

Safety and tolerability of intraputaminally delivered CER-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial

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Summary

Background: There is an urgent need for therapies that slow or reverse the progression of Parkinson's disease (PD). Neurotrophic factors can improve the function of degenerating neurons and protect against further neurodegeneration, and gene transfer might be a means to deliver effectively these factors to the brain. The aim of this study was to assess the safety, tolerability, and potential efficacy of gene delivery of the neurotrophic factor neurturin.

Methods: In this phase I, open-label clinical trial, 12 patients aged 35–75 years with a diagnosis of PD for at least 5 years in accordance with the UK Brain Bank Criteria received bilateral, stereotactic, intraputaminally delivered injections of adeno-associated virus serotype 2-neurturin (CERE-120). The first six patients received doses of 1.3×10^{11} vector genomes (vg)/patient, and the next six patients received 5.4×10^{11} vg/patient. This trial is registered with ClinicalTrials.gov, number NCT00252850.

Findings: The procedure was well tolerated. Extensive safety monitoring in all patients revealed no clinically significant adverse events at 1 year. Several secondary measures of motor function showed improvement at 1 year; for example, a mean improvement in the off-medication motor subscore of the Unified Parkinson's Disease Rating Scale (UPDRS) of 14 points (SD 8; $p=0.000121$ [36% mean increase; $p=0.000123$]) and a mean increase of 2.3 h (2; 25% group mean increase; $p=0.0250$) in on time without troublesome dyskinesia were seen. Improvements in several secondary measures were not significant, including the timed walking test in the off condition ($p=0.053$), the Purdue pegboard test of hand dexterity ($p=0.318$), the reduction in off time ($p=0.105$), and the activities of daily living subscore (part II) of the UPDRS ($p=0.080$).¹⁸ F-levodopa-uptake PET did not change after treatment with either dose of CER-120.

Interpretation: The initial data support the safety, tolerability, and potential efficacy of CER-120 as a possible treatment for PD; however, these results must be viewed as preliminary until data from blinded, controlled clinical trials are available.

Funding: Ceregene; Michael J Fox Foundation for Parkinson's Research.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder that is characterized by motor dysfunction (bradykinesia, rigidity, tremor, and gait impairment with postural instability) and degeneration of dopaminergic neurons in the substantia nigra pars compacta.¹ Current therapies, which are primarily based on dopamine replacement, provide excellent control of motor symptoms, particularly in the early stages of the disease. However, most patients will develop motor complications (wearing off and dyskinesia),² and there is no available treatment to retard or halt the progression of the disease. Accordingly, there is an urgent need for therapies that avoid motor complications and slow or reverse disease progression. Neurotrophic factors can improve the function of degenerating neurons and thus improve the symptoms of PD, protect against further neuro-degeneration, and retard disease progression. Glial-cell-derived neurotrophic factor (GDNF) can enhance the function of nigrostriatal dopaminergic neurons and improve motor behavior in monkeys that are rendered parkinsonian with 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP).^{3–5} In preliminary open-label trials, GDNF was infused through a single point-source into the putamen of patients with PD, and clinical benefits and increased activity in dopaminergic neurons on neuroimaging

were reported.^{6,7} However, doubleblind, placebo-controlled trials did not show a large beneficial effect of GDNF.^{8,9} These protocols might have been unable to show significant benefit because the methods of delivery were inadequate to distribute GDNF throughout the putamen.^{10–12} In this trial, we used an adeno-associated virus serotype 2 vector (AAV2; Genbank accession AF043303) to deliver NRTN (HUGO approved symbol), the gene that encodes neurturin, a naturally occurring structural and functional analogue of GDNF,¹³ to multiple sites throughout the putamen to achieve widespread, targeted delivery. Gene delivery requires no indwelling hardware and can provide continuous expression of the trophic factor throughout the targeted area after a single surgical procedure; therefore, gene delivery might be an effective way to deliver trophic factors to the striatum of patients with PD. Neurturin, like GDNF, has been shown to enhance the survival of dopaminergic neurons in rodent and primate models of PD.^{14–19} A series of studies was done to establish the safety and efficacy of AAV2–NRTN (CERE-120) before gene transfer was tested in patients with PD.^{14,17–21} Collectively, the results of these studies showed many benefits: injection of CERE-120 into the striatum gave controlled and predictable diffuse expression of NRTN throughout this structure; increasing doses of CERE-120 produced greater NRTN expression;^{18,21} CERE-120 gave accurate coverage of the targeted region of the striatum without expression in untargeted sites; the transgene persisted for at least 12 months, with maximum protein expression within about 1 month and no greater spread of protein at 1 year (Herzog C, et al, Ceregene, unpublished);¹⁸ the positive effects on the nigrostriatal dopamine neurons lasted for at least a year after a single injection (Herzog C, et al, Ceregene, unpublished);^{14,19} significant morphological and functional restoration of neurons was seen in several different rat and monkey models of PD, including aged and MPTP-lesioned monkeys;^{18,19} and CERE-120 expression did not cause adverse behavioral or histological events in any tissue or organ system for at least 1 year after treatment with doses that were hundreds of times greater than those that are required for efficacy or that were used in this trial (Herzog C, et al, Ceregene, unpublished).¹⁸ On the basis of these preclinical results, we did an open-label study over 12 months to test the safety, tolerability, and preliminary efficacy of bilateral, stereotactic intraputamenal injections of CERE-120 in patients with advanced PD who had motor complications that could not be adequately controlled with the available medical therapy.

Methods

Patients

Patients had PD as diagnosed in accordance with the UK Brain Bank Criteria²⁰ and had signed informed consent, that included permission to publish data from the study, before entry into the trial. The main entry criteria were being aged 35–75 years with a diagnosis of moderate to severe PD on the basis of clinical rating scales (defined as H and Y stage 3 or 4 and UPDRS motor score in the practically defined off condition of at least 30) for a minimum of 5 years, a good response to levodopa in the judgment of the investigator, motor complications that could not be satisfactorily controlled with medical therapy, at least 3 h of off time per day as assessed by entries in home diaries, stable doses of antiparkinsonian drugs in the preceding month, and no conditions that would make the patient unsuitable for surgery. Exclusion criteria were atypical parkinsonism, dementia that precluded giving informed consent or a mini-mental status examination (MMSE) score of less than 25, treatment with non-antiparkinsonian drugs or other investigational drug that might affect symptoms of PD within 60 days, previous neurosurgical treatment for PD, any brain abnormalities seen with MRI in the previous 12 months, any disorder that precludes surgery, chemotherapy, cytotoxic therapy or immunotherapy within 6 weeks, vaccination within 30 days, prior gene transfer therapy, drug or alcohol abuse, and clinically significant medical, psychiatric, or laboratory abnormalities, as judged by the investigator.

INSERT Figure 1- Schematic of the structure of CERE-120

AAV2 inverted terminal repeats (ITR) flank the *NRTN* expression cassette, which consists of the CAG promoter, the sequences encoding pre-proNGF and neurturin, and the polyadenylation (pA) signal from human growth hormone gene.

Procedures

The study was designed as a prospective, phase I, open label clinical trial to assess the safety, tolerability, and preliminary efficacy of two doses of intraputamenal CERE-120 (Ceregene, San Diego, CA, USA). The study was done at the University of California, San Francisco, and at Rush University Medical Center, Chicago, between May, 2005, and March, 2007. The study was approved by the institutional ethics and biological safety committees at each institution and by the US Food and Drug Administration. The protocol was reviewed by the Recombinant DNA Advisory Committee of the National Institutes of Health. An independent data safety monitoring board (DSMB) was chartered and met every month throughout the enrolment and dosage stages of the protocol, to review ongoing data from the study and to judge whether more patients should be enrolled and dosed. After all 12 patients had received CERE-120, the DSMB met every 3 months. CERE-120 was provided by Ceregene and manufactured under good manufacturing practice conditions. The CERE-120 vector genome contains the AAV2 inverted terminal repeats flanking a constitutive expression cassette that encodes human NRTN cDNA. To enhance secretion, the pre-prodomain of neurturin was replaced by that of the human nerve growth factor (figure 1). CERE-120 vectors were produced by standard triple transfection in 293 cells and purified by column chromatography.¹⁸ Six patients received a low dose (1.3×10^{11} vector genomes [vg]) of CERE-120, and six patients received a high dose (5.4×10^{11} vg). In the low-dose group, the first two patients were dosed a month apart, and thereafter pairs of subjects were dosed one month apart after the available safety data

was reviewed. When safety was established in the patients who had received the low dose, the same regimen was followed for the six patients in the high-dose group. With the exception of the dose of CERE-120, patients in the low-dose and high-dose groups were treated identically. Patients were assessed at baseline, then every week for 1 month after surgery, then every month for 3 months, and at 3-month intervals for the rest of the 12-month study period. Whenever possible, patients were kept on stable doses of antiparkinsonian drugs throughout the study, although adjustments were made if the patient's clinical situation warranted an increase or decrease in medication. Patients were anaesthetised with deep propofol sedation. A frame-based stereotactic technique and computer-assisted trajectory planning were used to give CERE-120 bilaterally into the putamen in four needle tracks on each side. Two deposits, each of 5 μ l, were given per needle track at 4 mm intervals along the dorsal–ventral plane, with a pause of 3 min between deposits. Both precommissural and postcommissural aspects of the putamen were targeted. Patients were monitored closely postoperatively and discharged after about 48 h. All patients had baseline and postoperative MRI studies. The overall primary outcome measure was safety. Safety assessments, which were done at baseline and at each postoperative visit, are reported here for the entire study period. Adverse events were sought through interviews, during which patients were encouraged to report any problems. Vital signs, electrocardiograms, and laboratory tests to assess electrolyte concentrations, haematological status, hepatic and renal function, the presence of serum antibodies to AAV2 and neuritin, and the presence of CERE-120 vector and neuritin protein in the serum and urine were done at baseline and at periodic intervals (baseline, day 2, week 1, and months 1, 3, 6, 9, and 12) throughout the trial. Mattis dementia rating scale, digit span (WAIS-III), letter–number sequencing (WAIS-III), trail making test (A and B), Wisconsin card sorting test, Boston naming test, controlled oral word association (FAS), animal fluency, judgment of line orientation test, Hopkins verbal learning test-revised, Beck depression inventory, and Lawton and Brody ADL–IADL, were done at baseline and at 3 and 12 months after surgery. A secondary outcome for the study was efficacy, which was defined as the change in Unified Parkinson's Disease Rating Scale (UPDRS)²² motor score (part III) during the practically defined off state between baseline and final visit. Patients were tested after they had been without oral antiparkinsonian drugs overnight and the effect of the medication had largely worn off. Before the start of the trial, we determined that a 25% or greater reduction in the off-medication UPDRS motor score at the end of the trial would be deemed clinically meaningful and sufficient to justify the risks of surgery. Another important secondary outcome measure was the amount of time spent in the on state without troublesome dyskinesia. This parameter was determined by a home diary completed by the patient at 30-min intervals during the waking day for the three consecutive days before each visit. Additional measures of motor function (or parkinsonian severity) assessed at baseline and at follow-up visits included the complete UPDRS, patient-completed diary assessments of motor function, timed motor tests (timed walking test, Purdue pegboard)²³, dyskinesia rating scale, clinical global impression of severity (in which the neurologist rates the overall severity of each patient's illness), clinical global impression of improvement scales, and quality of life measures: Parkinson's disease questionnaire (PDQ-39)²⁴ and short form-36 health-related quality of life questionnaire (SF-36).²⁵ Striatal fluorodopa uptake was measured with ¹⁸F-levodopa PET at baseline, 6 months, and 1 year; patients signed an additional informed consent form for this procedure. All PET scans were done at the University of British Columbia, Vancouver, Canada, with an ECAT 953B tomograph in 3D mode. Regions of interest were visually positioned over either the putamen or the parieto-occipital cortex for reference. The main outcome variable for the PET study was a change in uptake of putamenal¹⁸F-levodopa from baseline to final visit (month 12), with the striatal:occipital ratio of ¹⁸F-levodopa uptake calculated between 60 and 90 min after injection of the tracer.

Statistical analysis

Safety and tolerability were assessed descriptively by evaluation of all adverse events. Mean values and shift tables for laboratory evaluations were used to assess electrolyte concentrations, haematological status, and hepatic and renal function. Neurocognitive tests were analyzed as change from baseline at 1 year with a one sample *t* test or Wilcoxon signed-rank test, and with a one-way ANOVA against time, with Bonferroni correction. The changes at post-baseline visits were calculated for each assessment of motor function. Statistical testing was done to assess whether the absolute change and, where applicable, percentage change from baseline at 1 year was significantly different from zero in each dose and for both doses. For data that were normally distributed a one-sample *t* test was used, whereas for data that were not normally distributed a Wilcoxon signed-rank test was used. The Wilcoxon signed-rank test was used to test whether changes in the clinical global impression of severity score and clinical global impression of improvement score at 1 year were significantly different from baseline. Additionally, the global improvement score was categorized as improved (very much improved, much improved, or minimally improved) versus not improved (no change, minimally worse, much worse, or very much worse). A binomial test was used to determine whether the proportion of patients who worsened or improved was significantly different from 0.5.

INSERT Table 1 – Characteristics of Patients

INSERT Table 2 – Treatment-emergent adverse events

Role of the funding source

The study was sponsored by Ceregene and is registered with ClinicalTrials.gov (number NCT00252850). The sponsor assisted in the trial design, data collection, data analysis, and writing of the report. The authors had full

access to all the data in the study, responsibility for the final manuscript, and the decision to submit it for publication. Additional funding was provided by the Michael J Fox Foundation for Parkinson's Research, specifically for the scientific aspects of the protocol (eg, ¹⁸F-levodopa PET scans). The Michael J Fox Foundation had no role in the design, conduct, or analysis of the study or in writing the report.

Results

12 patients (nine men and three women) who were diagnosed with advanced idiopathic PD were enrolled. All patients had uncontrolled fluctuations in motor function and dyskinesia despite optimized antiparkinsonian therapy. Table 1 shows baseline demographic information. Patients spent a mean of 5.3 (SD 2.8) h per day in the off state, in which motor function was poor and not controlled with medical therapy, as assessed by entries in patient-completed home diaries. At baseline, the mean dose of antiparkinsonian medication was 1783 levodopa equivalent mg (range 400–6558 mg). All patients completed all protocol-defined visits for the study; however, two patients did not complete the final diary assessment, and one of these patients did not complete the final neuropsychometric assessment or the quality of life assessments. No clinically important adverse events (e.g., hemorrhage, infection, or infarction) occurred during surgery. One patient had a probable air embolism during drilling of the burr hole; in the semi-sitting position, he was noted to cough and had transient oxygen desaturation. The embolus was resolved by flooding the surgical field with saline and lowering the head; his blood pressure and heart rate remained stable and within normal range, and the procedure continued without further event. No serious adverse events occurred in any patient or at either dose of CERE-120 throughout the study. Table 2 lists adverse events during the study period, including all neurological and psychiatric events and any other adverse events seen in two or more patients. There were no ongoing adverse events at the end of the study that could be attributed to CERE-120 or to the surgical procedure, with the exception of increased T2 MRI signal along the surgical trajectory in one patient. Analysis with the Bonferroni method corrected for multiple comparisons showed no significant change in Mattis dementia rating scale ($p=0.540$), digit span ($p=0.780$), trail making test part A ($p=0.080$) or part B ($p=0.170$), Wisconsin card sorting perseverative responses ($p=0.250$) or errors ($p=0.310$), Boston naming test ($p=0.050$), animal fluency ($p=0.620$), letter fluency ($p=0.220$), judgment of line orientation test ($p=0.110$), Lawton and Brody activities of daily living scale score ($p=0.440$), Beck depression inventory ($p=0.710$), Hopkin's verbal learning test—learning ($p=0.200$), Hopkin's verbal learning test—recall ($p=0.020$ [but not significant when corrected for multiple comparisons]), or Hopkin's verbal learning test—discrimination ($p=0.030$ [but not significant when corrected for multiple comparisons]). When baseline scores were compared with scores at 1 year, no significant changes were found except for a decline in Hopkin's verbal learning test recall score ($p=0.00076$). There were no significant abnormalities seen in vital signs, electrocardiogram, or laboratory tests. Humoral immune responses to neurturin or AAV2 were measured by ELISA as changes in IgG titre compared with baseline. Changes were deemed meaningful if the titre increased by more than five times the baseline value, on the basis of the resolution of the assays used. No meaningful humoral responses to neurturin were seen. Two of the 12 patients (one in each group) had antibody titres to AAV2 at baseline. None of the patients in the low-dose group had meaningful changes in AAV2 serology throughout the study. Four of the patients in the high dose group had an increased titre of anti-AAV2 antibodies after being given CERE-120. Two of these patients had transient, mildly elevated titres (≥ 25 times), whereas the other two had increased titres (>25 times and >125 times) that peaked 6 months after treatment and tended towards baseline concentrations thereafter. The prevalence of anti-AAV2 antibodies correlated with the dose of CERE-120 given and not with pre-existing immunity to AAV2. Additionally, all serum antibody responses to AAV2 were asymptomatic. There was no evidence of viral shedding, as assessed by quantitative PCR with a lower limit of detection of 7 copies per 10 μ L, for the CERE-120 vector genome in serum or urine at day 2, week 1, or months 1, 3, 6, 9, and 12. No neurturin was detected in serum or urine, as measured by ELISA. T2 MRI signal changes were seen 1 month postoperatively along the trajectory path of the needle, but these were thought to be associated with the surgical procedure. Although the primary outcome measure and main focus of this study was safety, various secondary outcome measures were used to assess potential efficacy. At 12 months, the mean improvement compared with baseline in the off-medication UPDRS motor score was 14 points (SD 10, mean increase 36%; $p=0.017$) in the low-dose group and 14 points (7, 36%; $p=0.006$) in the high-dose group. Figure 2 shows the time course of change in off-medication UPDRS motor scores throughout the study. At both doses, improvement was seen in bradykinesia, rigidity, tremor, and postural instability. Modest improvements tended to occur as early as 1 month postdose and increased during the following months. Eight of the 12 patients had substantial ($>25\%$) improvement in off-medication UPDRS motor score at 12 months compared with baseline. One patient (L5), whose 12-month off-medication motor score was no better than at baseline, was later diagnosed as having multiple system atrophy (MSA) rather than PD. At 12 months, on-medication UPDRS motor scores, which show the patients' best function after taking their usual morning antiparkinsonian medications, did not change from baseline (mean decrease [improvement] of 0.5 points, SD 8; $p=0.840$). Total UPDRS scores (i.e., mentation and mood, activities of daily living, and motor function) and complications of therapy (e.g., severity of off time and extent of dyskinesia) were improved at 12 months compared with baseline (table 3). However, the improvement did not differ by dose. An important measure of the efficacy of PD therapy is the effect of the treatment on motor function throughout the day. Patient-completed diaries are a means to track a patient's motor function while under optimum antiparkinsonian medication at half-hour intervals throughout the day. At baseline, a mean of 5.3 h (SD 2.8) of off time and 9.3 h (2.4) of on time without troublesome dyskinesia per day

were reported in the 3-day diaries. At 12 months, patients reported a mean reduction from baseline of 1.9 h (SD 3.6, 95% CI –4.15–0.45, 35%; $p=0.105$) in off time and a mean increase of 2.3 h (3.1, 0.347–4.264, 25%; $p=0.025$) in on time without troublesome dyskinesia. Two patients (L6 and H4) did not complete diaries at 12 months, and their 6-month diary entries were carried forward for this analysis (the protocol did not call for diary assessments during the 9-month visit). Figure 3 shows the results of home diary assessments over the course of the study. The clinical global impression scale of severity and clinical global impression scale of improvement, in which a neurologist rates the overall severity of each patient's illness compared with other patients with PD and the improvement in the patient's illness as compared with baseline, respectively, were both improved at 12 months. There was a decrease in the mean clinical global impression scale of severity from 4.5 points (SD 0.67) to 3.8 points (0.97). The mean change from baseline was –0.8 points (SD 0.4, 95% CI –1.037 to –0.463; $p=0.004$). Furthermore, as assessed with the clinical global impression scale of improvement, 10 of 12 patients were improved at 12 months compared with baseline ($p=0.021$). Table 3 shows additional secondary outcome measures. Patients had ^{18}F -levodopa PET scans, a surrogate measure of nigrostriatal function, at baseline and at 6 and 12 months after surgery. At baseline, patients had lower ^{18}F -levodopa striatal uptake than healthy controls, and the uptake was more pronounced in the putamen than in the caudate nucleus, which is typical of PD. The uptake did not change on follow-up scans after treatment with CERE-120 for either the whole group or for patients in the dose groups. Specifically, for the 11 patients with baseline and 12 month PET data, the mean increase in ^{18}F -levodopa uptake from baseline in the combined (right and left) putamen at 12 months was 1.19% (SD 22, 70%, 95% CI –16.439–14.067; $p=0.866$).

INSERT Figure 2 – Off-medication UPDRS motor scores for all patients throughout the study

(A,B) Present absolute and percentage change in scores from baseline in the low dose cohort, and (C,D) in the high dose cohort. The broken line (B,D) at 25% denotes the magnitude of the response that was defined, a priori, to be a clinically meaningful change for an interventional treatment. The solid line denotes no change.

INSERT Figure 3- Patient-reported motor function from diaries

Each of the five panels shows the total time (within 24 hours) that the patient reported being in each state. Note that the on time without troubling dyskinesia that is described in the text is the combined times of on: no dyskinesia and on: mild dyskinesia in the figure.

INSERT Table 3 – Secondary outcome measures

Discussion

A therapy for PD that can provide symptomatic benefit without motor complications, can restore the function of degenerating neurons, and protects neurons from further degeneration is desperately needed. Trophic factors, such as neurturin, are candidate molecules to achieve these goals because they have the potential to enhance and protect the remaining striatal dopaminergic neurons. Trophic factors augment dopamine-mediated function in a more physiological manner than can be achieved with any other currently available means. Past efforts to use trophic factors to treat PD have been hampered by the inability to deliver these complex proteins to the targeted region of the brain in a safe and sustained manner without protein spread to untargeted areas. Gene transfer is a practical opportunity to achieve this goal. Indeed, extensive testing of CERE-120 in animals showed that gene transfer can reliably and selectively target expression of *NRTN* to the nigrostriatal region and that stable expression after a single treatment lasts for at least 1 year (Herzog C, et al, Ceregene, unpublished)^{17,18} with evidence of extended efficacy in several animal models of PD.^{14,19} Of equal importance is that extensive toxicology tests found no evidence of toxicity at any time point, despite the use of doses that are hundreds of times higher (by brain weight) than those required to test efficacy or than those used in our human trial.^{14,17,19} In this open-label, phase I study, 12 patients were followed-up for 12 months after intraputaminial transfection of CERE-120. Delivery of *NRTN* was found to be safe and well tolerated. Although there were no serious adverse events, only a small number of patients were studied, and further testing in greater numbers of patients and for longer is required. Most of the adverse events that did occur were those commonly associated with stereotactic brain surgery, and none was deemed to be clinically significant or unanticipated. In previous studies of dopaminergic cell transplant, off-medication dyskinesia (a form of disabling dyskinesia that persists even after levodopa is stopped) was seen in as many as 50% of patients;¹ this began as early as 1–3 months after implantation²⁶ and was so disabling in some patients that it required further surgical intervention. In the present study, no patient developed off-medication dyskinesia. Observations about efficacy in this small, open-label study must be treated as preliminary; however, many of the secondary measures suggest that CERE-120 might have had beneficial effects on motor function in both dose groups. An entry criterion for enrolment in this study was that patients had advanced disease with symptoms that could not be further improved with available medical therapies. 1 month after treatment, patients began to show improvement in the UPDRS motor subscale score evaluated when anti-parkinsonian medications had largely worn off, which was the secondary motor endpoint defined a priori as the main indicator of efficacy for the trial. Notably, the improvement increased during the next several months and persisted throughout the duration of the study. This pattern of improvement is consistent with the known biology of neurotrophic factors in general and the specific biology of neurturin. The persistence of benefit is also consistent; the AAV2 vector system has an established capability to confer long-term and stable gene expression. The beneficial

effects mirror the pattern of those seen in MPTP-lesioned monkeys that were treated with CERE-120.¹⁹ Improvements were also seen in other secondary outcome measures, such as home diary assessments of off time and on time without troublesome dyskinesia, activities of daily living, global rating measures of disease severity, and timed motor tests of gait and dexterity. Most of these changes were clinically significant, and they might have important day-to-day benefits for patients. Nevertheless, not all improvements in secondary measures, including objective assessments of timed walking and hand dexterity, reached statistical significance; thus, caution must be exercised when interpreting the preliminary findings from this unblinded and uncontrolled study. Several treatments for PD that have appeared promising in open-label studies have failed to show efficacy under more rigorous controlled and blinded conditions.^{8,9} The improvement in the UPDRS motor scores in the practically defined off state persisted in all but four patients. One of these patients (L5) was eventually diagnosed as having MSA rather than idiopathic PD, and over time he developed more prominent dysautonomia, facial and limb dystonia, and a substantially diminished response of rigidity, bradykinesia, and gait disturbance to levodopa in association with imaging evidence indicative of MSA. Although at baseline the patient's UPDRS motor score improved from 60 to 25 on treatment with levodopa and other antiparkinsonian medications (58% response), at the time of the final visit there was only a 16% change in the scores after antiparkinson drugs were given. On the basis of the clinical and neuroimaging findings during the course of a year, in retrospect, it became clear that the patient had been misdiagnosed as having idiopathic PD and, in fact, had MSA all along. The widespread loss of neurons in the striatum and pallidum that occurs in MSA might explain why no sustained improvement was seen in this patient. The severity of motor impairment at baseline seemed to predict which subjects might better respond to treatment with CERE-120. The pretreatment off-medication motor scores of the four patients who had less than 25% improvement in off-medication motor scores at 1 year post-treatment were significantly worse ($p=0.014$) than the pretreatment off-medication motor scores of the eight patients whose improvement exceeded 25% (figure 2). Although we need to confirm these associations in a larger trial, disease severity and, specifically, the presence of dopamine unresponsive features might need to be taken into account when the entry criteria for patients are defined in future studies. There were no significant differences between dose groups for any endpoint. This is probably because both doses adequately treated the targeted region of the brain during the time period; furthermore, with only six patients per dose, the study was not powered to detect any difference between the two doses. No change in striatal uptake from baseline was seen with ¹⁸F-levodopa PET in either dose group at the 12 month assessment. This negative finding could point to a lack of biological effect of CERE-120. Because this was a phase I, open-label study, any suggestion of possible efficacy on some of the motor measures that were previously outlined could be spurious and indicate a placebo response, investigator bias, or other factors. Nevertheless, the lack of change seen on PET might just show the inability of this method to detect CERE-120-induced changes. Although ¹⁸F-levodopa PET has been used as a marker for dopamine nigral neuron degeneration in patients with PD because it correlates generally with PD disease progression, it has not been shown to predict reliably disease severity on a case-by-case basis; nor does a higher ¹⁸F-levodopa signal reliably correlate with an improved treatment-related clinical outcome.^{27,28} Rather, ¹⁸F-levodopa PET images only a relatively narrow aspect of dopamine activity and storage. Any increase in signal is primarily indicative of the increased dopamine storage that occurs through the specific dopamine pathway that contains aromatic amino acid decarboxylase. Importantly, this pathway is not normally rate-limiting for dopamine synthesis but it becomes increasingly important as PD progresses and the normal tyrosine hydroxylase pathway becomes increasingly inadequate. The increase in signal seen on ¹⁸F-levodopa PET will, therefore, be enhanced only by factors that also enhance aromatic amino acid decarboxylase function or the vesicular storage of dopamine. Many aspects of dopamine neuronal synthesis and storage can be improved with no effect seen on ¹⁸F-levodopa PET signaling. Indeed, most animal data suggest that the robust neurotrophic effects of CERE-120 occur through the upregulation of tyrosine hydroxylase (i.e., the normal rate-limited pathway for dopamine). If this holds true in patients with PD, a higher signal would not necessarily be seen with ¹⁸F-levodopa PET, although it would certainly be desirable to have an imaging method that can identify the presence and extent of neurturin expression and its effects on the function of nigrostriatal dopaminergic neurons. Results from an ongoing, multicentre, sham-surgery-controlled, double-blind phase II trial will help to clarify the clinical effects of CERE-120 and imaging correlates with more certainty than was possible in this preliminary study. The AAV2 vector system is also being used to deliver the gene that encodes glutamate decarboxylase to the subthalamic nucleus of patients with PD with the goal of enhancing GABA-mediated inhibition.²⁹ Another approach is to improve levodopa conversion to dopamine by delivering the gene that encodes aromatic amino acid decarboxylase to the striatum with the AAV2 viral vector.³⁰ These strategies are all in open-label studies and aim to ameliorate the motor symptoms of PD. By contrast, the basis for delivering neurturin is the potential for a neurorestorative effect aimed not only at improvement of the symptoms but also the deceleration or reversal of disease progression. This study only investigated the ability of CERE-120 to provide potential symptomatic improvement in motor function and did not assess potential neuroprotective or neurorestorative effects. In conclusion, we have shown that delivery of the gene that encodes neurturin with the AAV2 vector to target the nigrostriatal system is feasible and well tolerated. This open-label study also provides preliminary evidence for the safety and potential symptomatic efficacy of the transfer of genes that encode trophic factors as a treatment for PD. A larger, prospective, controlled, double-blind, multicentre, clinical trial is needed to assess further the findings from this preliminary study, and such a study is now underway (ClinicalTrials.gov, number NCT00400634).

Contributors

WJM wrote the first draft of the paper, and all authors contributed to subsequent versions of the manuscript. WJM, CWO, and RTB did the overall data analysis. DACW did the analysis of the neurocognitive data. AJS supervised the PET scanning and did the analysis of the PET data. WJM, JLO, PAS, PSL, CWO, and RTB designed the study. WJM, JLO, LV, PAS, PSL, RAEB, and RT carried out the study, whereas RTB provided regulatory and general oversight support.

Conflicts of interest

RTB is an officer of Ceregene, which developed CERE-120 and sponsored this clinical trial; as such, he receives a salary and has been awarded stock options. CWO is a consultant for Ceregene and receives consulting fees and has also been awarded stock options. None of the other authors report conflicts of interest.

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