gene targeting is needed.

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Supplemental content

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Alzheimer disease (AD) is the most common cause of dementia worldwide and is associated with loss of cholinergic neurons in the nucleus basalis of Myenert (NBM).[1,2] Cholinesterase inhibitors remain the primary treatment offered to patients with AD but provide relatively modest symptomatic improvement in some patients.

Nerve growth factor (NGF) regulates the functional state of cholinergic neurons in the basal forebrain.[3,4] It is produced in the cerebral cortex and retrogradely transported as the NGF/tropomyosin receptor kinase A signaling complex to the basal forebrain.[5] After lesions of cortical cholinergic projections, NGF prevents the death of cholinergic neurons[6-10] and restores learning.[8] Moreover, NGF reverses cholinergic atrophy and improves cognitive performance in aged rats.[11,12] The neuroprotective actions of NGF on basal forebrain cholinergic neurons persist in nonhuman primates with lesions[9] and who are aged.[13,14] Based on these findings, NGF has been considered a potential therapy for cholinergic preservation in AD, although its impact on other aspects of AD-associated pathophysiological processes, such as amyloidogenesis and neurofibrillary tangle formation, remains uncertain. Because NGF does not cross the blood brain barrier, techniques of gene delivery have been used to administer it in several preclinical[13-15] and clinical[16] studies. The first human study to our knowledge, an open-label trial with 8 participants using ex vivo NGF gene delivery, demonstrated a reduction in the rate of disease progression by 36% to 51% during 2 years on the Mini-Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-Cog 11). Additionally, fludeoxyglucose F18-labeled positron emission tomography ([^{18}F]-FDG-PET) scans in 4 participants treated with NGF demonstrated widespread interval increases in brain metabolism 6 to 8 months after treatment. Gene delivery of NGF using adeno-associated viral vector (serotype 2) (AAV2-NGF), was initially studied in a dose-escalating phase 1 clinical trial in patients with mild to moderate AD.[17,18] The phase 1 study demonstrated the long-term safety and feasibility of a surgically-based NGF therapeutic approach in patients with mild to moderate AD.[17] Here we report results from a placebo-controlled (sham surgery) 24-month study of 49 participants with mild to moderate AD treated with AAV2-NGF to assess feasibility, safety, and tolerability and to explore efficacy.

Key Points

Question Can stereotactically guided gene transfer of nerve growth factor using adeno-associated viral vector (serotype 2) (AAV2-NGF) provide long-standing neurotrophic support to cholinergic neurons in patients with mild to moderate Alzheimer disease and prevent decline on cognitive measures as well as standard Alzheimer disease biomarkers?

Findings In this multicenter randomized clinical trial, AAV2-NGF was safe and well-tolerated through 2 years. Magnetic resonance imaging and fludeoxyglucose F18-labeled positron emission tomography imaging and neuropsychological testing showed no evidence of efficacy.

Meaning This trial further demonstrates the feasibility of sham-surgery–controlled stereotactic gene delivery studies in patients with Alzheimer disease; it remains to be determined, through future pathological examination of brains, whether AAV2-NGF was accurately targeted to the nucleus basalis of Meynert and whether nerve growth factor protein spread sufficiently from sites of administration to influence its cholinergic cellular targets.

Methods

AAV2-NGF is a gene transfer vector that was developed to deliver NGF to the cholinergic neurons of the NBM.[19,20] It is delivered via stereotactic neurosurgery, genetically programming neurons in the NBM to express full-length human NGF. A total dose of 2.0×10^{11} vector genomes of AAV2-NGF was delivered by bilateral stereotactic injections into the basal forebrain region containing the NBM. The dose used in this study was the same as the high-dose cohort in the phase 1 study.[17] The selection of this dose was based on the goal of achieving effective gene delivery and NGF expression in the NBM, the wide margin of safety based on nonclinical studies, and favorable safety profile of this dose in phase 1.

Participants

The trial was registered at clinicaltrials.gov (NCT00876863). The full trial protocol is available in Supplement 1. The study was approved by the human subjects review committee and conducted at 10 sites listed in the acknowledgments. Recruitment took place from November 5, 2009, to December 19, 2012. Analyses began in February 2015. Written informed consent was obtained from all participants (Supplement 2). Participants were deemed capable of providing informed consent if, on screening evaluation, they could repeat the basic concept of the study and the major potential risks. Participants were consented in accordance with state law governing the designation of a surrogate decision-maker and in compliance with federal, state, and institutional review board requirements. In cases of impaired capacity (2 participants in each arm), a legally authorized representative provided surrogate consent. A total of 88 participants were assessed for eligibility; 39 participants did not pass screening, and 49 participants were randomized to receive either AAV2-NGF or sham surgery. A total of 26 participants were allocated to the treatment arm and 23 participants, to the placebo arm (**Figure 1**).

The primary enrollment criterion was a diagnosis of mild to moderate AD as determined by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria.[21] Additional criteria included individuals aged 55 to 80 years, a score of 4 or less on a modified Hachinski Ischemia Scale,[22] and an MMSE[23] score between 17 to 26. The most common reason for screen failure was an MMSE score below cutoff.

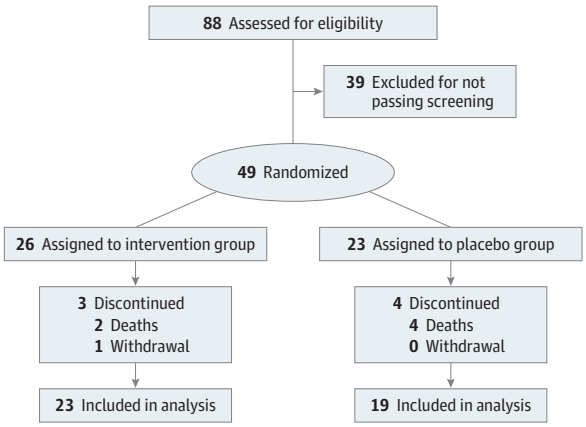
Participants had no other significant neurologic or medical abnormalities contraindicating surgery, magnetic resonance imaging (MRI)/PET imaging, or study participation. In addition, all participants were permitted to have been stable on standard-of-care medications (ie, acetylcholinesterase inhibitors or memantine) for AD before and continue with medication during the study. The demographics

of the participants enrolled are described in **Table 1**. The mean (SD) age for the 49 participants was 68.0 (6.2) years with a range of 55 to 79 years. The mean (SD) MMSE score at screening was 22.1 (3.2) for the placebo group and 21.2 (3.8) for the treatment group. All participants were receiving stable cholinesterase inhibitors and memantine at time of enrollment. All remained receiving stable doses of these medications throughout the trial. There was no statistical difference between the 2 arms with respect to apolipoprotein E ε4 carrier status: 16 (76%) in the control group vs 17 (68%) in the treatment group (odds ratio [OR], 0.57; 95% CI, 0.136-2.23; P = .38). Apolipoprotein E ε4 carrier status did not affect response to treatment.

Dosing Procedures

In this study, there was a surgical team (unblinded) and a research team (blinded). The surgical team was responsible for presurgical evaluation and providing medical clearance for surgery. The neurosurgeons (D.B. and other nonauthors) performed the study surgical procedures and were responsible for clinical care of participants while they were hospitalized as well as postoperative follow-up. In addition, the neurosurgeon were responsible for reviewing all brain MRIs and computed tomography scans for safety purposes throughout the course of this study to maintain blinding.

Figure 1. Patient Flow Diagram



The research team was responsible for recruitment, obtaining informed consent, and for performing the study visits. The research team remained blinded to a participant's treatment group assignment throughout the study.

In the treatment group, AAV2-NGF was administered in a single stereotactic neurosurgical procedure under general anesthesia as previously described.[17] Participants were admitted to the hospital the day before surgery. On the morning of surgery, the participant was administered general anesthesia with endotracheal intubation to prevent inadvertent movement during surgery. An MRI-compatible stereotactic head frame and fiducial localizer was attached and secured to the skull. A contrast-enhanced MRI scan was then acquired and used to calculate precise coordinate for the injection targets in the basal forebrain. Images were reformatted on a Stealth Station (Medtronic, Inc), and trajectories were planned to avoid sulci, ventricles, and end approximately 5 mm from the pial surface. AAV2-NGF was manually injected via specially designed stereotactic needles in each of 2 sites along a single stereotactic tract in each hemisphere targeting the NBM. In the sham surgery group, partial burr holes were covered with titanium plates and the scalp closed. Postoperative MRI scans confirmed targeting and were reviewed for safety.

Clinical Assessments

The primary end point was the change from baseline in the ADAS-Cog 11[24] to the total score at 24 months posttreatment. The change from baseline at 24 months in ADAS-Cog 11 was assessed using a mixed-model repeated-measures analysis model to test the treatment effect with baseline ADAS-Cog 11 score and other potential variables as covariates. Secondary end points (change from baseline at 24 months) included Clinical Dementia Rating–sum of boxes (CDR-SOB),[25] modified Clinical Global Impression of Change,[26] MMSE, Neuropsychiatric Inventory,[27] and Alzheimer Disease Cooperative Study-Activities of Daily Living.[28] After surgery, participants entered the treatment observation period, which lasted 24 months. Participants were evaluated on day 1, week 2, and month 1, 3, 6, 9, 12, 15, 18, 22, and 24. Safety was assessed by reports of adverse events, physical and neurological examination, laboratory tests, and MRI results. All safety data were regularly reviewed by the Data Safety Monitoring Committee. Participants were invited to enroll into an extended observation phase at the completion of the 24-month study period.

Table 1. Baseline Demographics (N = 49)[a]

Characteristic	Mean (SD)		
	Placebo Group (n = 23)	Treatment Group (n = 26)	*P* Value
Age, y	68.0 (0)	68.0 (6.4)	.74
Men, No. (%)	14 (54)	14 (61)	.38
APOE ε4 carrier, No. (%)	16 (69%)	17 (65%)	.69
ADAS-Cog 11	20.3 (8.2)	20.4 (7.6)	.74
CDR-SOB	4.7 (2.0)	4.8 (2.1)	.94
mCGIC	4.91 (1.0)	4.81 (1.1)	.55
MMSE	22.1 (3.2)	21.2 (3.8)	.42
NPI	8.8 (8.9)	8.6 (9.4)	.76
ADCS-ADL	65.7 (7.9)	65.2 (11.0)	.66

Abbreviations: ADAS-Cog 11, Alzheimer's Disease Assessment Scale–cognitive subscale; ADCS-ADL, Alzheimer's Disease Cooperative Study–Activities of Daily Living; APOE, apolipoprotein E; CDR-SOB, Clinical Dementia Rating–sum of boxes; mCGIC, modified Clinical Global Impression of Change; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

[a] There were no statistically significant differences between baseline demographics in the 2 arms of the study.

Table 2. Safety Summary[a]

Characteristics	Placebo Group (n = 23)	Treatment Group (n = 26)	Overall (N = 49)
No. of AEs	279	349	628
Mild AEs, No. (%)	199 (71)	232 (66)	431 (68)
Moderate AEs, No. (%)	64 (23)	95 (27)	159 (25)
Severe AEs, No. (%)	16 (5)	22 (6)	38 (6)
Participants with ≥1 AEs	23	26	49
No. of SAEs	13	16	29
Participants with SAE, No. (%)	8 (30)	7 (30)	30.6

Abbreviations: AE, adverse events; SAE, serious adverse events.

[a] There were 6 deaths (placebo: 4 vs treatment: 2). None of the SAEs were deemed related to study procedures. There were no clinically meaningful changes in laboratory test results.

If the analysis indicated efficacy, all participants in the placebo group would be invited to enroll into an open-label arm.

FDG PET Methods

[^{18}F]-FDG-PET scans were obtained at baseline and at month 6, 12, and 24. The [^{18}F]-FDG-PET scans were used to provide an assessment of the impact of AAV2-NGF on regional brain metabolic activity in the bilateral posterior cingulate gyri, an area of the brain particularly affected in AD.[29] Briefly, region of interest averages for each scan were scaled by the posterior cerebellum to provide relative metabolic data by region and to correct for differences in injected FDG dose and scanning site, as previously described.[17]

MRI Methods

Magnetic resonance imaging at baseline and at month 1, 12, 24, and 60 were acquired using General Electric, Philips, or Siemens 1.5 and 3.0 T scanners. Site personnel scanned participants longitudinally on the same scanner using a consistent protocol and quality checks confirmed that parameters held constant. Volumetric MRI analysis was performed on 3-dimensional T1-weighted volumes acquired sagittally with imaging parameters modeled on the nonaccelerated T1-weighted sequence from the Alzheimer Disease Neuroimaging Initiative. NeuroQuant (CorTechs Laboratories) image preprocessing and automated segmentation was used to measure whole-brain, hippocampus, and lateral ventricle volumes.[30,31] Total brain volume, total ventricular volume, hippocampal volume, and rates of change were calculated for analysis of treatment effect after study completion.[32,33]

Blood, Serum, and Urine Assessments

Blood samples were collected at baseline and at each post-baseline visit for standard clinical chemistry and hematology panels, as well as AAV2 and NGF antibody titers. Apolipoprotein E genotyping was performed using real-time polymerase chain reaction restriction fragment length polymorphism analysis. Genomic DNA from blood was extracted using QIAamp DNA blood maxi kit (Qiagen) and *apolipoprotein E* genotyping performed using Applied Biosystems The TaqMan SNP Genotyping assay was run on a Bio-Rad CFX96.

Statistical Methods

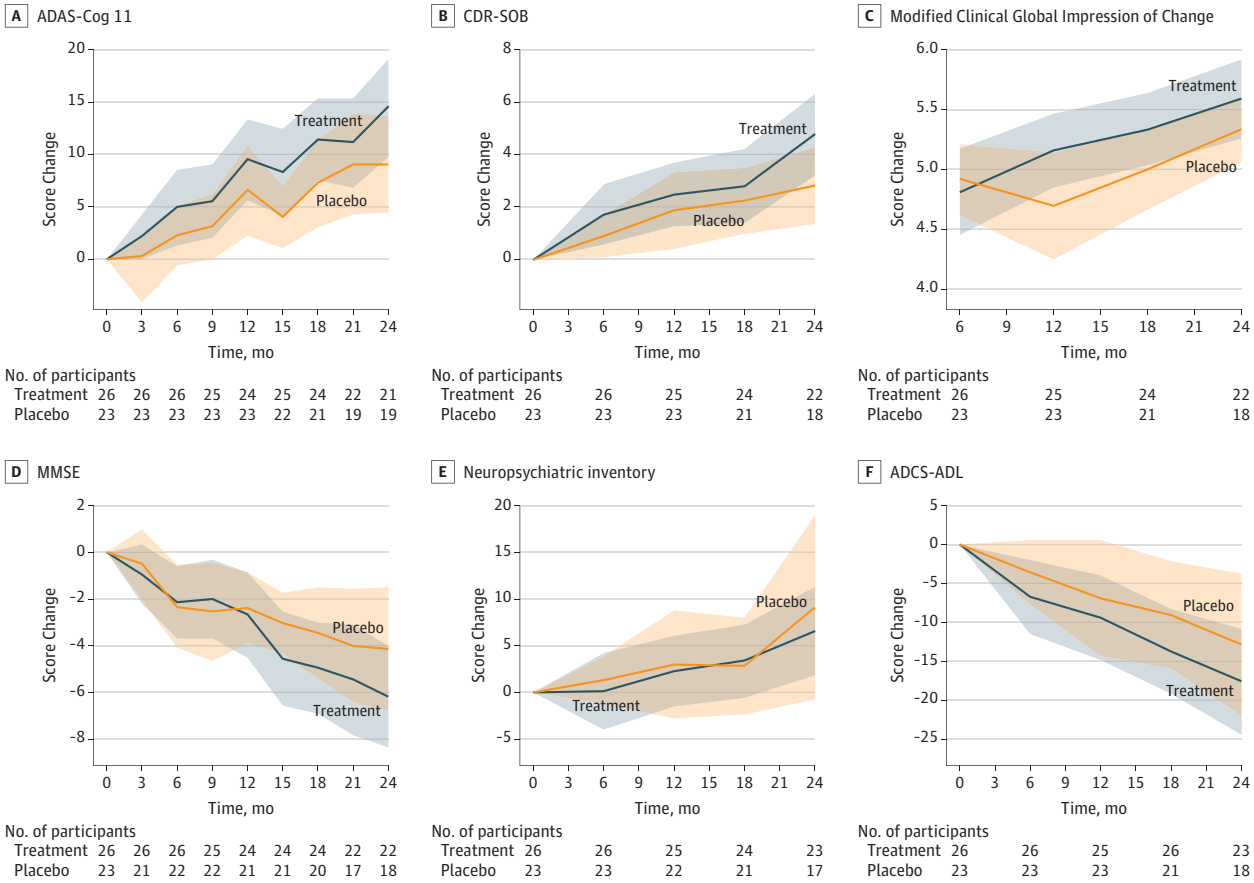
Efficacy analyses of all primary and secondary measures were based on a modified intention-to-treat population. Mixed-model repeated-measures analyses were used to assess between-group differences in change scores from baseline to month 24. The dependent variable in each mixed-model repeated-measures analysis was change from baseline. Mean change from the baseline and 95% confidence intervals are presented for each outcome measure. The following covariates were included in models testing efficacy: baseline age, sex, education, presence of apolipoprotein E ε4 allele, baseline MMSE score, and baseline hippocampal volume. Safety analyses were based on summary listings of adverse events, with Fisher exact test used for pairwise comparisons. Safety analyses were based on the full intention-to-treat population. The convenience sampling method was used for this study and was sufficiently powered to assess feasibility, safety, and tolerability but was not adequately powered to assess efficacy.

Results

Safety and Tolerability

No differences between the AAV2-NGF and placebo groups were found on laboratory test results, vital signs, or physical examinations. Five participants (10.2%) demonstrated nominal increases in anti-AAV2 antibodies, but these differences were not considered clinically significant. No participants developed anti-NGF antibodies. Adverse event collection began at screening. All adverse events reported were deemed by investigators as either not related or unlikely related to study drug; as a group, none showed any evidence for an AAV2-NGF dose relationship. A total of 628 adverse events (431 mild, 159 moderate, 38 severe) were reported (349 in the treatment group, 279 in the placebo group) (**Table 2**). The most common adverse events were headache (19 of 26 [73%] in the treatment group vs 16 of 23 [70%] in the placebo group; OR, 0.845; 95% CI, 0.203-3.504; $P > .99$) and dizziness (10 of 26 [38%] in the treatment group vs 9 of 23 [39%] in the placebo group; OR, 1.027; 95% CI, 0.278-3.774; $P > .99$). The most common serious adverse event was coronary artery occlusion (3 of 26 [11%] in the treatment group vs 0 of 23 (0%) in the placebo group; OR, 0; 95% CI, 0-2.680; $P = .28$); these occurred during 9 to 18 months after gene delivery and were therefore unlikely to be related to stereotaxic surgery. The second most common serious adverse event was convulsions in (2 of 26 [8%] the treatment group vs 1 of 23 (4%) in the placebo group; OR, 0.551; 95% CI, 0.008-11.301; $P > .99$).

Figure 2. Primary and Secondary End Points



A, Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-Cog 11). There was no benefit from adeno-associated viral vector (serotype 2)–nerve growth factor on ADAS-Cog 11 at 24 months. The mean change was 14.52 (95% CI, 9.86-19.18) vs placebo 9.11 (95% CI, 4.46-13.76); $P = .17$. B, Clinical Dementia Rating–sum of boxes (CDR-SOB) change score. The treatment group increased by 4.75 (95% CI, 3.20-6.30) points, while the placebo group increased by 2.81 (95% CI, 1.34-4.28) points ($P = .09$). C, The treatment group's modified Clinical Global Impression of Change (mCGIC) score increased by mean of 5.59 (95% CI, 5.26-5.92) points, while placebo increased by 5.33 (95% CI, 5.06-5.60) points ($P = .21$). D, The treatment group's Mini-Mental State Examination (MMSE) scores declined 6.18 (95% CI, 4.00-8.36) points vs 4.17 (95% CI, 1.50-6.84) points in the placebo group ($P = .16$). E, The treatment group's Neuropsychiatric Inventory score increased by 6.61 (95% CI, 1.85-11.37) points vs 9.18 (95% CI, −0.71 to 19.07) in the placebo group ($P = .95$). F, The treatment group's Alzheimer Disease Cooperative Study–Activities of Daily Living (ADCS-ADL) score change declined 17.65 (95% CI, 10.81-24.49) points vs 12.94 (95% CI, 3.75-22.13) in the placebo group ($P = .61$). With the exception of Neuropsychiatric Inventory, the treatment group tended to perform worse on all other clinical outcome measures. The error bands (shading) are the 95% confidence intervals.

Most adverse events were mild in severity (431 [68%] of the most common adverse events were mild; 159 [25%] were moderate; and 38 [6%] severe). There were no ongoing adverse events that could be attributed to AAV2-NGF. No treatment-related alterations in laboratory parameters were observed. All reported serious adverse events, and any adverse events that occurred in more than a single participant are listed in Table 1. There were 6 deaths (4 in placebo group, and 2 in treatment group); none were deemed related to drug or study procedures.

Primary Efficacy Measure

The primary end point was the change from baseline in the ADAS-Cog 11 to total score at 24 months posttreatment (**Figure 2**A). An increase in ADAS-Cog 11 indicated worse global cognition. No significant difference was noted between the treatment and placebo groups (mean [SD] change, 14.52 [4.66] vs 9.11 [4.65]; $P = .17$).

Secondary Outcome Measures

Secondary end points (change from baseline at 24 months) included CDR-SOB, modified Clinical Global Impression of Change, MMSE, Neuropsychiatric Inventory, and Alzheimer Disease Cooperative Study–Activities of Daily Living. Increased scores on CDR-SOB, modified Clinical Global Impression of Change, and Neuropsychiatric Inventory indicate worsening cognition and behavior. Increased scores on MMSE and Alzheimer Disease Cooperative Study–Activities of Daily Living indicate improved cognition and functioning, respectively. The results of the secondary outcome measures at month 24 are summarized in **Table 3** and Figure 2, with values being reported as mean change (95% CI). On the CDR-SOB, the treat-

Table 3. Change on Outcome Measures at 24 Months (N = 49)

Outcome Measure[a]	Mean Change (95% CI) Placebo Group (n = 23)	Treatment Group (n = 26)	*P* Value
ADAS-Cog 11[b]	9.11 (4.46 to 13.57)	14.52 (9.86 to 19.18)	.17
CDR-SOB	2.81 (1.34 to 4.28)	4.75 (3.20 to 6.30)	.09
mCGIC	5.33 (5.06 to 5.60)	5.59 (5.26 to 5.92)	.21
MMSE	−4.17 (−6.84 to 1.50)	−6.18 (−8.36 to 4.00)	.16
NPI	9.18 (−0.71 to 19.07)	6.61 (1.85 to 11.37)	.95
ADCS-ADL	−12.94 (−22.13 to 3.75)	−17.65 (−24.49 to 10.81)	.61

Abbreviations: ADAS-Cog 11, Alzheimer's Disease Assessment Scale–cognitive subscale; ADCS-ADL, Alzheimer Disease Cooperative Study–Activities of Daily Living; CDR-SOB, Clinical Dementia Rating–sum of boxes; mCGIC, modified Clinical Global Impression of Change; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

[a] In almost all outcome measures, there was a trend toward worsening in the treatment arm.

[b] There was no statistical difference in ADAS-Cog 11 at 24 months between treatment and placebo arms. Mean (SD) change was 14.52 (4.66) for treatment vs 9.11 (4.65) for the placebo group (*P* = .17).

ment group increased by 4.75 (95% CI, 3.2-6.3) points while the placebo group increased by 2.81 (95% CI, 1.34-4.28) points (*P* = .09) (Figure 2B). On the modified Clinical Global Impression of Change, the treatment group increased by mean of 5.59 (95% CI, 5.26-5.92) points, while placebo increased by 5.33 (95% CI, 5.06-5.60) points (*P* = .21) (Figure 2C). For the MMSE, the treatment group declined 6.18 (95% CI, 4.0-8.36) points vs 4.17 (95% CI, 1.50-6.84) (*P* = .16) (Figure 2D). On the Neuropsychiatric Inventory, the treatment group increased by 6.61 (95% CI, 1.85-11.37) points vs 9.18 (95% CI, −0.71 to 19.07) in the placebo group (*P* = .95) (Figure 2E). Finally, the treatment group's Alzheimer Disease Cooperative Study-Activities of Daily Living declined 17.65 (95% CI, 10.81-24.49) points vs 12.94 (95% CI, 3.75-22.13) points in the placebo group (*P* = .61) (Figure 2F).

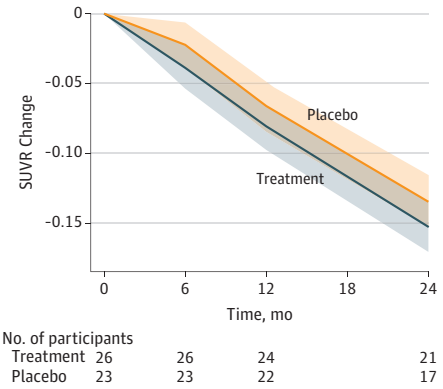
PET Imaging Results

[^{18}F]-FDG-PET were compared at month 24 between treatment and placebo. The ^{18}FDG PET scans were used to provide an assessment of AAV2-NGF on regional brain metabolic activity in the bilateral posterior cingulate gyri as measured by regional standardized uptake value ratio. In the treatment group, change in bilateral posterior cingulate gyri decreased by 0.152 (95% CI, 0.128-0.176) standardized uptake value ratio, while in the placebo group, it decreased by 0.142 (95% CI, 0.124-0.160) standardized uptake value ratio (*P* = .47). There was no statistical difference between the 2 groups at 24 months (**Figure 3**).

Volumetric MRI Imaging Results

Hippocampal volume declined 0.41 (95% CI, 0.33-0.49) cubic cm in the treatment group, while in placebo, it declined by 0.43 (95% CI, 0.36-0.50) cubic cm at month 24 (*P* = .95). In the treatment group, ventricular volume increased by 52.16 (95% CI, 47.15-57.17) change units, while it increased by 44.84 (95% CI, 34.90-54.78) change units in the placebo group (*P* = .64) (**Figure 4**A and B). The change units for ventricle volume is a summation of baseline volume with percent deformation at follow-up. These differences all did not have statistical significance.

Figure 3. Bilateral Posterior Cingulate Change in Regional Cerebral Blood Glucose Metabolism



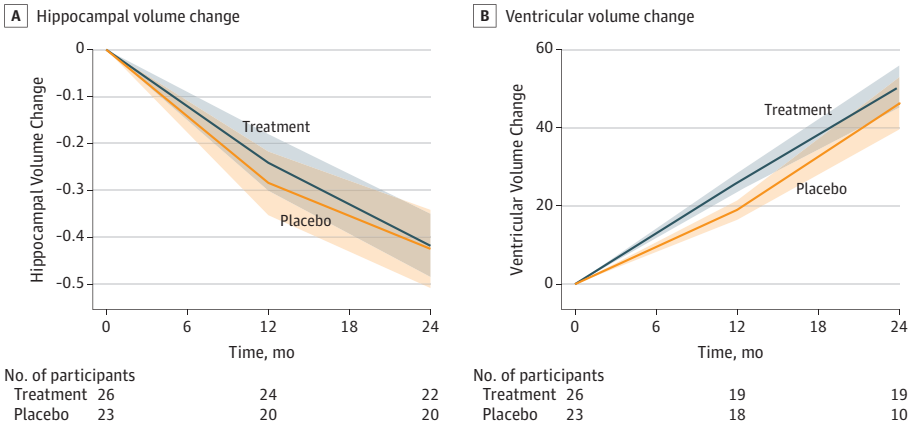
Change in bilateral posterior cingulated gyri decreased by 0.152 (95% CI, 0.128-0.176) standardized update value ratio (SUVR). In the placebo group it decreased by 0.142 (95% CI, 0.124-0.302) SUVR.

Discussion

This phase 2 study demonstrated that AAV2-NGF delivery is feasible, safe, and well tolerated in patients with mild to moderate AD. However, AAV-NGF2 had no benefit on cognition at 24 months after treatment. Analysis of secondary outcomes likewise showed no treatment benefit. Magnetic resonance imaging as well as [^{18}F]-FDG-PET scans did not demonstrate differences between treatment and placebo. Additionally, there were no differences in serious adverse event rates between the treatment and placebo groups.

The study was underpowered to detect efficacy because of the small sample size; our objective was to determine the feasibility of conducting a double-blinded, sham surgery–controlled clinical trial in AD, and this was confirmed. The likelihood of detecting benefit may have been influenced by vector mistargeting to the intended brain region: a preliminary analysis of 3 brains from the phase 1 AAV2-NGF trial suggests that at least two-thirds of NGF injection sites were mistargeted (analyses are ongoing).

Figure 4. Magnetic Resonance Imaging Volumetric Measures



A, Hippocampal volume declined 0.41 (95% CI, 0.39-0.49) cm^3 in the treatment group, while in the placebo group, it declined by 0.43 (95% CI, 0.36-0.50) cm^3 at month 24. B, In the treatment group, ventricular volume increased by 52.16 (95% CI, 47.15-57.17) change units, while it increased by 44.84 (95% CI, 34.91-54.78) change units in the placebo group.

It is also possible that NGF, if adequately targeted, would be ineffective in improving AD cognition because the pathology in patients with symptomatic AD is too far advanced. Finally, NGF treatment may fail because trophic factor treatment directed solely to the cholinergic component of neurodegeneration is insufficient to alter the clinical course of AD.

Limitations

This study had limitations. First, amyloid PET imaging or cerebrospinal fluid analysis was not used to assure the exclusion of individuals with non-AD diagnoses. Second, the sample size greatly curtailed the power to detect all but a large effect size. Similarly, the small sample size was too small to conclude that NGF worsened outcomes compared with untreated patients.

Conclusions

The study design elements used in this trial will inform future studies of other neurotrophins, such as brain-derived neurotrophic factor,[34,35] that are being considered for AD therapy. We were able to successfully use a placebo control for the intervention used in this clinical trial. Specifically, the use of sham surgery, which included general anesthesia, variable operating room time, and scalp and skull incisions, makes this sham a reasonable control for the operative procedure and avoided placing an intracerebral needle. This knowledge will be helpful for planning future clinical trials in AD that will involve surgical interventions.

ARTICLE INFORMATION

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Study concept and design: Rafii, Tuszynski, Barba, Siffert, Aisen.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Rafii, Tuszynski, Thomas, Rissman.
Critical revision of the manuscript for important intellectual content: Rafii, Tuszynski, Barba, Brewer, Rissman, Siffert, Aisen.
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Administrative, technical, or material support: Rafii, Tuszynski, Barba, Brewer, Rissman, Siffert.
Study supervision: Rafii, Rissman, Siffert, Aisen.

Conflict of Interest Disclosures: Dr Tuszynski founded Ceregene, Inc but dissociated from the company in 2013 and had no conflict of interest subsequent to that time. Dr Siffert was an employee of Ceregene, Inc and owned stock, is a full-time employee of Nestle Health Science, and is a shareholder in Alcobra Pharma and AveXis. Dr Brewer is a consultant for Elan, Bristol-Myers Squibb, Avanir, Novartis, Genentech, and Eli Lilly and holds stock options in CorTechs Labs, Inc and Human Longevity, Inc. Dr Aisen is a consultant to NeuroPhage, Merck, Roche, Novartis, Lundbeck, Biogen, Probiodrug, Pfizer, Anavex, and Abbvie and receives grants from Eli Lilly and Company, Janssen, the Alzheimer's Association, and the National Institutes of Health. No other conflicts were reported.

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