



Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial

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Summary

Background In an open-label phase 1 trial, gene delivery of the trophic factor neurturin via an adeno-associated type-2 vector (AAV2) was well tolerated and seemed to improve motor function in patients with advanced Parkinson's disease. We aimed to assess the safety and efficacy of AAV2-neurturin in a double-blind, phase 2 randomised trial.

Methods We did a multicentre, double-blind, sham-surgery controlled trial in patients with advanced Parkinson's disease. Patients were randomly assigned (2:1) by a central, computer generated, randomisation code to receive either AAV2-neurturin ($5 \cdot 4 \times 10^{11}$ vector genomes) injected bilaterally into the putamen or sham surgery. All patients and study personnel with the exception of the neurosurgical team were masked to treatment assignment. The primary endpoint was change from baseline to 12 months in the motor subscore of the unified Parkinson's disease rating scale in the practically-defined off state. All randomly assigned patients who had at least one assessment after baseline were included in the primary analyses. This trial is registered at ClinicalTrials.gov, NCT00400634.

Results Between December, 2006, and November, 2008, 58 patients from nine sites in the USA participated in the trial. There was no significant difference in the primary endpoint in patients treated with AAV2-neurturin compared with control individuals (difference $-0 \cdot 31$ [SE 2·63], 95% CI $-5 \cdot 58$ to $4 \cdot 97$; $p=0 \cdot 91$). Serious adverse events occurred in 13 of 38 patients treated with AAV2-neurturin and four of 20 control individuals. Three patients in the AAV2-neurturin group and two in the sham surgery group developed tumours.

Interpretation Intraputamin AAV2-neurturin is not superior to sham surgery when assessed using the UPDRS motor score at 12 months. However, the possibility of a benefit with additional targeting of the substantia nigra and longer term follow-up should be investigated in further studies.

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Introduction

Parkinson's disease is a common neurodegenerative disorder characterised clinically by bradykinesia, rigidity, tremor, and gait dysfunction, and pathologically by degeneration of dopamine neurons in the substantia nigra pars compacta. Present therapies provide satisfactory disease control for most patients, particularly in the early stages. However, chronic levodopa treatment is associated with motor complications, does not control potentially disabling features such as falling and dementia, and fails to prevent disease progression.¹ Thus, many patients suffer disability despite available medical and surgical treatments. More effective treatments that improve clinical disease control and slow progression are urgently needed.

Neurotrophic factors might improve neuronal function and protect against neurodegeneration. Glial-cell-derived neurotrophic factor (GDNF) protects dopamine neurons in in-vitro and animal models of Parkinson's disease.^{2,3} Neurturin is a naturally occurring structural and functional analogue of GDNF⁴ that improved dopaminergic activity in aged monkeys⁵ and also protected dopamine neurons in animal models of Parkinson's disease.⁶⁻⁹ Results from open-label trials have shown benefits of continuous

infusion of GDNF into the putamen in patients with advanced Parkinson's disease.^{10,11} However, these results were not confirmed in double-blind studies,^{12,13} possibly because the trophic factor was not adequately distributed throughout the target region.^{14,15}

Gene delivery has the potential to provide diffuse distribution and long-lasting expression of a therapeutic protein in one surgical procedure, and gene delivery of neurturin and GDNF provides long-term histological and behavioural benefits in primate models of Parkinson's disease.^{5,9,16,17} Adeno-associated type-2 (AAV2)-neurturin is a vector that has been genetically engineered to express and secrete the human gene for neurturin.⁸ The AAV2 vector does not induce an inflammatory reaction, has been used safely in clinical trials, and provides long-lasting transgene expression.¹⁸ An open-label, 12-month phase 1 trial of bilateral stereotactic intraputamin injections of AAV2-neurturin in patients with advanced Parkinson's disease showed that the treatment was safe, well tolerated, and associated with benefits in motor functions.¹⁹

We therefore aimed to assess the safety and efficacy of stereotactic surgery with injections of AAV2-neurturin versus sham surgery in patients with advanced Parkinson's disease in a double-blind, randomised trial.

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Methods

Patients

We did a double blind, randomised, multicentre trial. Men and women who were of any ethnic group, were 35–75 years of age, and had idiopathic Parkinson's disease according to the UK Brain Bank Criteria²⁰ with disease duration of at least 5 years were eligible. Inclusion criteria were a good response to levodopa in the opinion of the treating investigator and levodopa-induced motor complications that could not be satisfactorily controlled with medical therapy; at least 2 h per day of off time (poor motor function) according to home diaries; a score of at least 30 on the motor section (part 3) of the unified Parkinson's disease rating scale (UPDRS) in the off state (range 0–108, with higher scores suggesting more severe disease); and stable doses of antiparkinsonian drugs for at least 1 month before enrolment. Exclusion criteria were atypical or secondary parkinsonism, mini-mental state examination score of 26 or less, previous neurosurgical treatment for Parkinson's disease, and clinically significant medical, psychiatric, or laboratory abnormalities.

An independent data monitoring committee, comprising three movement disorder neurologists, two functional neurosurgeons, a neuroscientist familiar with gene therapy, and a statistician, monitored the study. A central neuroradiologist reviewed all MRI scans and reported findings directly to the data monitoring committee. They met quarterly and ad hoc to review all safety data (unmasked to treatment group) and had the authority to halt the study at any time, but were not directly involved in the study conduct.

The protocol, including the ethics of sham surgery, was approved by the institutional review boards at each of the participating institutions and by the recombinant DNA advisory committee of the National Institutes of Health, where the protocol was publicly reviewed, discussed, and endorsed. Patients signed written informed consent forms before enrolment that were approved by the institutional review boards.

Randomisation and masking

Patients were randomly assigned (2:1) to receive treatment with bilateral stereotactic intraputamin injections of AAV2-neurturin (CERE-120; Ceregene, San Diego, CA, USA) or a sham procedure by a central, computer generated, randomisation code. All patients and study personnel with the exception of the neurosurgical team were masked to treatment assignment. Patients were assessed by a masked treating investigator at baseline and follow-up. A separate masked investigator did the UPDRS assessments. Masking was not broken until the last enrolled patient completed the 12-month visit.

Procedures

Stereotactic surgery was done with neuroimaging to plan injection trajectories. Patients were anaesthetised with deep propofol sedation and a treatment kit was then

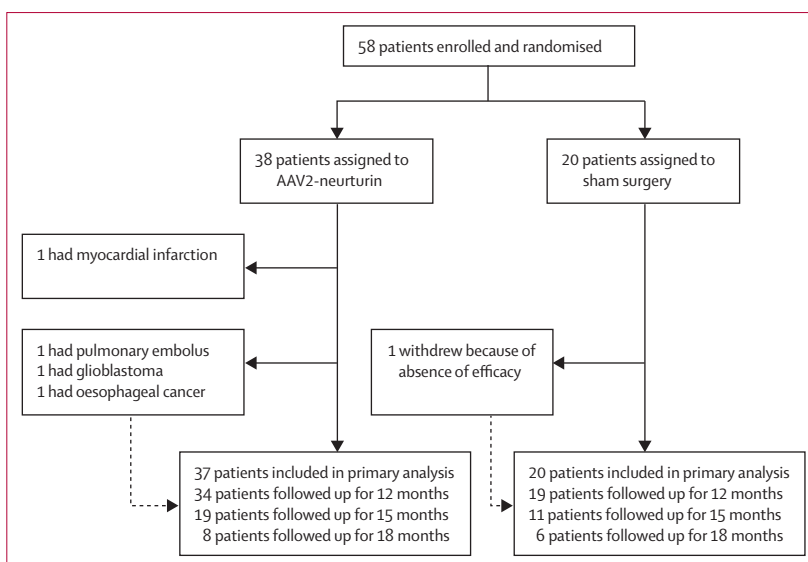


Figure 1: Trial profile

	AAV2-neurturin (n=38)	Sham surgery (n=20)
Age (years)	60.1 (7.6)	57.3 (8.3)
Men	28 (74%)	15 (75%)
Duration of Parkinson's disease from diagnosis (years)	9.5 (3.4)	10.0 (4.9)
Hoehn and Yahr stage in the off state	3.03 (0.5)	3.05 (0.8)
UPDRS score in the off state		
Part 1	2.3 (1.6)	2.7 (1.5)
Part 2	18.5 (4.9)	18.8 (5.1)
Part 3	38.7 (8.5)	39.0 (9.4)
UPDRS scores in the on state		
Part 1	1.6 (1.4)	1.9 (1.7)
Part 2	8.3 (5.8)	8.8 (6.1)
Part 3	17.1 (7.5)	16.6 (7.6)
Home diary assessment of motor state		
On without troublesome dyskinesia (h/day)	10.7 (2.8)	9.5 (2.5)
On with troublesome dyskinesia (h/day)	1.0 (1.4)	1.3 (1.9)
Off (h/day)	5.2 (2.7)	6.1 (2.7)
Levodopa, immediate release (mg)	712.5 (492.8)	720.0 (459.4)
Levodopa dose equivalents (mg)*	1046.9 (551.8)	1108.7 (640.7)

Data are mean (SD) or number (%). UPDRS=unified Parkinson's disease rating scale. *100 mg levodopa was considered equivalent to 3 mg apomorphine, 10 mg bromocriptine, 133 mg carbidopa/levodopa controlled release, 1 mg pergolide, 1 mg pramipexole, 3 mg ropinirole, 1.33 mg rotigotine.

Table 1: Demographics and baseline characteristics

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opened to establish assignment group. For patients assigned to active treatment, a gene transfer procedure was done with AAV2 as a vector to deliver DNA-encoding neurturin to the putamen. AAV2-neurturin in a total brain dose of 5.4×10^{11} vector genomes was administered

bilaterally through frontal burr holes with four tracts per side and two deposits per tract separated by about 5 mm. Instructions given to each neurosurgeon and their staff stated that “The four ventral targets should be distributed evenly throughout the anterior–posterior extent of the putamen and relatively centred in the lateral dimension. At least two targets should be posterior to the anterior commissure and at least one target should be anterior to the commissure. The rostral targets are achieved by raising the needle by 4 mm along the same trajectory. Targets should avoid the edges of the putamen and any extremely narrow area at the posterior end of the putamen (ie, a tail). Trajectory planning should avoid crossing the ventricles, sulci, and vascular structures. The ventral targets should be separated from each other by 5–7 mm and should lie

within 0–4 mm superior to the intercommissural plane. CERE-120 [AAV2-neurturin] will be given to each ventral target location with a volume of 5 µL per target at a rate of 2 µL/min. After each deposit, the needle is kept in place for 3 min to allow drug distribution and avoid backflow. This procedure is to be repeated for each of the remaining needle tracks—ie, a total of four needle tracks per putamen to a total volume of 40 µL per hemisphere.” Patients assigned to sham surgery underwent an identical procedure except partial thickness burr holes that did not penetrate the inner table of the skull were made and intracranial injections were not done.

Patients were assessed at baseline and months 1, 3, 6, 9, and 12 after surgery and every 3 months thereafter until the final patient enrolled had completed the

	AAV2-neurturin (n=37)	Sham surgery (n=20)	AAV2-neurturin–sham surgery	p value
Primary endpoint				
UPDRS part 3 score in the off state	-7.21 (1.56, -10.34 to -4.09)	-6.91 (2.12, -11.16 to -2.66)	-0.31 (2.63, -5.58 to 4.97)	0.91
Secondary endpoints				
UPDRS score in the off state				
Part 1	-0.44 (0.29, -1.02 to 0.13)	1.17 (0.39, 0.39 to 1.96)	-1.62 (0.49, -2.59 to -0.64)	0.0017
Part 2	-3.40 (0.86, -5.11 to -1.68)	-2.17 (1.16, -4.5 to 0.17)	-1.23 (1.44, -4.13 to 1.66)	0.40
UPDRS score in the on state				
Part 1	0.24 (0.30, -0.37 to 0.84)	0.76 (0.41, -0.06 to 1.58)	-0.52 (0.51, -1.55 to 0.50)	0.31
Part 2	-0.93 (0.68, -2.3 to 0.43)	1.68 (0.93, -0.18 to 3.53)	-2.61 (1.15, -4.91 to -0.31)	0.027
Part 3	0.03 (1.10, -2.18 to 2.23)	-1.75 (1.49, -4.74 to 1.25)	1.77 (1.85, -1.94 to 5.49)	0.34
Home diary assessment of motor state (h/day)				
On state without troublesome dyskinesia	1.25 (SD 0.50, 0.25 to 2.25)	0.34 (SD 0.68, -1.03 to 1.7)	0.91 (SD 0.86, -0.8 to 2.63)	0.29
On state with troublesome dyskinesia	-0.36 (SD 0.24, -0.84 to 0.12)	-0.07 (SD 0.33, -0.72 to 0.59)	-0.29 (SD 0.41, -1.1 to 0.52)	0.47
Off state	-1.21 (0.43, -2.07 to -0.35)	0.16 (0.59, -1.02 to 1.33)	-1.36 (0.73, -2.83 to 0.1)	0.067
Timed walking test*				
Off state (s)	-2.65 (4.36)	-3.00 (1.67)	NA	0.56
On state (s)	0.38 (0.99)	0.60 (0.75)	NA	0.50
Purdue pegboard test—most affected hand				
Number of pegs in the off state	0.85 (0.34, 0.17 to 1.54)	0.92 (0.46, -0.01 to 1.85)	-0.07 (0.58, -1.22 to 1.09)	0.91
Number of pegs in the on state	0.62 (0.29, 0.03 to 1.21)	-0.14 (0.40, -0.95 to 0.66)	0.76 (0.50, -0.23 to 1.76)	0.13
Purdue pegboard test—least affected hand				
Number of pegs in the off state	0.91 (0.31, 0.29 to 1.54)	1.18 (0.43, 0.33 to 2.04)	-0.27 (0.53, -1.33 to 0.79)	0.61
Number of pegs in the on state	0.21 (0.29, -0.37 to 0.79)	0.11 (0.40, -0.68 to 0.91)	0.09 (0.49, -0.89 to 1.08)	0.85
Dyskinesia rating scale†				
Off state	-0.08 (0.05)	0.05 (0.15)	NA	0.15
On state	-0.22 (0.10)	-0.10 (0.22)	NA	0.69
PDQ-39 single index score	-3.83 (1.77, -7.39 to -0.28)	3.06 (2.44, -1.84 to 7.96)	-6.89 (3.08, -13.06 to -0.72)	0.029
SF-36 physical health composite score	2.25 (1.14, -0.05 to 4.55)	1.04 (1.48, -1.94 to 4.03)	1.21 (1.88, -2.56 to 4.98)	0.52
CGI severity of illness‡	-0.51 (0.15)	-0.50 (0.15)	NA	0.92
CGI global improvement‡				
Masked rater	3.34 (0.16)	3.75 (0.14)	NA	0.11
Investigator	3.60 (0.17)	3.70 (0.18)	NA	0.67
Patient	3.69 (0.26)	4.05 (0.34)	NA	0.36
Levodopa dose equivalent (mg)	101.97 (SD 362.93, -48.20 to 241.29)	-6.75 (SD 601.55, -193.66 to 200.24)	NA	0.45

Data are least squares mean (SE, 95% CI) or least squares mean (SE) unless otherwise stated. p values derived from ANCOVA unless otherwise stated. UPDRS=unified Parkinson's disease rating scale. NA=not available. PDQ-39=Parkinson's disease questionnaire-39. SF-36=short form-36. CGI=clinical global impression. *p values based on Wilcoxon-Mann-Whitney test. †p values based on Cochran-Mantel-Haenszel row mean score test.

Table 2: Changes in primary and secondary outcome measures from baseline to 12 months

12-month study. At each visit up to 12 months, patients were assessed with the UPDRS²¹ in the practically-defined off state (around 12 h after the last dose of antiparkinsonian drug) and in the best on state (best response to morning dose of antiparkinsonian drug). The motor subscale (part 3) of the UPDRS was also done in the practically-defined off state at each visit after 12 months for the subset of patients who continued to have masked assessments until the last patient enrolled had completed the 12-month study. Home diary assessment of motor state, timed motor tests, the dyskinesia rating scale, and the clinical global impression assessment were done at baseline, 6 months, and at each visit thereafter. Quality of life was assessed with the Parkinson's disease questionnaire (PDQ)-39²² and short form (SF)-36²³ at baseline and 12 months. Safety assessments were done at each visit. Antiparkinsonian drugs were maintained at a constant level throughout the study.

The primary endpoint was change in UPDRS motor score (part 3) in the practically-defined off state from baseline to 12 months. Secondary outcomes were change from baseline to final visit in UPDRS motor score in the on state, UPDRS subscores, timed motor tests, dyskinesia rating, home diary rating of motor function, PDQ-39 score, SF-36 score, and clinical global impression score. We also did a prespecified analysis of the primary and secondary outcome measures that compared change from baseline to 18 months for all patients who had masked assessments after month 12 (months 15 and 18) using the last visit carried forward for those who had assessments only at month 15. Safety was assessed by spontaneous reports of adverse events, physical examination, laboratory tests, and MRI. All safety data were regularly reviewed by the data monitoring committee.

Statistical analysis

For 90% power to detect a difference of 10 (SD 10) points between groups on the UPDRS motor subscale, with 2:1 random allocation using a two-sided *t* test with $\alpha=0.05$, a sample size of 51 patients would be required. Primary and secondary endpoints were analysed by ANCOVA, with treatment group as a main effect and baseline UPDRS motor score in the practically-defined off state as a covariate. All randomly assigned patients who had at least one post-baseline assessment were included in efficacy analyses. Last observation carried forward was done to account for missing data. For confirmatory purposes, we also did prespecified repeated measures analyses with linear mixed models; these analyses use the observed data from each patient with no imputation for missing data. All randomly allocated patients were included in the safety analysis. Patient characteristics at baseline and at specified timepoints are summarised with SD, but estimates of treatment effects and of differences between treatment groups are summarised with SE. No statistical adjustments were made for multiple comparisons.

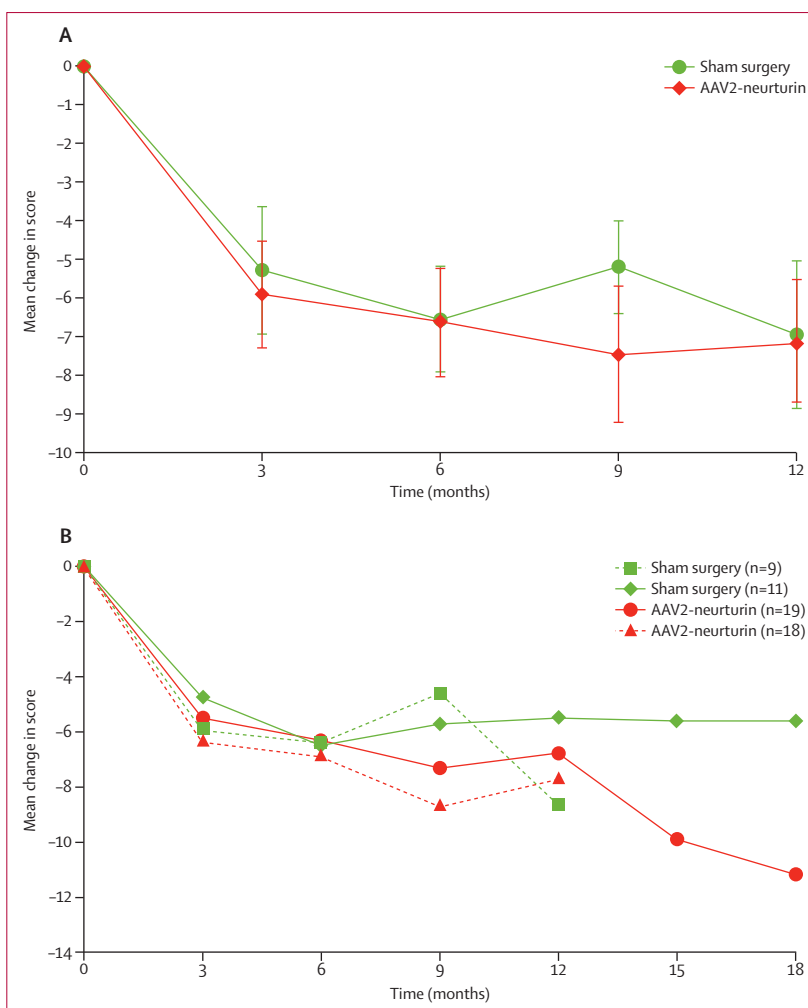


Figure 2: Change in unified Parkinson's disease rating scale part 3 score in the off state

(A) Mean change from baseline for all patients with at least one assessment after surgery. (B) Mean change from baseline in the subgroup of patients who had masked visits after month 12 (n=30), with last observation carried forward for those who had completed assessments only to month 15 (n=16). Solid lines represent patients who were followed up for longer than 12 months. Dotted lines represent patients who were followed for only 12 months. Bars=SE.

This trial is registered with ClinicalTrials.gov, NCT00400634.

Role of the funding source

Ceregene funded the study and was responsible for supplying AAV2-neurturin, collecting data, clinical monitoring, and statistical analysis. A grant was also provided by the Michael J Fox Foundation for Parkinson's Research to help pay costs. All authors were responsible for the study design, data analysis, data interpretation, writing of the report, and the decision to submit for publication. All authors had complete access to the database, which contained all data in the study, after the database was locked, did independent statistical analyses, and vouch for the completeness and accuracy of the data.

Results

Between December, 2006, and November, 2008, 58 eligible patients from nine sites in the USA participated in the trial (figure 1). 38 patients were assigned to AAV2-neurturin and 20 to sham surgery. There were no significant differences in demographics and baseline characteristics between groups (table 1). 53 patients completed all scheduled visits of the 12-month study (figure 1).

Table 2 shows the results of the primary and secondary outcome measures at 12 months. UPDRS part 3 score was 38.65 (SD 8.46) at baseline and 31.46 (10.45) at 12 months in the AAV2-neurturin group and 39.00 (9.44) at baseline and 32.05 (13.97) at 12 months in the sham surgery group (p for difference between groups 0.91).

Figure 2A shows the mean change from baseline in UPDRS motor off score at each visit. Several secondary outcome measures favoured AAV2-neurturin treatment at 12 months: the activities of daily living subscale (part 2) of the UPDRS in the on state (p=0.027), mental subscale (part 1) of the UPDRS in the off state (p=0.0017), and PDQ-39 single index score (p=0.029). There was also weak evidence of improvement in off time in home diary assessments (p=0.067). No secondary outcome measure favoured the sham surgery group. Results were not influenced by baseline UPDRS score (data not shown). A subset of patients were given fluorodopa PET scans at baseline (pretreatment) and at 12 months, and no significant difference in uptake was observed between the two groups or timepoints (webappendix).

See Online for webappendix

	AAV2-neurturin (n=19)	Sham surgery (n=11)	AAV2-neurturin-sham surgery	p value
Primary endpoint				
UPDRS part 3 score in the off state	-11.96 (1.87, -15.8 to -8.7)	-4.34 (2.48, -9.52 to 0.75)	-7.61 (3.16, -14.1 to -1.13)	0.023
Secondary endpoints				
UPDRS score in the off state				
Part 1	-0.34 (0.43, -1.01 to 0.2)	1.40 (0.57, 0.22 to 1.88)	-1.74 (0.72, -3.21 to -0.27)	0.022
Part 2	-3.37 (1.36, -5.1 to -1.24)	0.91 (1.79, -4.46 to 0.79)	-4.28 (2.25, -8.9 to 0.35)	0.069
UPDRS score in the on state				
Part 1
Part 2
Part 3	-2.98 (1.27, -2.5 to 1.37)	-0.86 (1.67, -4.74 to 0.53)	-2.12 (2.10, -6.42 to 2.18)	0.32
Home diary assessment of motor state (h/day)				
On state without troublesome dyskinesia	2.72 (SD 0.85, 0.96 to 4.46)	-0.25 (SD 1.13, -2.57 to 2.06)	2.97 (SD 1.43, 0.03 to 5.91)	0.048
On state with troublesome dyskinesia	-0.94 (SD 0.25, -1.46 to -0.42)	-0.26 (SD 0.33, -0.94 to 0.43)	-0.68 (SD 0.42, -1.54 to 0.18)	0.11
Off state	-1.92 (0.73, -3.41 to -0.42)	0.23 (0.97, -1.75 to 2.22)	-2.15 (1.23, -4.67 to 0.37)	0.0907
Timed walking test*				
Off state (s)	-8.11 (3.25)	-0.55 (0.81)	NA	0.021
On state (s)	0 (1.00)	0.09 (0.58)	NA	0.53
Purdue pegboard test—most affected hand				
Number of pegs in the off state	1.50 (0.52, 0.43 to 2.56)	1.33 (0.68, -0.08 to 2.73)	0.17 (0.87, -1.6 to 1.94)	0.85
Number of pegs in the on state	0.96 (0.41, 0.12 to 1.8)	0.93 (0.54, -0.17 to 2.04)	0.03 (0.68, -1.37 to 1.42)	0.97
Purdue pegboard test—least affected hand				
Number of pegs in the off state	0.62 (0.64, -0.7 to 1.94)	0.80 (0.86, -0.96 to 2.55)	-0.18 (1.09, -2.42 to 2.07)	0.87
Number of pegs in the on state	-0.09 (0.45, -1.01 to 0.83)	0.84 (0.60, -0.39 to 2.06)	-0.92 (0.77, -2.49 to 0.64)	0.24
Dyskinesia rating scale†				
Off state	0 (0.11)	-0.18 (0.18)	NA	0.96
On state	-0.26 (0.19)	0.18 (0.30)	NA	0.41
PDQ-39 single index score
SF-36 physical health composite score
CGI severity of illness†	-0.32 (0.15)	-0.27 (0.20)	NA	0.19
CGI global improvement†				
Masked rater	3.32 (0.19)	3.82 (0.23)	NA	0.13
Investigator	3.21 (0.21)	4.27 (0.27)	NA	0.0089
Patient	3.53 (0.40)	5.00 (0.38)	NA	0.031
Levodopa dose equivalent (mg)	110.93 (SD 1094.03, -553.40 to 781.25)	706.74 (SD 829.90, 36.42 to 1371.07)	NA	0.23

Data are least squares mean (SE, 95% CI) or least squares mean (SE) unless otherwise stated. Includes patients with follow-up visits at 15 and 18 months with last observation carried forward. p values derived from ANCOVA unless otherwise stated. UPDRS=unified Parkinson's disease rating scale. NA=not available. PDQ-39=Parkinson's disease questionnaire-39. SF-36=short form-36. CGI=clinical global impression. *p values based on Wilcoxon-Mann-Whitney test. †p values based on Cochran-Mantel-Haenszel row mean score test.

Table 3: Changes in primary and secondary outcome measures from baseline to 18 months

30 patients had double-blind assessments after month 12 and were included in the analyses at 18 months; last visit carried forward was done for the 16 patients who had visits at only 15 months (figure 1). In this subgroup, the UPDRS part 3 score was 35.84 (SD 7.72) at baseline and 24.63 (7.88) at 18 months in the AAV2-neurturin group

and 40.18 (7.24) at baseline and 34.55 (10.60) at 18 months in the sham surgery group (p for difference between groups 0.023; table 3). Figure 2B shows the mean change from baseline in UPDRS motor off scores at each visit in this subgroup. Significant improvement at 18 months was also noted with AAV2-neurturin compared with sham surgery for the mental subscale (part 1) of the UPDRS in the off state (p=0.022), on-time without troublesome dyskinesia (p=0.048), timed walking in the off state (p=0.021), and clinical global impression of improvement rated by the treating investigator (p=0.0089) and the patient (p=0.031; table 3). There was weak evidence of improvement with AAV2-neurturin for the activities of daily living subscale of the UPDRS in the off state (p=0.069) and daily off time (p=0.091). No secondary outcome measure favoured the control group at 18 months. The results of the analyses from the repeated measures regression model were similar to the results of the prespecified analyses (data not shown).

Table 4 shows relevant adverse events that affected the nervous system or events that occurred in more than 10% of patients. 37 serious adverse events occurred in 13 of 38 patients treated with AAV2-neurturin and four of 20 control individuals. Two patients in the AAV2-neurturin group died (one myocardial infarction at 47 days and one pulmonary embolism at 91 days postoperatively); these deaths were not judged to be related to AAV2-neurturin. Tumours occurred in three patients in the AAV2-neurturin group (one glioblastoma, one oesophageal adenocarcinoma, and one adenocarcinoma of the prostate) and two in the sham surgery group. Quantitative PCR assay on biopsied tissue was negative for AAV2-neurturin in each case. The glioblastoma was anatomically remote from the site of gene delivery and, upon closer examination, had been present on MRI before study entry. For these reasons, these tumours were not thought to be related to AAV2-neurturin by the study investigators or the data monitoring committee, although this possibility cannot be completely excluded. Serious adverse events in the AAV2-neurturin group that were related to the surgical procedure included confusion, seizure, haemorrhage, cerebral oedema, and caudate nucleus infarct (all in one patient), transient mental change (in one patient), and urinary retention (in two patients). In all cases, symptoms were transient and there was no residual neurological deficit. In control individuals, serious adverse events included parotid gland tumour, apocrine gland adenocarcinoma, chest pain, dyspnoea, fall, humerus fracture, cervical spine instability, and gastroenteritis. None was attributed to the study intervention.

Neither neurturin protein nor neurturin antibody was detected in the serum of any patient. An increase in titre of serum antibody was noted in ten patients treated with AAV2-neurturin (seroconversion or amplification); the increased titres were transient in two patients, decreased over time but remained above baseline in four, and were persistent in four. No clinical

	AAV2-neurturin (n=38)	Sham surgery (n=20)
Headache	26 (68%)	10 (50%)
Nausea	13 (34%)	6 (30%)
Post-procedural pain	11 (29%)	7 (35%)
Dyskinesia	9 (24%)	6 (30%)
Parkinson's disease worsening	8 (21%)	4 (20%)
Insomnia	8 (21%)	2 (10%)
Incision site complication	6 (16%)	2 (10%)
Postoperative headache	5 (13%)	5 (25%)
Back pain	5 (13%)	2 (10%)
Pain in extremity	5 (13%)	0 (0%)
Rash	5 (13%)	0 (0%)
Fall	4 (11%)	2 (10%)
Constipation	4 (11%)	3 (15%)
Urinary tract infection	4 (11%)	2 (10%)
Micturition urgency	4 (11%)	1 (5%)
Depression	4 (11%)	3 (15%)
Hallucination	3 (8%)	0 (0%)
Arthralgia	3 (8%)	3 (15%)
Tremor	2 (5%)	2 (10%)
Freezing phenomenon	2 (5%)	1 (5%)
Balance disorder	2 (5%)	0 (0%)
Brain oedema	2 (5%)	0 (0%)
Cognitive disorder	2 (5%)	0 (0%)
Anxiety	2 (5%)	4 (20%)
Hypaesthesia	1 (3%)	4 (20%)
Dystonia	1 (3%)	1 (5%)
Paraesthesia	1 (3%)	1 (5%)
Aphasia	1 (3%)	0 (0%)
Cerebral haemorrhage	1 (3%)	0 (0%)
Convulsion	1 (3%)	0 (0%)
Intracranial haemorrhage	1 (3%)	0 (0%)
Haemorrhagic cerebral infarction	1 (3%)	0 (0%)
Hemiparesis	1 (3%)	0 (0%)
Hypersomnia	1 (3%)	0 (0%)
Lethargy	1 (3%)	0 (0%)
Memory impairment	1 (3%)	0 (0%)
Confusional state	1 (3%)	2 (10%)
Abnormal dreams	1 (3%)	0 (0%)
Delirium	1 (3%)	0 (0%)
Parkinsonian gait worsening	0 (0%)	1 (5%)
Procedural hypertension	0 (0%)	2 (10%)
Agitation	0 (0%)	1 (5%)

Data are number of patients (%). Only events that affected relevant nervous system functions or that occurred in more than 10% of patients are listed.

Table 4: Adverse events

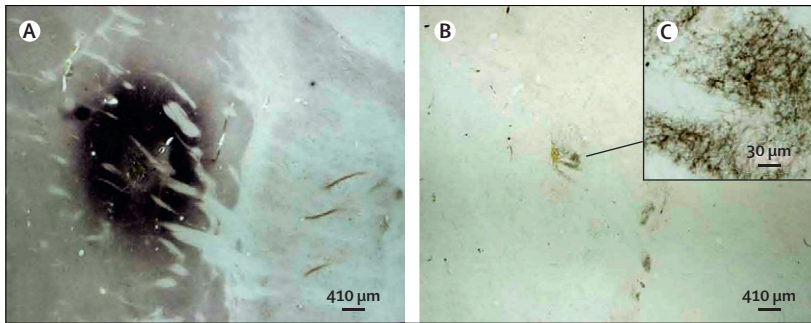


Figure 3: Immunohistochemistry sections from the putamen of a patient with Parkinson's disease who had AAV2 gene delivery of neurturin

(A) Neurturin staining in the putamen. (B) Adjacent section in the putamen stained for tyrosine hydroxylase. Although some tyrosine hydroxylase staining was seen in areas of neurturin expression, it was sparse. (C) Higher power (10 \times) photomicrograph.

manifestation, adverse event, or other correlates to this antibody response were reported.

Histological analysis was done on the brains of two patients who were treated with AAV2-neurturin.²⁴ Both had pathological evidence of Parkinson's disease with marked degeneration of the nigrostriatal tract. Staining for neurturin protein covered around 15% of the targeted putamen by volume, but only isolated neurturin deposits were detected in melanin-containing neurons in the substantia nigra. Small, circumscribed areas of tyrosine hydroxylase staining (a biomarker for dopaminergic activity) were seen within regions of neurturin staining in the putamen (figure 3). A complete description of the histological findings are described in a separate report.²⁴

Discussion

We report the results of the first double-blind, controlled phase 2 trial of a gene therapy for Parkinson's disease (panel). Patients who received AAV2-neurturin did not show significant improvement with respect to the primary outcome measure at 12 months compared with those who received sham surgery. At 18 months, AAV2-neurturin treatment was associated with modest, but significant, benefits in the primary outcome compared with controls, but the sample size was small. Several secondary measures favoured AAV2-neurturin at 12 and 18 months but none favoured the sham procedure at either timepoint. Clinically significant differences in the UPDRS motor score (part 3) have been estimated to be about 2.5 points for minimal, 5.2 for moderate, and 10.8 for large effects.²⁵ The difference between groups at 18 months was about 7.6 UPDRS points, and could be classed as moderate on the basis of these criteria. However, this was not the primary analysis and the sample size was small, so these data should be interpreted with caution. Because a large number of analyses and comparisons were made with no adjustment, the data should not be over-interpreted.

Many serious adverse events occurred in patients who received AAV2-neurturin, but most were associated with the surgery and were anticipated. Although the tumours that occurred were not thought to be caused

by AAV2-neurturin treatment, the potential for a trophic factor to induce or accelerate tumour growth must be considered and patients need to be followed up for longer periods of time both in extensions of this study and in future trials.

Although the distribution of neurturin protein in the putamen was restricted in histological assessments, its trophic influence can be much more extensive than can be detected with antibody labelling, and the level of expression reported here, or even less, is associated with benefits in rodent and monkey models of Parkinson's disease within the timeframes used in this study.^{5,7-9,26} However, the benefits of neurturin depend on transport of the protein (or DNA) from the striatum to the substantia nigra, because the protein needs to be expressed in neuronal cell bodies to induce upregulation of repair genes. Although neurturin distribution in the striatum of the patients who had an autopsy was comparable with that in animal models, neurturin expression in the substantia nigra was substantially less than is seen in animal models with comparable striatal expression.²⁴ We believe that the reduced neurturin expression in the substantia nigra of our patients was caused by the degeneration and loss of function of the nigrostriatal pathway that occurs in Parkinson's disease. Impaired transport of the trophic factor to the substantia nigra in patients with Parkinson's disease might have delayed the onset and limited the magnitude of the reported benefit. Benefits emerging after 12 months suggest the need for a longer time period for sufficient amplification of the neurturin signal to induce clinical benefit. This hypothesis could explain why the rate of change in the active and sham groups was similar for 12 months but diverged thereafter. At the 12 month timepoint, the subgroup of patients treated with AAV2-neurturin who were followed up for 18 months seemed to have similar effects to the subgroup of patients treated with AAV2-neurturin who were assessed for only 12 months (figure 2B). That multiple secondary endpoints favoured AAV2-neurturin but none favoured sham surgery further suggests that there was some beneficial effect of AAV2-neurturin. For these reasons, direct injections into the substantia nigra might need to be done in future studies, possibly in combination with higher putaminal doses, to obtain clinically meaningful benefits of gene delivery of neurturin, particularly within a 12 month time period. Mistargeted protein in the hypothalamus or other midbrain structures could induce adverse effects including weight loss and psychiatric problems, but no evidence of this has been seen with AAV-neurturin in any of our animal studies.

Although once thought controversial, the use of sham brain surgery is now regarded as essential to control for placebo effects in surgical interventions for Parkinson's disease.^{27,28} The failure of multiple double-blind trials of surgical therapies for Parkinson's disease to confirm the results of open-label trials illustrates this point.^{12,13,29,30} In this study we did not see the benefits we reported in our

open-label trial.¹⁹ A placebo effect might have influenced the results at 18 months, but this seems unlikely because of the masking and randomisation. The sham surgery technique was designed to mimic active treatment with low risk (because the dura was not breached and the brain not penetrated).

Gene transfer enables long-term gene expression; thus, continued monitoring of the long-term safety profile of AAV2-neurturin treatment is important, particularly because we did not use a regulatable gene.³¹ No adverse events have been directly attributed to AAV2-neurturin so far; however, our safety database for AAV2-neurturin is still small (50 patients have been followed up for 2–5 years after treatment). Although the absence of any clear safety issues is encouraging, it is not definitive evidence of safety, and follow-up of larger numbers of patients over extended periods of time is needed. Patients in this study have been enrolled into an open-label extension study and will be followed up for a minimum of 5 years.

Preclinical findings make a compelling case for assessment of neurotrophic factors such as neurturin in Parkinson's disease. A trophic factor might provide benefits by improving function of residual neurons and by protecting them from ongoing neurodegeneration. However, Parkinson's disease affects multiple brain regions beyond the nigrostriatal system, and there is no scientific basis for thinking that localised gene delivery of a trophic factor will meaningfully improve the non-dopaminergic features of the disease.³² Nonetheless, neurological dysfunction secondary to dopamine degeneration is a major feature of Parkinson's disease, and AAV2-neurturin offers the potential to restore dopaminergic function in a physiologic manner without the motor complications associated with levodopa or the adverse effects associated with deep brain stimulation.³³ We therefore believe that continued investigation of a gene therapy that primarily targets the dopamine system is worthwhile and anticipate that this approach might one day be extended to non-dopaminergic regions. Our study supports the feasibility of gene delivery in Parkinson's disease. Although we did not meet the primary endpoint at 12 months, histological findings suggest that we might not have delivered sufficient protein to the substantia nigra in this time period.²⁴ Clinical benefits at 18 months suggest that longer-term assessments (ie, >12 months after treatment) might be needed to identify the maximum benefit that can be achieved with AAV2-neurturin. For these reasons, in a new study the substantia nigra will be directly targeted, a higher dose will be injected into the putamen, and patients will be followed-up for longer time periods to assess whether this approach offers benefits for patients with advanced Parkinson's disease (ClinicalTrials.gov identifier NCT00985517). If the results are positive, we will investigate AAV2-neurturin as a treatment for patients with milder Parkinson's disease.

Panel: Research in context

Systematic review

We searched PubMed without date restrictions up to August, 2010, for studies testing brain delivery of the trophic factors glial-cell-derived neurotrophic factor (GDNF) or neurturin to patients with advanced Parkinson's disease. We used the following search terms: GDNF, Neurturin, and Parkinson's disease. We found two open-label trials of GDNF delivered by catheter into the striatum^{10,11} and two double-blind trials of GDNF delivered into the ventricle or the striatum.^{12,13} We also found one open-label study using a viral vector to deliver neurturin to the striatum.¹⁹ We assessed the quality of the evidence on the basis of whether the study was open label or double blind.

Interpretation

Positive results with GDNF were reported in the open-label studies^{10,11} but these results were not confirmed in the two double-blind studies.^{12,13} The open-label study of gene delivery of neurturin as a treatment for Parkinson's disease met its primary endpoint of safety.¹⁹ The present study did not meet its primary endpoint of efficacy but did report positive results in the subgroup of patients who were assessed for up to 18 months, suggesting that there might be a delay in transport of the trophic factor to the substantia nigra because of the extent of degeneration of the nigrostriatal tract. This study emphasises the importance of double-blind trials in assessment of novel surgical therapies for Parkinson's disease and suggests that future gene delivery studies should be done with direct injections into the substantia nigra as well as the putamen.

Contributors

WJM, RTB, and CWO wrote the first draft of the manuscript. CSD undertook the statistical analyses. All authors reviewed, commented on, and approved the manuscript. As chair of the steering committee, CWO had the final responsibility for the manuscript content.

Steering committee

C W Olanow (chair), A Lozano, C S Davis, W J Marks Jr, F Bloom, and K Kieburtz.

Conflicts of interest

All authors listed as study investigators were reimbursed for budgeted expenses related to this study by their respective institution, which had negotiated a budget with Ceregene; the institution (not the investigator) was paid directly. No author was paid to write this paper. WJM is a study investigator and a paid consultant and lecturer for Medtronic. RTB and JS are employees of Ceregene and have been granted stock options. CSD received fees for statistical consultation and for some of the analyses for the study and is a paid consultant for Ceregene. AL is a paid member of Ceregene's scientific advisory board and owns stock options. NB, RW, and RA are study investigators and paid consultants to Ceregene after the database was locked. JV is a study investigator; a paid consultant to Medtronic, Boston Scientific, St Jude Medical, and Cleveland Medical; and a lecturer for University of Alabama Lecture Courses. MStA is a study investigator; is a paid consultant to Biogen, Osmotica, General Electric, Neurologix, and Synosia; has received grants from IMPAX, Neurtaltus, Novartis, Schering-Plough, and the Parkinson's Study Group; and is a paid lecturer for Allergan, Boehringer Ingelheim, GlaxoSmithKline, Novartis, and Teva Pharmaceuticals. DT, LV, RB, BG, GB, and PAS are study investigators. JJ is a study investigator; is a scientific board member of the Michael J Fox Foundation for Parkinson's Research and The Neurotoxin Institute; is a paid consultant for Allergan, Chelsea Therapeutics, EMD-Serono, Lundbeck, Merz Pharmaceuticals, and Teva Pharmaceuticals; is a paid lecturer for the American Academy of Neurology; and has received royalties from Elsevier, Lippincott Williams and Wilkins, and UpToDate. RS is a study investigator and a board member, paid consultant, and lecturer for Medtronic and has received grants from St Jude Medical. MT is a study investigator; is a paid consultant for St Jude Medical and Medtronic; is a paid lecturer for Medtronic, Allergan, GlaxoSmithKline, Boehringer Ingelheim, and Novartis; and has received grants from Medtronic, St Jude Medical, Abbott, Allergan, and Teva Pharmaceuticals. MStE is a study investigator; a paid

consultant for Teva Pharmaceuticals, Merck-Schering-Plough, Novartis, Adamas, and Ipsen Group; a paid lecturer for Novartis and Medtronic; and an officer for the Movement Disorder Society. PSL is a study investigator, is a paid consultant to Ceregene after the database was locked, and has received grants from Genzyme. JLO is a study investigator and has received grants from Medtronic and Allergan and lecture fees from Allergan. JN is a study investigator and paid consultant for Xenoport, Impax Laboratories, Neurogen, Synosia, Neuroderm, Merck, Lilly and Medtronic, Elan Pharmaceuticals, Adnex Pharma, and Lundbeck; has received grants from Schering-Plough (now Merck); and has received lecture fees from Novartis and Teva Pharmaceuticals. KK has received research grant support from the National Institutes of Health, the Michael J Fox Foundation, Medivation, Neurosearch, and Pfizer; serves as a consultant to the US Food and Drug Administration, the Veteran's Administration, the National Institutes of Health, Abbott, Biogen Idec, Boehringer Ingelheim, EMD Serono, FoldRx, Impax, Ipsen, Isis, Lilly, Lundbeck, Merz, Novartis, Orion, Otsuka, Prestwick, Schering-Plough, Sienna Biotech, Synosia, Solvay, Teva Pharmaceuticals, UCB Pharma, and Xenoport; and provides legal consulting to Pfizer and the Welding Rod Litigation Defendants. JHK is a paid member of the scientific advisory board of, owns stock options from, and has received grant support from Ceregene. CWO is a paid member of the scientific advisory board of and owns stock options in Ceregene and is a paid consultant for Novartis, Orion, Teva Pharmaceuticals, Lundbeck, Merck-Serono, and Abbott.

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