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Direct delivery of an investigational cell therapy in patients with Parkinson's disease: an interim analysis of feasibility and safety of an open-label study using DBS-Plus clinical trial design

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ABSTRACT

Objective To evaluate the interim feasibility, safety and clinical measures data of direct delivery of regenerating peripheral nerve tissue (PNT) to the substantia nigra (SN) in participants with Parkinson's disease (PD).

Methods Eighteen (13 men/5 women) participants were unilaterally implanted with PNT to the SN, contralateral to the most affected side during the same surgery they were receiving deep brain stimulation (DBS) surgery. Autologous PNT was collected from the sural nerve. Participants were followed for safety and clinical outcomes for 2 years (including off-state Unified Parkinson's Disease Rating Scale (UPDRS) Part III assessments) with study visits every 6 months.

Results All 18 participants scheduled to receive PNT implantation received targeted delivery to the SN in addition to their DBS. All subjects were discharged the following day except for two: post-op day 2; post-op day 3. The most common study-related adverse events were hypoaesthesia and hyperaesthesias to the lateral aspect of the foot and ankle of the biopsied nerve (6 of 18 participants experienced). Clinical measures did not identify any hastening of PD measures providing evidence of safety and tolerability. Off-state UPDRS Part III mean difference scores were reduced at 12 months compared with baseline (difference=−8.1, 95% CI −2.4 to −13.9 points, p=0.005). No complications involving dyskinesias were observed.

Conclusions Targeting the SN for direct delivery of PNT was feasible with no serious adverse events related to the study intervention. Interim clinical outcomes show promising results meriting continued examination of this investigational approach.

Trial registration number NCT02369003.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The loss of functional neurons in the substantia nigra is a hallmark of Parkinson's disease. Finding a way to stabilise or slow the neurodegeneration of these neurons continues to be an ongoing effort.

WHAT THIS STUDY ADDS

⇒ The procedure of deploying autologous reparative peripheral nerve tissue to the substantia nigra is feasible and has not led to serious adverse events related to the implantation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ While unilateral delivery appears to be a feasible and safe investigational procedure, expanding the scope of deployments to bilateral substantia nigra deliveries may help in determining the appropriate amount of reparative peripheral nerve tissue to use for future trials.

INTRODUCTION

No disease modifying therapies currently exist for Parkinson's disease (PD). Investigational biologic or cell therapies for treating neurological disorders typically require the direct delivery of a novel agent into a specific anatomic target or targets within the central nervous system. Many clinical trials focusing on PD have used a direct delivery method to evaluate the potential of growth factors,[1–5] gene therapies,[6–8] fetal brain tissue[9 10] or stem cells[11] for their ability to have a potential impact on therapeutic outcomes. Direct delivery methods are associated with surgical risks and present organisational, procedural and ethical challenges for the participants and research teams, especially when performed first in human, stand-alone procedures in preliminary clinical trials.[12] The substantial

need for disease altering therapies for PD is the major driving force for this line of work.[13]

Our approach focuses on developing a potential disease modifying intervention based on cell therapeutic principles. The potential mechanism of action of cell-based therapies is postulated to be either a triggering event, where the implanted cells do not survive, but trigger a lasting reaction within the host brain, or a living therapy, where the cells survive long term and perform a designated function to influence the host environment. Living therapies for neurodegenerative disorders can involve cell replacement strategies, such as the transplantation of fetal dopaminergic neurons,[9 10] or cellular support strategies that facilitate repair and neuroprotection.[1 2 7 14] For our approach, we chose to use autologous peripheral nerve tissue (PNT) as a strategy to provide cellular support to the diseased and dying neurons within the region of the substantia nigra (SN). Peripheral nerves demonstrate a robust ability to repair themselves following injury through the production of many cell-growth and cell-survival factors, as well as cell-to-cell interactions, leading to axonal regeneration and return of function (reviewed in [15 16]).

Our surgical protocol design integrates the direct delivery of PNT with deep brain stimulation (DBS), a standard of care, US Food and Drug Administration (FDA)-approved, surgical approach to the symptomatic treatment of advanced PD.[17–19] This integrated platform, which we have termed DBS-Plus, offers multiple advantages, and some associated limitations and challenges, which are addressed in this report.

The major goals of this study were to evaluate the safety of the DBS-Plus surgical approach and the potential consequences of the direct delivery of a cell therapy intended to integrate into the delivery area and survive for a long period of time.

We have previously reported our proof-of-concept results[20] and corresponding feasibility and 1 year safety data[21] in eight participants trialling PNT implantation to the SN and DBS targeting to the subthalamic nucleus (STN). The current report builds on our previous experience and provides feasibility, safety and clinical outcome data from 18 new participants trialling PNT implantation to the SN and DBS primarily targeting the globus pallidus interna (GPi). The results provide a midpoint interim evaluation from the original trial designed with a 2-year study duration. Complete results, including imaging, neurocognitive and clinical outcomes, will be reported after completion of the 2-year trial by all subjects.

Box 1 Inclusion and exclusion criteria

Inclusion criteria

Undergoing deep brain stimulation (DBS) of the subthalamic nucleus or globus pallidus interna
Between the ages of 40–75
Able to give informed consent
Show a positive response to Levodopa
Show no significant cognitive deficit per a formal neurocognitive exam
Be able to tolerate the surgical procedure

Exclusion criteria

Any condition that would make the subject a poor candidate for DBS of any target
Under the age of 40 or over the age of 75
Unable to give informed consent
Previous Parkinson's disease surgery or intracranial surgery

METHODS

Study design

Investigator initiated, open-label, single-centre, phase I trial focusing on the feasibility and safety of the direct delivery of PNT. Patients deemed to be qualified candidates for DBS and who had agreed to receive DBS were informed about the study and asked to participate (figure 1 and box 1). The trial design also includes

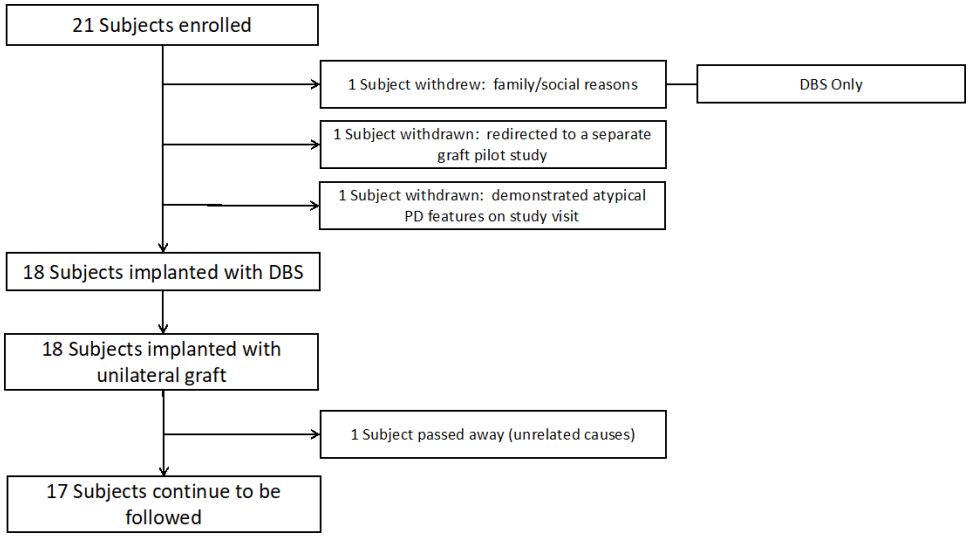


Figure 1 Enrolment and surgical flow. DBS, deep brain stimulation; PD, Parkinson's disease.

reporting of clinical evaluations and outcome data to assist in the determination of those measures that may prove clinically meaningful for evaluating disease progression and the potential for disease modification.

Study approvals and monitoring

The University of Kentucky Institutional Review Board approved the study, and data from the trial were regularly reviewed every 4 months by the Center for Clinical and Translation Science Data and Safety Monitoring Board at the University of Kentucky. All participants provided written informed consent. The trial protocol was developed and was carried out in accordance with the FDA's Same Surgical Procedure Exemption, 21 CFR 1271.15(b). Go/nogo requirements were based on adverse event (AE) reporting and adjudication. If three or more AEs were considered to be severe, and related to the study, then the study would have to be halted until further investigation and evaluation.

Imaging

MRI

Preoperative MRIs (3T) were obtained before surgery for targeting purposes. Thin cut CT images were obtained following Cosman-Roberts-Wells (CRW, Integra, Princeton, New Jersey, USA) frame placement and then fused to the preoperative MRIs for stereotactic planning. Postoperative MRIs (1.5T) were obtained within 36 hours of the implantation surgery during the participant's inpatient stay. MRIs were evaluated clinically by neuroradiology faculty.

Single photon emission computed tomography (SPECT)

All participants underwent ^{123}I-ioflupane SPECT scans at two time points, preoperative and 24 months postoperative.

Image acquisition

Four hours after injection of 5.6 (0.3) mCi, Mean (SD), of ^{123}I-ioflupane (DaTscan), SPECT images of the head were acquired using a gamma camera (Symbia, Siemens, USA).

Image analysis

Both qualitative and quantitative analyses of the basal ganglia comparing preoperative and 24-month postoperative timepoints were performed. For quantitative analysis, specific binding ratio (SBR) was calculated for the left and right striata, using DaTQUANT software (V.2.0, GE Healthcare, Boston, Massachusetts, USA), according to the formula: SBR = ((region count density)/(occipital region count density))− 1. The preoperative evaluations are presented here, and the 24-month comparisons will be reported at the conclusion of the study. Consensus between the qualitative assessment and identification of a normal striatal signal pattern serves as an exclusionary criterion for the clinical diagnosis of PD.[22]

Surgical implantation

The DBS-Plus protocol is designed to allow the direct delivery of an experimental agent within the same operative setting as DBS without interfering with the DBS procedure. In the current trial, PNT was implanted after the bilateral DBS electrodes had been placed, tested, secured to the skull and connected to the lead extensions. The specific details have been previously described.[20 23]

In brief, all stereotactic procedures were performed under general anaesthesia using a CRW frame. DBS and PNT trajectories were planned individually using stereotactic planning software (IPlan V.3.0 Stereotaxy, BrainLab, Munich) to enter through a cortical gyrus in the region of the coronal suture and lateral enough to avoid the lateral ventricles. Participants received bilateral DBS electrodes: 17 to the GPi and 1 to the STN. Microelectrode recordings were used to aid in target localisation. All trajectories were targeted to avoid intraparenchymal vasculature visible on MPRAGE sequences. Entry zones for the PNT implant cannula were lateral to the DBS electrode entry zone and traversed the same burr hole as the electrode. Implant cannula was simply a standard guide tube with a 5-mm long side window.[20]

PNT was implanted unilaterally into the SN contralateral to the most affected side based on Unified Parkinson's Disease Rating Scale (UPDRS) Part III scores. The centre portion of the SN, based on susceptibility weighted imaging (SWI) hypodensity, was used for targeting the centre of the PNT deployment. Implant cannula capacities were 5 mm in length with a 1.5 mm diameter. The PNT consisted of five to ten 1 mm segments cut from fascicles harvested and dissected from the distal component sural nerve that had been transected during the first stage of the multistage DBS surgery.[23] In accordance with the FDA's Same Surgical Procedure Exemption, PNTs were not exposed to any chemical or biological elements beyond sterile saline, and the only manipulations were dissection, resizing and reshaping. Immunosuppressants were not used because the PNT is autologous tissue.

Analysis of implant cannula trajectory and implant placement

Implant cannula trajectories were easily identified on postoperative MRI MPRAGE and SWI sequences. Implant locations were also identified and designated by placing an object marker within the mid portion of the implant delivery zone (2.5 mm from the distal terminus). Accuracy of implant targeting was determined by localising the implant marker in relationship to the SN as identified both visually and with the anatomical 3D overlay within Elements (Brainlab, Munich). Because the SN lies within a plane orthogonal to the Euclidian planes designated by the anterior commissure-posterior commissure line (AC-PC) line and the coronal plane, the figures generated for use in depicting the implant sites were oriented along the long axes of the SN (figure 2).

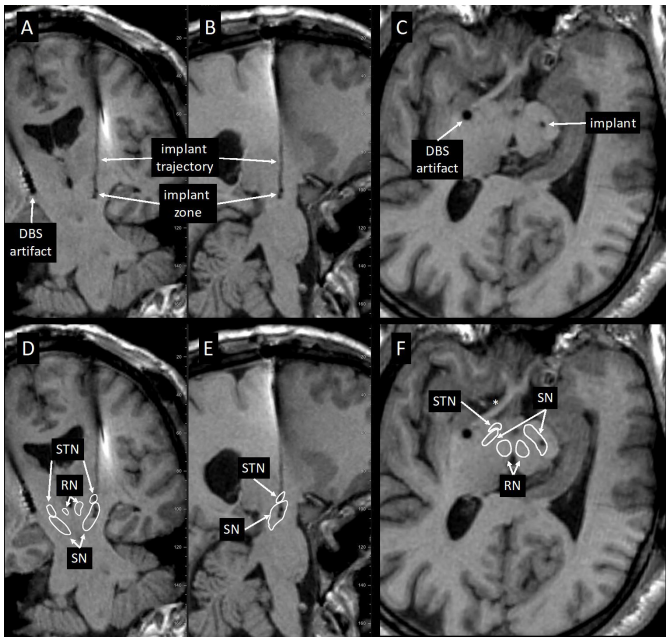


Figure 2 Postoperative MPRAGE scans displaying deep brain stimulation (DBS) electrode and graft cannula (implant trajectory and zone) tracks. DBS electrode and graft cannula placement in the coronal (A), parasagittal (B) and orthogonal to the trajectory (C) planes. Outlines of the substantia nigra (SN), subthalamic nucleus (STN) and red nucleus (RN) superimposed on the images (D–F) show implant zone relative to the SN.

Safety and feasibility

Safety data for each participant were collected starting from the first stage of surgery to the end of the study. Feasibility data were collected for each participant from the time of enrolment to the end of the study and were broken down into procedural and compliance data. Procedural feasibility was determined by the successful completion of the grafting portion of the protocol. Compliance feasibility was determined by the participant's ability to successfully complete the study from consent through the 12-month midpoint.

Postoperative evaluation

After DBS and PNT implantation, participants were admitted overnight and then followed clinically through routine postoperative visits, programming visits and for their postoperative study visits. Medication was reported as total daily levodopa equivalent dose (LED).[24] For UPDRS testing preoperatively, while in the practically defined off-state, participants stopped antiparkinsonian medications at least 12 hours before undergoing UPDRS testing (>24 hours for long-acting medications). After surgery, participants, if on PD medication, underwent a minimum 12-hour withdrawal from antiparkinsonian medications and turned off the DBS stimulator for 12 hours before testing.

Clinical measures

Clinical measures included UPDRS (all components) preoperatively and at 6-month intervals postoperatively assessed by experienced raters blinded to location. Additional clinical measures included a comprehensive neurocognitive evaluation, Parkinson's Disease Questionnaire (PDQ) 8 measures, the Non-Motor Symptoms Scale and formal gait analysis. These measures were collected at baseline and at 24 months and will be presented at the completion of the study.

Data analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Kentucky. Missing study visits (0 missing/18 visits at baseline, 0/18 at 6 months, 3/18 at 12 months) were not included as part of the analysis. Missing item data: scale items with missing data were imputed using mean substitution for that item across all participants at the corresponding time point. UPDRS Part IV questions 35–38 and 40–42 required a 'yes' or 'no' answer, which was scored with a 1 for yes or 0 for no. To address the missing data points for yes/no questions, we used the single imputation method. We randomly selected a participant, with a random number generator, who had a response and used that selected participant's response as the missing measurement. Results are reported as mean (SD) unless reported as 95% CI.

Descriptive statistics were calculated for clinical outcomes. Linear mixed models were used to investigate changes in UPDRS III scores across time. Mixed models are appropriate where the independent observation assumption is violated, in this case through the inclusion of repeated measures, and are able to make use of all available data through the use of maximum likelihood estimation. An α level of 0.05 was used for significance of the omnibus test of time. Follow-up pairwise comparisons were controlled for multiple comparisons using the Tukey method. Data analysis was conducted using PROC MIXED as part of SAS V.9.4 (SAS Institute) and JMP Pro V.14.0 (SAS Institute).

RESULTS

The data from 18 participants (table 1) are presented in this report and include the components of feasibility, safety, imaging, implant location and clinical outcome measures.

Feasibility

We evaluated several aspects of study feasibility ranging from trial design to likelihood of successful surgery

Table 1 Participant demographics

Characteristic	Value
Age, years, mean (SD)	63 (8)
PD duration in years, mean (SD)	10 (4)
Assigned birth sex	13 male/5 female

PD, Parkinson's disease.

procedural completion. An overall summary is presented in figure 1. Twenty-one participants were enrolled; three of these were withdrawn on subsequent preoperative study visits: two because of the identification of additional, exclusionary clinical information and one because of family and social issues. All three went on to have successful DBS without PNT implants. All remaining participants (n=18) underwent the staged[20 23] DBS-Plus protocol (mean time between stages: 7 days, range 3 days to 14 days). All participants were successfully implanted with a DBS system and subsequently received therapeutic programming and treatment. With respect to the PNT implantation protocol, all participants successfully received an implant without any procedural complications. In each case, the sural nerve was identified and transected during stage I, and the distal segment was identified and harvested during stage II. The amount of harvested tissue was adequate to provide enough tissue for fascicle dissection and implantation with enough left over for collection and storage in our tissue biobank.

The duration of the DBS-Plus surgical procedure, from incision to closure, averaged 208 min (30 min). All participants completed the 6-month evaluation and 15/18 completed the 12-month evaluation. One participant died, 7 months after surgery, from medical complications from a bowel obstruction. Two participants missed their evaluations for non-study related medical reasons (a hip fracture; a device-related infection).

Safety

The major objectives of this study were to assess the safety of the DBS-Plus surgical approach as well as the long-term safety and tolerability of the implanted PNT. With respect to the DBS-Plus procedure, there were no intraoperative complications from either the DBS portion of the surgery, or from the sural nerve transection during stage I, or from the nerve harvesting and delivery during stage II. The stage II procedure was well tolerated: 16/18 (89%) participants were discharged on post-op day one, one on day two and one on day three. Comparatively during the same time frame at our centre, for 33 consecutive patients with PD, 27/33 (82%) had a length of stay (LOS) of 1 day. Mean LOS for DBS-Plus=1.2 days; LOS for DBS only=1.6 days.

AEs (table 2) were adjudicated according to relatedness to the PNT intervention component of the protocol, which included the implant procedure and the implant itself. There were no serious AEs related to the study component. The only reported AEs related to the study intervention were an infection of the ankle incision following the second procedure and hypoaesthesia and hyperaesthesias of the lateral foot distal to the sural nerve tissue harvest site. The infection was superficial and was treated successfully with oral antibiotics. Altered sensitivity of the foot was reported in 6 of 18 participants and was an expected event given the nature of the procedure.

Imaging

^{123}I-ioflupane/SPECT imaging: all participants had scan results demonstrating loss of striatal signal intensity consistent with a presynaptic decrease in ligand binding (figure 3A) below levels of healthy control subjects (figure 3B). Thus, no subjects were excluded from PD diagnosis based on normal ^{123}I-ioflupane/SPECT findings.

Post-op MRI: clinical neuroradiological evaluations were performed on all 18 participants and reported typical postoperative changes associated with the DBS surgery—mostly mild pneumocephalus in several participants. One participant was observed to have a 1cc haemorrhage surrounding the DBS electrode but did not complain of any clinical symptoms or display any deficits associated with the finding; the participant was discharged on post-op day 1.

Assessment of PNT location

Implant locations were identified on the post-op MRIs and then mapped into the midbrain anatomical space in relation to the SN, red nucleus and STN (figure 4A). A composite of the centre point of the implant zones for each participant (figure 4B) shows that all implant zones were either fully within the SN (n=16) or on the border (n=2; lateral border of left SN). Within the SN, the grouping of implants was centred within the middle third (A–P), in the mid-half to upper-half (D–V)—except for one in the lower half, and distributed evenly (M–L).

Clinical measures

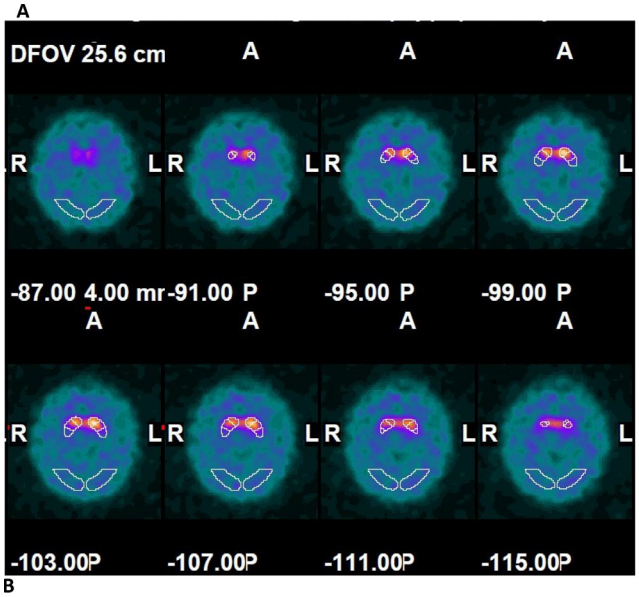
Table 3 shows secondary outcome analyses. Mean total LED was 899 (531) mg/day at baseline, 362 (301) mg/day 6 months after surgery, and 379 (259) mg/day 12 months after surgery. Following surgery, 2 of 18 participants reported continuing dyskinesias from presurgery although both participants showed improvement.

For UPDRS Part III practically defined off-state, scores decreased at 6 months after surgery and continued to be stably lower at 12 months after surgery. The overall main effect of time was significant $F(2,17) = 9.73$, $p=0.002$.

Table 2 Adverse events rated as possibly, probably or definitely related to the study

MedDRA V.23.1 primary term	# Reported/total (%)	Number of participants (affected/total)
Postoperative wound infection of the ankle	1/7 (14%)	1/18
Hypoaesthesia of foot/ankle	5/7 (71%)	5/18
Hyperaesthesia of foot/ankle	1/7 (14%)	1/18

A



B

	Mean (Z-score)	95% Confidence Interval	N
Striatum Ipsilateral SBR	-4.2	(-4.4, -3.9)	18
Striatum Contralateral SBR	-3.8	(-4.2, -3.8)	18

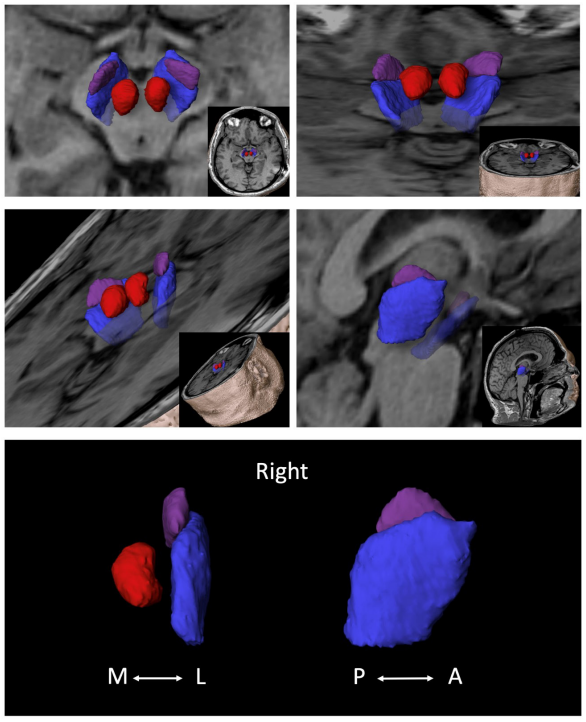
Figure 3 (A) Multiple depths through the axial plane show abnormal ^{123}I-ioflupane binding in the outlined striatal regions in one study participant. Values indicate different depths oriented based on A (anterior), P (posterior), L (left) and R (right). (B) Baseline comparison of the mean specific binding ratio (SBR) (ipsilateral and contralateral to the intended peripheral nerve tissue implant location) to the DaTQUANT normal database.

There were significant decreases between baseline and 6 months (difference=−8.0, 95% CI −2.6 to −13.3 points, t=3.81, p_{adj}=0.004) and between baseline and 12 months (difference=−8.1, 95% CI −2.4 to −13.9 points, t=3.64, p_{adj}=0.005). The difference from 6 to 12 months was not significant (difference: 0.2, 95% CI −6.1 to +5.7 points, t=0.08, p_{adj}=0.996). To further explore the effects of unilateral implantation on the laterality subscores of the UPDRS, we compared lateral UPDRS items (items 20–26) at baseline and 12 months for the body sides contralateral and ipsilateral to the PNT graft. Mean (SD) lateral scores at baseline were 16.2 (5.4) points (contralateral side) and 10.1 (5.0) points (ipsilateral side). At 12 months, mean (SD) scores were 11.2 (3.9) points (contralateral side) and 9.5 (5.0) points (ipsilateral side). Mean differences at 12 months compared with baseline were −5.0 (95% CI −8.0 to −2.0) points for the contralateral side and −1.2 (−3.1 to +0.7) points for the ipsilateral side.

DISCUSSION

In this report, we present a 12-month interim assessment of the feasibility, safety, with respect to subject participation and follow-through, and include available clinical outcome measures.

A



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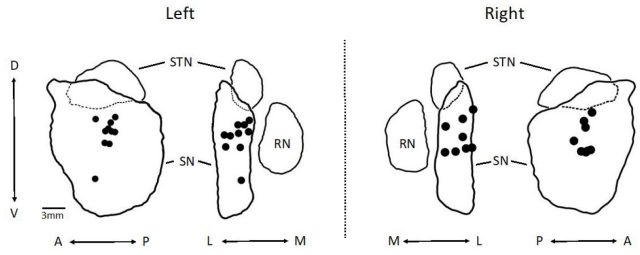

Figure 4 (A) 3D object depiction of the target zone. Using MPRAGE images, space-filling objects of the subthalamic nucleus (STN) (purple), substantia nigra (SN) (blue), red nucleus (RN) (red) depicted in different planes are shown. (B) Peripheral nerve tissue (PNT) placement within the SN on the left (n=10) and right (n=8) are shown. Black dots represent the centre point of deployment as determined from analysis of post-op MRI sequences. Composites were organised from the analysis of individual PNT placements using Elements software. Outlines are of objects shown in (A). A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

Feasibility

The requirements for participation were not burdensome as demonstrated by the high rate of completion of the evaluation visits. The PNT implant procedure did not interfere with the DBS surgery or therapy. All participants who were scheduled for surgery were able to receive bilateral DBS implant, followed by PNT placement, and all were successfully programmed in the clinic postoperatively.

Safety

The safety profile of the DBS-Plus procedure was favourable with respect to the surgical requirement of tissue harvesting and direct delivery to the SN. The postoperative LOS was no different than for DBS only patients implanted during the same time interval as the study

Table 3 Participant outcomes

	Baseline mean (SD)	6 Months mean (SD)	12 Months mean (SD)
Secondary outcome evaluation			
UPDRS Part III (OFF medication)*	38.5 (11.4)	N/A	N/A
UPDRS Part III (OFF medication/OFF stimulation)*	N/A	30.5 (12.9)†	30.9 (9.9)†
Modified Hoehn & Yahr (OFF-state)‡	3.0 (0.7)	2.5 (0.4)	2.6 (0.7)
Combined therapy evaluation			
UPDRS Part I§	3.4 (2.2)	3.1 (2.8)	3.5 (2.1)
UPDRS Part II¶	18.3 (6.7)	13.3 (5.6)	13.9 (5.8)
UPDRS Part III (ON medication)**	18.3 (9.4)	N/A	N/A
UPDRS Part III (ON medication/ON stimulation)**	N/A	15.4 (6.4)	14.3 (6.2)
Complications of therapy evaluation			
UPDRS Part IV††	6.8 (3.2)	3.1 (2.0)	3.1 (2.1)
Dyskinesia items 32, 33, 34	1.9 (2.2)	0.2 (0.6)	0.1 (0.3)
Modified Hoehn & Yahr (ON-state)‡‡	2.1 (0.5)	2.1 (0.5)	2.2 (0.4)

For all scales, higher scores indicate more severe parkinsonism.
*Motor examination while off therapy (range 0–108).
†P=0.002 main effect of time. Significant decrease at 6 months and 12 months versus baseline.
‡Measured while off therapy (range 0–5).
§Mentation, behaviour and mood (range 0–16).
¶Activities of daily living while on therapy (range 0–52).
**Motor examination while on therapy (range 0–108).
††Complications of therapy (range 0–23).
‡‡Measured while on therapy (range 0–5).
UPDRS, Unified Parkinson's Disease Rating Scale.

(data not shown). No new neurological deficits occurred from the DBS surgery or the grafting procedure. Non-study related AEs were similar to those seen commonly with DBS surgery or were unrelated altogether.

From a surgical perspective, the sural nerve transection during stage I and harvesting during stage II are practically the same as performing a sural nerve biopsy twice, with the second biopsy occurring through the same incision 2 weeks following the first biopsy. There was no evidence of infection or poor wound healing related to this procedure.

Imaging

Radiological evaluations revealed expected postoperative changes typically related to DBS surgery. Postoperative images demonstrated accurate targeting of the electrodes. Based on previous intranigral deliveries,[25–27] we targeted the midpoint of the rostral-caudal span of the SN. As shown in the composite of deposit locations, we successfully delivered to the SN. The dimensions of the PNT deposit were a cylinder approximately 5 mm in height and 1.6 mm in diameter. Depositing PNT at the midpoint of the medial-lateral axis (SN width ~4 mm) provided an opportunity for elements from the PNT to reach unhealthy dopamine neurons either within the interstitium or within the nigrosomes of the SN pars compacta.[28 29]

Clinical outcome

Clinical measures are important tools to assess the potential for worsening due to progression or an adverse response to PNT, or for possible improvements potentially related to the graft, grafting procedure (insertional effect) or placebo effect. As a phase I trial, this study is not designed or powered to determine clinical efficacy, but rather to evaluate clinical parameters and their ability to provide evidence for the possibility of disease modification. Overall, the clinical measures did not show any direct evidence of worsening or disease progression and performance scores remained generally stable. Although the complication of runaway dyskinesias has been associated with grafting of fetal dopaminergic tissue,[30] we did not observe worsening of dyskinesias or development of new dyskinesias in our participants. Analysis of the UPDRS Part III off-state scores revealed a significant reduction in scores at 6 and 12 months compared with baseline. This finding not only provides strong evidence that the PNT implants did not hasten the disease progression, but also suggests that the procedure may hold some promise in the reduction of this outcome measure. Although a 12-hour washout period for medication and stimulation for the off testing may not be fully adequate to allow participants to reach a fully therapy-free state, keeping a standardised 12-hour protocol provides consistency and is not overly

burdensome. Use of the UPDRS Part III as an endpoint in future studies will also require a strict definition of what constitutes a clinically important difference.

Evaluation of the clinical outcome measures demonstrates both strengths and weaknesses. The main utility of the majority of the clinical measures is to monitor for safety by having the ability to detect the possibility of acute worsening of disease symptoms. Considering we are surgically targeting the SN, the UPDRS III scores in the off-state remain the best tool for the assessment of changes in the motor components of the disease. Non-motor symptoms and manifestations most likely result from degeneration and cell loss from other nuclear regions.[31–34]

What are the next steps for PNT implantation?

Based on the outcomes reported here, we support a plan to move this project toward testing in a randomised, double-blind trial to assess the efficacy of PNT deployment to the SN. Furthermore, we think the DBS-Plus platform is a useful strategy for studying PNT implantation at this stage of development. However, despite the encouraging results reported here, fundamental issues remain to be resolved. Specifically, would additional PNT deliveries to the SN affect outcomes while remaining feasible and safe? As part of our methodical design, with safety and feasibility at the forefront, our next step is a 'dose-finding' study—multideployment unilaterally or bilaterally of PNT. The results from these types of studies would guide us in determining whether or not single-location, unilateral implantations, as described here, would be the dosage of PNT most appropriate to use in future efficacy trials. We chose to start with single delivery into the unilateral SN with the expectation that this approach would be the most feasible to perform with the least risk compared with multipass or bilateral deliveries. Therefore, our next steps will be to assess multilocation delivery of PNT to the SN unilaterally and bilaterally.

Summary

The results of this paper provide supporting evidence for the feasibility and safety of direct delivery of PNT into the SN of participants with PD at the time of DBS surgery. In our experience, the DBS-Plus approach offers many advantages for investigating novel interventional therapies involving patients with PD with advanced disease. We aim to provide transparency to allow this procedure or its components to be openly available to any interested teams. If successful, this type of approach could be used clinically in conjunction with standard DBS protocols. It could also be investigated for earlier implementation with the possibility of rescuing more of the 'at risk' cell population.[35]

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Contributors Author contributions are listed below based on these three elements:**1) Research project:** A. Conception, B. Organization, C. Execution; **2) Analysis:** A. Design, B. Execution, C. Review and Critique; **3) Manuscript:** A. Writing of the first draft, B. Review and Critique. JEQ: 1B, 1C, 2A, 2B, 3A; JTS: 1A, 1B, 2C, 3B; JAG: 1B, 1C, 3B; CJM:2B, 3B; REK: 1C, 2C, 3B; MJC: 1C, 2C, 3B; ZG: 2C, 3B; GAG: 1A, 1B, 3B; CvH: 1A, 1B, 1C, 2A, 2B, 3A and is the guarantor for the overall content.

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Form B: Medical IRB Research Description

Continuation of a Pilot Study to Evaluate the Safety and Feasibility of Implanting Autologous Peripheral Nerve Grafts into the Substantia Nigra of Subjects with Parkinson's Disease Undergoing Deep Brain Stimulation Surgery and Treatment

1. Background: PD results from the progressive degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNpc). No current treatment halts or reverses the ongoing degeneration or loss of cell function. PD is a complex, chronic, neurodegenerative disorder that occurs worldwide and affects up to one million Americans (Parkinson's Disease Foundation, 2011). It is well established that the symptoms of PD, tremor, rigidity, and slowness of movement, result from the progressive degeneration of dopaminergic neurons within the SNpc deep in the midbrain. Unfortunately, the cause of the degeneration remains unknown. While existing pharmaceutical and surgical therapies have some impact on the symptoms of PD, there is no current therapy that stops or reverses the ongoing loss of cell function, deterioration, and ultimate cell death. Early preventative intervention is difficult because symptoms of PD do not begin until 75-80% of the dopaminergic neurons have lost measurable function. However, there is evidence to suggest that a proportion of these cells are off-line and non-functional but not dead.

This pilot study is designed to follow up on a previous, preliminary study and test the long-term safety and feasibility of the implantation of autologous peripheral nerve grafts into the substantia nigra pars compacta (SNpc) of participants with PD undergoing deep brain stimulation (DBS) surgery. Peripheral nerve tissue contains Schwann cells which produce growth factors that have been demonstrated to support the survival and function of dopaminergic neurons.

Participants will serve as their own donor for the tissue, which will be implanted at the time they undergo DBS surgery.

Transplantation and Growth Factors: The two main strategies aimed at preventing disease progression have been to either transplant cells or tissues in an attempt to replace the lost dopaminergic neurons or to deliver growth factors that can restore the injured neurons before they die. Clinical trials involving the transplantation of dopaminergic tissues into the brain have not been able to demonstrate clinical benefit (Olanow et al, 2003). Additionally, the source of the transplant material, fetal tissue, has raised considerable ethical issues regarding the viability of this therapeutic strategy (Hoffer and Oslon, 1991). On the other hand, studies involving the application of various growth factors to dopaminergic tissues have shown more promising results. Pre-clinical studies have identified several growth factors, including GDNF (Gash et al, 1996), BDNF (Tsukahura et al 1995), and CNTF (Clatterbuck et al, 1993), that are able to support the survival and maintenance of dopaminergic neurons. Additionally, pilot studies investigating the direct delivery of GDNF into the brains of patients with PD have shown safety as well as some clinical improvement (Slevin et al, 2005, Gill et al, 2003). Nevertheless, strategies utilizing growth factor delivery raise additional questions, such as which growth factors should be used and how should they be obtained, delivered, and dosed. Currently purified growth factors are very difficult to manufacture and access is limited by substantial patent restrictions. As a way to overcome these obstacles, this proposal is designed to investigate the use of an autologous source of cellular tissue from the peripheral nervous system (PNS) that has been shown to produce and express several important growth factors.

Peripheral nerve tissue and Schwann Cells: The predominant cell type in the PNS is the Schwann cell (SC). Under normal conditions, SCs support and maintain axonal function and metabolism. In response to injury, SCs upregulate the production of growth factors that play a major role in the repair of the damaged nerve. The growth factors produced include those that have been shown to promote and maintain dopaminergic neurons in experimental conditions: GDNF (Henderson et al, 1994), BDNF (Meyer et al, 1992) and NT-3 (Funakoshi et al, 1993). The central hypothesis of this proposal is that the harvesting of the peripheral nerve graft will initiate the production of growth factors through the injury response, and that the transplanted graft will allow the SCs to associate with the degenerating dopaminergic neurons and deliver the expressed growth factors to restore and maintain the injured neurons over time.

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The primary objective of this pilot study is to demonstrate safety of the approach: introducing a minor modification of a standard, FDA approved neurosurgical procedure in use for over a decade to implant autologous peripheral nerve into the central nervous system. As such, the study is designed to pose minimal risk and minimal inconvenience to the subjects. Additionally, the test paradigm is performed strategically to not interfere with the surgery or delivery of the scheduled clinical DBS therapy. The scientific basis for this study is that the implanted peripheral nerve tissue is naturally well suited to provide multiple growth factors that have been shown experimentally to support the survival and function of dopaminergic neurons. **Central to this proposal is the hypothesis that the implanted tissue will physiologically deliver growth factors to restore to normal function the afflicted dopaminergic neurons found in PD.**

DBS for PD was approved for use in 2002 and has been shown to be an effective treatment for patients with advancing symptoms that are refractory to medication management (Follett et al, 2010). A recent study has also shown that DBS for PD is better than medical management alone for patients with advanced disease (Weaver et al, 2009). Nevertheless, any surgical procedure carries a risk of adverse events. Specific risks of DBS surgery include, intracranial hemorrhage, stroke, development of a new neurological deficit, and complications related to hardware malfunction. A retrospective study evaluating over 300 DBS procedures for movement disorders concluded that DBS is safe and effective, and that even though the overall reported incidence of adverse events is relatively high (43.3%), most are not serious, but are transient and do not pose a significant risk (Kenney et al 2007). The authors also reported the incidence of life threatening vascular adverse events as uncommon (1.6%). Thus, this pilot study will be performed in subjects who have already elected to undergo a safe procedure that has a known adverse event profile.

This study is innovative in several ways, including the clinical focus, the scientific basis, and the procedural approach.

First, the clinical focus of the project is directed toward the rescue and regeneration of damaged dopaminergic neurons. Most clinical studies of PD involve symptomatic treatments, but do not directly attack the underlying pathophysiology. In an attempt to reverse the pathologic process, peripheral nerve tissue will be implanted into the area of cell loss. Peripheral nerve tissue was selected because it has been shown to produce a host of growth factors that provide support for the dopaminergic neurons that are at risk for further degeneration.

Second, the project is novel scientifically in its approach to promote cell survival. While some studies using a single growth factor have shown modest clinical benefits on PD (Slevin et al, 2005, Gill et al, 2003), there are several problems with the approach. One problem is that the delivery is currently limited to a single growth factor. Another is that the delivery requires a chronically implanted device or gene therapy approach. Finally, the target for growth factor delivery is not necessarily in the region of the cell loss. This project has been designed specifically to overcome these issues. Peripheral nerve tissue is an ideal source for donor tissue. As stated previously, in response to nerve injury, peripheral nerve tissue increases its production of growth factors to initiate and maintain nerve repair. The SC is the main source for these growth factors, many of which support dopaminergic cell survival and function. It is likely that multiple factors will be better able to rescue and sustain the impaired neurons. Additionally, the autologous nerve tissue will be implanted directly into the SNpc, the anatomical region of the PD-injured dopaminergic cells. In this way, a natural source of growth factors will be delivered directly into a major area of cell damage.

Third, the project is novel in its methodological approach. The most significant feature of the protocol is the combined timing of the graft harvesting and implantation with the scheduled deep brain stimulation surgery. In this institution DBS surgery is routinely performed and is accomplished in two stages. Stage I is a preparatory surgery and is performed under general anesthesia. It includes the placement of the pulse generator, tunneling of the lead extensions, and the placement of the two frontal burr holes to be used as access for the microelectrode recording. It is during this surgery that the peripheral nerve graft will be prepared for harvesting from the sural nerve. This is accomplished

through a standard neurosurgical approach used for biopsy of the sural nerve and typically takes approximately 15 minutes. Stage II surgery is performed 5 days later and includes the placement of the DBS electrodes utilizing frame-based stereotactic guidance and microelectrode recordings to confirm targeting. The targeting and confirmation for the stimulating electrodes also confirms the targeting of the substantia nigra, the target of the peripheral nerve graft. The peripheral nerve graft will then be harvested and implanted into one substantia nigra *only after* both electrodes have been placed successfully. In this way, the study portion of the procedure does not interfere with the scheduled DBS surgery or treatment.

Overall, this study integrates several novel concepts based on clinical, scientific, and technical grounds.

The concept of tissue transplantation into the CNS has been explored as a therapy for the treatment of multiple conditions, including PD (Freed et al, 2001) Multiple Sclerosis (Stangel 2004) and Spinal Cord Injury (Levi *et al.*, 2002). Several animal studies have demonstrated the feasibility of transplantation of various tissues into the CNS, including embryonic ventral mesencephalic cell suspensions (Brundin et al, 1986) and solid grafts (Strömberg et al, 2000, van Horne et al, 1991), adrenal medulla grafts and co-grafts (Watts et al, 1995), carotid body cell aggregates (Arjona et al, 2003), fetal cerebellar (Bankiewicz et al 1991) and amniotic tissue (Bankiewicz 1994), as well as purified Schwann cells (Pizzorusso et al, 1994).

Studies in both humans and animals have shown the safety of the transplantation of purified SCs into the brain and spinal cord (Pizzorusso *et al.*, 1994; Kohama *et al.*, 2001; Stangel, 2004; Saberi *et al.*, 2008). In the study by Strangel, autologous SCs were transplanted into lesions in the right frontal lobe of the brains of MS patients. While the treatment did not provide any significant clinical benefit in this trial, the transplantation was determined to be safe with "no complications and neither inflammation nor abnormal cell growth." Additionally, Stangel notes that the benefit to an autologous transplantation of SCs is that each patient is able to serve as his/her own donor, minimizing the need for immunosuppression. This study will utilize grafts of peripheral nerve tissue, which contain SCs, rather than using isolated SC suspensions. This choice is based on the finding that continued contact between the SCs and the extracellular matrix (ECM) of the peripheral nerve tissues is very important for SC function. Chernousov et al (2008) have shown that laminin and collagen, proteins of the ECM, regulate SC function and survival and help to promote neurite outgrowth and cellular repair.

The safety of autologous peripheral nerve grafts into the CNS is supported by the results of investigations in both non-human primate models (Levi *et al.*, 2002) and in patients with PD (Watts *et al.*, 1997). Following positive results in a study examining the efficacy of intra-striatal co-grafts of autologous adrenal medulla and sural nerve in macaque models of PD (Watts *et al.*, 1995), the research team placed such co-grafts in the caudate and putamen of five human subjects with advanced PD (Watts *et al.*, 1997). The surgery proved to be without complications and was ultimately deemed both safe and successful. The safety of transplantation into the SNpc has been demonstrated by Mendez et al (2002) by placing cell suspensions bilaterally into the SN, as well as the putamen, without any perioperative or long term complications. To date, results from the first seven participants in our initial pilot study (IRB 12-1021-F6A) have only shown adverse events (Urinary retention, hypomania, superficial cellulitis, cough, and headache) in line with standard DBS surgeries. Therefore, evidence from the literature suggests that stereotactically grafting peripheral nerve tissue into the SNpc will also prove to be a safe procedure for PD patients.

An inquiry to the FDA regarding the need for permission or oversight yielded a response stating that the study does not meet criteria for the need for FDA approval or oversight based on the protocol design in which the patients donate their own tissue, the tissues are not modified or enhanced prior to implantation, and the implantation occurs during a procedure that the subjects have already elected to undergo for clinical reasons. A copy of this letter can be found at appendix A.

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Data from this pilot proposal will be used to generate a programmatic investigation of this approach with respect to impact on symptoms, optimization of graft size and placement, and mechanism of action. If successful, this treatment strategy could significantly change the treatment of PD and could have an impact on other neurodegenerative disorders as well.
In summary, this pilot study will provide safety data that can be used to generate a larger phase III clinical trial. If successful, it would herald the development of a new treatment for PD in which patients are able to provide their own tissue as a source of growth factors that could arrest or reverse the ongoing cellular loss that is responsible for their devastating motor dysfunction.

2. Objectives: The first specific aim is to assess the feasibility and safety of the combined peripheral nerve graft/DBS surgical procedure. The second specific aim is to evaluate the long term clinical safety of the peripheral nerve implant.

3. Study Design: We are proposing the implantation of autologous peripheral nerve grafts into the SNpc of participants with PD undergoing deep brain stimulation (DBS) surgery. This approach is designed to test the safety of a new therapeutic strategy, in which Schwann cells (SC) within peripheral nerve grafts serve as a source of neurotrophic factors, which have been shown experimentally to support the survival and growth of the neurons that degenerate in PD.

This study will investigate a cellular treatment that could provide trophic support for diseased neurons and possibly halt or reverse the degeneration. We will evaluate the feasibility and safety of the procedure as well as the long term safety of the combined treatment. By design, participants will undergo the nerve graft preparation and implantation during the DBS surgery. Following a standard protocol, DBS surgery is performed by the PI in two stages: a preparatory stage I procedure, followed five days later by a second surgery (stage II) to implant the stimulating electrodes into the designated target. The peripheral nerve graft preparation and implantation is designed to impose minimal risk and discomfort to the participants and is performed during these two stages. The graft preparation is performed utilizing a standard neurosurgical approach used for sural nerve biopsies during the stage I DBS surgery, performed under general anesthesia. The harvesting and implantation of the nerve graft is performed during the stage II DBS surgery. Clinical evaluations for safety will begin directly following graft implantation and continued at regular postoperative time points while the participant is admitted as an inpatient. Participants also undergo a standard post-op CT or MRI scan that is used to verify electrode placement and check for the possibility of hemorrhage.

Participants will be followed closely clinically at regular time points for 24 months after implantation. They will be evaluated for possible neurological sequela through scheduled neurological exams, including use of a Unified Parkinson's Disease Rating Scale (UPDRS), during follow up appointments. Additional evaluations will occur during routine visits for DBS programming, adjustments, and optimization. Preoperatively and at the 24 month visit, formal neuropsychological and non-motor symptoms and quality of life exams will be administered. Participant adverse event profiles will be compared to those of routine DBS PD patients from our program as well as to the adverse event profiles reported in the literature for the same DBS procedure.

The anticipated outcome is that the procedure can be performed in a safe and efficient manner, adding not more than 30-45 minutes to the length of each stage of the DBS procedure. It is further anticipated that the grafts will prove to be clinically safe over the 24-month follow-up period with no greater adverse events compared to DBS procedures without graft placement. This study poses minimal risk as the additional procedures are "piggy-backed" to a major neurosurgical procedure that is routinely performed for patients with PD. If successful, this treatment approach would be widely available to any patient undergoing DBS surgery for PD with minimal risk and minimal cost.

4. As a pilot project, the primary outcome is safety. The peripheral nerve grafts will be implanted in patients with PD who are concurrently undergoing DBS surgery in the FDA-approved targets for PD, the subthalamic nucleus (STN) or the internal globus pallidus (GPi). Thus, only patients who have already been approved for bilateral DBS surgery will be eligible for this study. The peripheral nerve

graft will only be placed after all elements of the DBS system have been implanted. This not only eliminates the need for a separate surgery, but also ensures that the patient will receive the full benefits of DBS therapy regardless of the outcome of this safety trial.

5. Study Population: Sixteen subjects with a diagnosis of PD who have been selected and consented for DBS of the STN or GPi will be asked if they would like to participate in the study. In this way, the ability to participate in the study will not play a factor in the decision to undergo DBS therapy. Participants will be between 40 and 75 years of age and will include men, women, and minorities.

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Inclusion Criteria:

- Undergoing DBS of the STN or GPi
- Between the ages of 40-75
- Able to give informed consent
- Show a positive response to sinemet
- show no significant cognitive deficit per a formal neuropsychological exam,
- be able to tolerate the surgical procedure

Exclusion Criteria:

- Any condition that would not make the subject a candidate for DBS of the STN or GPi
- Under the age of 40 or over the age of 75
- Unable to give informed consent
- Previous PD surgery or intracranial surgery

6. Subject Recruitment Methods and Privacy: Potential participants will be approached in the clinic setting after they have elected to undergo DBS surgery for PD. As participants will be recruited from patients who have elected to receive DBS therapy, refusal to participate in this study will not prevent them from getting their recommended and selected treatment for their PD symptoms. No advertising will be performed.

7. Informed Consent Process: Prior to entering the study, the risks and benefits of participating will be explained to the patient by a member of the study team authorized to obtain informed consent. Written informed consent from the patient will be placed in each patient's medical record and documentation of the informed consent process will be placed in the patient's progress notes. A signed informed consent form will be retained by the investigator. The study participant will receive a copy of the informed consent form. Only subjects with intact cognition and consent capacity will be allowed to participate.

8. Research Procedures: A Summary of Study Procedures can be found below. All tests and procedures listed below are being done for the purpose of the research study only. More detailed information about some of the procedures is shown below.

		SURGERY		FOLLOW-UP				
	Screening	(Stage 1)	(Stage 2)	24-48 hour post op	Month 6	Month 12	Month 18	Month 24
Study Consent	X							
Medical History	X							
Medication History	X				X	X	X	X
UPDRS	X				X	X	X	X
PDQ-8	X							X
Non-motor symptoms scale	X							X
Gait assessment	X				X	X	X	X
Exposure and Prep of the peripheral		X						

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Nerve								
Harvesting and implanting of the nerve graft			X					
Protein analysis of peripheral nerve tissue		X	X					
Record DBS Settings					X	X	X	X
MRI	X			X				X
Neuro-psych Evaluation	X							X
Adverse Event assessment	X	X	X	X	X	X	X	X
Videotaping	X				X	X	X	X

As part of the standard preoperative DBS protocol, subjects will be evaluated using the unified Parkinson's disease rating scale (UPDRS) and undergo a formal neuropsychological evaluation. Subjects will then be scheduled for their two stage DBS surgeries. Stage I surgery is preparatory, is performed under general anesthesia, and includes the placement of the pulse generator, tunneling of the lead extensions from the pulse generator pocket incision just below the collar bone to the scalp behind the ear, and the placement of the two frontal burr holes to be used as access for the microelectrode recording and stimulator placement performed during the second stage. It is during this surgery that a sural nerve graft will be prepared for harvesting for the second stage of the surgery. This is accomplished through a standard neurosurgical approach used for biopsy of the sural nerve. A 3-4 cm incision is made longitudinally just above and behind the lateral aspect of the ankle. The sural nerve lies in the subcutaneous space, is easily identified, and is then isolated and transected distally. A small silk suture is loosely placed around the proximal segment, 2 cm from the distal transaction. The suture serves as a marker for identification for harvesting during the second stage. Transecting the nerve initiates the injury response of growth factor production. Stage II surgery includes the placement of the DBS electrodes utilizing frame-based stereotactic guidance and microelectrode recordings to confirm targeting. The DBS electrodes will be placed according to standard clinical practice. The peripheral nerve grafts will then be harvested and implanted into one SNpc after both DBS electrodes have been placed successfully. For this component, the previously created incision in the ankle region is re-opened under local anesthesia, the marking stitch is identified, and the sural nerve is transected 2 cm proximal to the previous distal transaction. The nerve segment is then placed in normal sterile saline and trimmed for loading into the transplant cannula. The graft, ~0.8mm in diameter and ~1cm in length is then deposited stereotactically into the SNpc. All incisions are then closed in the standard, surgical, subcuticlular fashion. In this way, the study portion of the procedure will not interfere with the scheduled DBS surgery or treatment.

Participants will then be followed by our clinical routine for their DBS treatment, including a normal postoperative one to two day hospital stay, a one and two week post-operative visit, and multiple neurological clinical visits for initial stimulation programming and optimization. A routine head CT or MRI scan will be obtained within 24 hours after the completion of the second surgery. Subjects will undergo formal UPDRS evaluations at 6, 12, 18, and 24 months and a repeat neuropsychological exam will be performed at 24 months. All adverse events will be reported and documented. Results will be compared to the known adverse event profile associated with bilateral DBS of the STN for PD as documented in the literature. The expectation is that the grafting of peripheral nerve tissue to the SNpc will have an adverse event profile that is no different than that of DBS surgery alone.

In this study, subjects will be monitored for both perioperative and long term postoperative adverse events. Clinical monitoring will be performed by the movement disorders neurosurgeon, neurologist, and the clinical study coordinator, a movement disorders neurology PAc experienced in DBS evaluation and programming. Pre-operative clinical baseline data will include a UPDRS evaluation, formal neuropsychological evaluation, a quality of life assessment (PDQ-8), non-motor symptom questionnaire,

gait assessment, and a co-morbidity profile obtained from the pre-operative medical history. The perioperative period is arbitrarily designated as the time from day of the first stage surgery to two weeks following the day of the second stage surgery. This allows for routine clinical assessments to take place during patient contact while in the hospital setting and during the routine two week post-operative visit. During this time period, adverse events will be recorded from clinical reporting and chart review as well as from specific queries by the clinical coordinator at the two week post-op visit. A routine post-operative scan, MRI or CT will be performed within 48 hours of the conclusion of the second stage of the surgery. Adverse events will be categorized as either related to PD, DBS surgery, or not related to either. In addition, adverse events will be categorized as mild, moderate, or severe, as defined by the Medical Dictionary for Regulatory Activities, version 11.0. Serious adverse events will be designated as any event that results in death, disability, or prolonged or new hospitalization or that is life threatening or that requires medical or surgical intervention.

Postoperative data will be collected at 6, 12, 18, and 24 months from the time of the second stage surgery. Data collection will take place during scheduled clinic visits and will include UPDRS scores, medication dosages, recording of DBS settings and programming changes, gait analysis, as well as a query of adverse events dating from the last clinical visit. At the 24 month visit, the formal neuropsychological non-motor symptom, and the PDQ-8 exams will be repeated. As a pilot safety study, the participant adverse event profile, quality of life scores, and neuropsychological evaluations will be compared to those reported in the literature. Additionally, subject scores on the UPDRS parts III and part IV (motor and complications off therapy) and medication modifications will be evaluated for possible clinical benefit from graft placement. Off medication assessments will be performed at least three hours but possibly as much as twelve hours off medication (time from last PD medication dose). This time frame has been deemed acceptable by the IRB at this institution in a previous study involving participant assessment off PD medications (Slevin et al, 2005).

We will request permission from all subjects to videotape them at screening and then post-operatively, at follow-up visits. Subjects may be videotaped during examinations in order to visually document changes in their exams over time.

We will also ask the subjects if they agree to be contacted in the future with regards to their willingness to participate in additional follow up visits or in future research studies about how to prevent, detect or treat Parkinson's disease.

POTENTIAL FUTURE USE

Banking of tissue specimens

A small sample (approximately ½ inch) of the peripheral nerve obtained during the first and second stages of the DBS surgery will be frozen and kept at the University of Kentucky under the direction of Dr. Craig van Horne for research purposes. The nerve tissue collected will not be needed for the diagnosis or management of the participant's health and will not interfere with the graft that will be implanted during the main part of the study. The results of any testing done on the samples will not be shared with the participant.

No additional tissue will be taken. If participants agree, the sample obtained for this research study may be tested immediately or may be frozen and examined later. Donated samples may be tested for markers that might indicate how the nerve graft is working. No genetic testing will be performed on collected samples.

Samples will be kept until we receive written notice from the participant that they wish to have their sample destroyed. Subjects will be advised that destruction of their sample may not be possible because the sample may no longer exist. Participation in this part of the study is optional. Participants can refuse to take part in the banking of their peripheral nerve and still take part in the main study.

Form B: Medical IRB Research Description

9. Resources: The research procedures will be carried out at the UK Chandler Medical Center, and the Kentucky Neuroscience Institute. The patients will be followed by Doctors van Horne and Slevin and their research team which includes physicians and certified nurses and clinical research coordinators during the study. Emergency medical equipment, medications and supplies will be at the physician's disposal should the patient have an acute untoward reaction. The UK Center for Translational Science will provide Quality Assurance monitoring throughout the life of this study.

10. Potential Risks: The most significant health risk of this study is related to the surgical procedure at the time of graft placement. The overall risk of a major adverse event (considered to be catastrophic hemorrhage) of graft placement is assumed to be no greater than that of a single needle pass through the brain, reported to be approximately one in 500 or 0.2% (Kenny et al, 2007). Additional risks are associated with the graft harvesting procedures. The surgical risk is minimal and this part of the protocol is considered a minor surgery and employs a surgical approach that is used for sural nerve biopsies. Discomfort is minimized by performing the initial portion of the procedure under general anesthesia. The incision is ~3cm in length located above and behind the lateral aspect of the ankle.

The sural nerve is a sensory nerve that supplies sensation to the lateral aspect of foot and heel. Typically, only one fascicle of the nerve is removed which usually causes no detectible sensory deficit. Larger biopsies have reported to lead to either temporary sensory loss or permanent loss of sensation to a small dermatomal patch on the outer aspect of the foot and/or heel.

Additionally, the grafting procedure has been designed to occur at the time of DBS surgery – so as not to require a separate surgery. The timing of the grafting procedure is scheduled to take place after the DBS electrodes have been placed so as to not interfere with the implantation and installment of the DBS electrodes. There could be a technical difficulty during the portion of the procedure that involves graft harvesting or implantation that would prevent adequate graft placement. Thus, if there is a problem with graft harvesting or placement, participants will still receive their elected DBS therapy but would not receive the graft.

Although this study does involve grafting, participants will donate their own tissue. This eliminates the risk of graft rejection and obviates the need for immunosuppressant medications typically used with other transplant studies.

Release of videotaped neurological session: In order to maintain the scientific and educational integrity of the videotaped sessions we are unable to blur out the subject's entire body. We will however, blur out their eyes and nose which will only allow the viewer to see their mouth. The risks associated with allowing the release/showing of the subjects' videotaped sessions include full body and mouth recognition by the viewer.

Subjects will be provided with the option of whether or not to allow the release of their videotaped sessions. Their election will be documented in the signed informed consent.

11. Safety Precautions: Provisions to guard against the potential risks and discomforts discussed in section 9 are as follows: Every precaution to prevent a direct study injury will be taken by medical personnel and the investigators. The research subject will be followed by physicians, fellows, registered nurses and other research staff members for the duration of the subjects' hospitalization. Routine care will be provided by the hospital staff. Emergency medical equipment, medications and supplies will be at the physician's disposal should the subjects have an acute untoward reaction. The subjects will be monitored for clinical adverse experiences throughout study therapy. Throughout the study, all adverse events will be monitored and recorded**.** The UK Center for Translational Science will provide Quality Assurance monitoring throughout the life of this study

12. Benefit vs. Risk: As the scientific basis of this study to restore the degenerating dopaminergic neurons associated with PD, the potential benefit would be an improvement in the participant's symptoms due to graft function. This would benefit the participant by reducing the amount of therapy

(medication and or stimulation) needed for symptom control. In the long term, participants could also experience a stabilization of the progression of their disease.

13. Available Alternative Treatment(s): Should a subject decline the opportunity for participation in the study they will continue on with their DBS surgery, and FDA approved treatment for medically refractory PD. There are no other current combined treatment options that incorporate DBS. Please note that DBS therapy includes continued medical therapy to optimize the treatment of PD symptoms.

13. Research Materials, Records, and Privacy: The investigative team maintains the right to keep, preserve, use and dispose of the findings of this investigation in accordance with University of Kentucky Records Management and IRB policies and guidelines. Investigational records from this study will be maintained in a confidential manner; subject names will not be associated with any published results. All clinical information obtained will be considered to be part of the patient's medical chart and will be treated as such according to standard HIPPA guidelines and regulations. Additionally, clinical information relating to the study will be copied, de-identified, coded, and placed in a separate binder. Binders will be kept in a locked cabinet in the office of the study coordinator.

14. Confidentiality: We will keep private all research records that identify the subject to the extent allowed by law. We will make every effort to prevent anyone who is not on the research team from knowing that the subject gave us information, or what that information is.

Subject information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. The subject will not be personally identified in these written materials. We may publish the results of this study; however, we will keep subject name and other identifying information private.

Videos taken of a subject during study related neurological testing sessions may be shown at local and national meetings, conventions, conferences for scientific and educational purposes only. Release of the subjects' videos will not be made if the subject has not authorized it.

In order to process the subjects study payments we will need to collect their social security number. The subject does not have to give us this number however, refusing to provide us with their social security number may result in their not receiving study payment. The subject can refuse to provide their social security and still be enrolled into the study.

The study information collected from the subjects participation in the study will be collected and stored in paper charts and video. They follow the principles of the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

Subject charts, video, and any other items containing confidential items will be stored in a safe place overnight and not left on the desk. Charts will not be left in an area where others might have access to them.

The subject should know, however, that there are some circumstances in which their information may have to be shown to other people. For example, the law may require us to show their information to a court.

Officials from the Food and Drug Administration (FDA) and the University of Kentucky may look at or copy pertinent portions of records that identify the subject.

15. Payment: Subjects will be compensated for time and travel. They may receive up to $500.00 for taking part in this study. Subjects will receive $100.00 compensation following study visits at the 6, 12, 18, and 24 month visits.

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16. Costs to Subjects: The subject and/or their insurance company, Medicare or Medicaid will be responsible for the costs of all care and treatment they receive during this study that they would normally receive for your condition. These are costs that are considered medically reasonable and necessary and will be part of the care they receive if they do not take part in this study. Subjects will be responsible for all costs associated with their DBS surgery.

There will be no charge to the subject for their participation in this study. The study-related procedures, and study visits will be provided at no charge to the subject or their insurance company.

Costs associated with treating any injury suffered while participating in this study will be the responsibility of the subject or their insurer. Subjects will be advised to ask their insurer if they have any questions regarding their insurer's willingness to pay these costs; this includes contacting Medicare or Medicaid if they are covered by Medicare, or Medicaid.

A co-payment/deductible may be required by their insurer or Medicare/Medicaid even if the insurer or Medicare/Medicaid has agreed to pay the costs. The amount of this co-payment/deductible may be substantial.

Subjects do not give up their legal rights by signing this form.

17. Data and Safety Monitoring: Monitoring for adverse events will be conducted in real-time by the study investigators and study coordinators. Risks involved with this study are considered greater than minimal risk. For this reason, we will utilize the standing independent Data Safety Monitoring Board (DSMB) as chartered by the University of Kentucky Center for Clinical and Translations Science (CCTS) to monitor the safety of this study. The DSMB will meet semiannually or as needed, and will review subject recruitment, AE's, side effects, laboratory results, dropouts, protocol violations, and inclusion/exclusion criteria. More frequent meetings will take place if side effects or other problems are prevalent.

18. Subject Complaints: Subjects will be encouraged to address any complaint to any member of the study team including the PI. They will be told that they can, at any time, call the Office of Research Integrity at the University of Kentucky at (859) 257-9428 or toll free at 1-866-400-9428.

19. Research Involving Non-English Speaking Subjects or Subjects from a Foreign Culture: N/A

20. HIV/AIDS Research: N/A

21. PI-Sponsored FDA-Regulated Research: N/A. This study is exempt from IND and FDA monitoring.