IMPLANTATION OF SPHERAMINE ® IN ADVANCED PARKINSON'S DISEASE (PD)

Roy A.E. Bakay [1], Cathy D. Raiser [2], Natividad P. Stover [2], Thyagarajan Subramanian [3], Michael L. Cornfeldt [4], Alfred W. Schweikert [4], Richard C. Allen [4], and Ray Watts [2]

[1] Department of Neurosurgery, Rush Presbyterian-St. Luke's Medical Center, Chicago, IL, [2] Department of Neurology, Emory University, Atlanta, GA, [3] Departments of Neurology and Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, [4] Cell Therapy, Titan Pharmaceuticals Inc., Somerville, NJ

TABLE OF CONTENTS

1. ABSTRACT

Evaluation of the safety and efficacy of unilateral stereotactic implantation of cultured human retinal pigment epithelial (hRPE) cells attached to microcarriers (Spheramine®) in patients with advanced PD in an open label pilot study. Six patients with advanced PD (3 males; 3 females; mean age 52.2 years; mean duration of PD 10.2 years; mean Hoehn and Yahr stage "off" 3.75) were assessed at baseline and post-operatively using the modified CAPIT. Each patient underwent MRI-guided stereotactic transplantation of 325,000 hRPE cells attached to microcarriers in 5 tracts, 5 mm apart in the post-commissural putamen contralateral to the most affected side. Immunosuppression was not used. The UPDRS Motor (UPDR-M) score in the practically defined "off" state was the primary outcome measure. At 6 months post-op, the mean UPDRS-M (off) score improved to 35 (34%) from a pre-op baseline mean of 52 (p < .001). Secondary outcome measures improved including the total UPDRS (33%), Timed Motor Tests (on, 14%; off, 23%), PDQ39 QOL (30%), and Schwab and England score (on, 11%; off, 30%). Bilateral improvements have been observed in motor symptoms, with the greatest effect seen contralateral to the implants. Three of six patients currently have lower Dyskinesia Rating Scale scores than at baseline, while the scores of the other three are unchanged from baseline values. No "off-state" dyskinesias have been observed. Thus Spheramine® implantation therapy appears to be safe and well tolerated for 6 months post-implantation.

2. INTRODUCTION

Parkinson's disease (PD) is characterized primarily by the degeneration of the dopaminergic neurons in the dorsomedial aspect of the substantia nigra pars compacta (SNc). These neurons give rise to long axonal projections into the caudate nucleus and the putamen and make short dendritic connections to the neurons in the subtantia nigra pars reticulata (SNr). The pathophysiology of PD has been attributed to a greater than 80% decrease in dopamine content within the striatum (particularly in the post-commissural putamen) secondary to the degeneration of SNc neurons (1). This results in disinhibition of the subthalamic nucleus (STN), which then results in increased basal ganglia output from the globus pallidus (GPi) and the SNr (2). The net result is the inhibition of cortical motor neurons. Pharmacological therapeutic strategies for PD are currently directed at replacement of dopamine in the brain either by exogenous supplementation of dopamine precursor (levodopa), dopaminergic agonists, drugs which prevent the breakdown of dopamine, or medicines that modulate other neurotransmitters which oppose the effects of dopamine, i.e. glutamate or acetylcholine.

The discovery that levodopa ameliorates parkinsonian symptoms revolutionized the treatment of PD and continues to be the most efficacious available treatment for PD (3). However, disease progression and chronic treatment with levodopa leads to multiple disabling side effects including motor fluctuations, dyskinesia, and dystonia in the majority of PD patients within 5 to 7 years after starting treatment (4). Although many attempts have been made to produce long-acting preparations of levodopa and dopamine agonists, disabling side effects continue to be a major problem in patients with PD (5). Continuous mechanical systemic administration of levodopa can prevent the occurrence of these disabling side effects (4,6,7). However, these methods of administration are currently impractical and unacceptable to patients with PD.

A unique series of investigations have explored the possibility of long-term continuous replacement of striatal dopamine using dopaminergic cell transplantation. Initial studies by Backlund et al. showed a lack of efficacy of stereotactic transplantation of adrenal medullary

dopaminergic cells into the striatum. (8) Nevertheless, this opened the way for clinical studies of central nervous system (CNS) transplantation. Since then several other types of dopaminergic cells have been transplanted successfully into PD patients in attempts to ameliorate parkinsonian symptoms. The greatest clinical benefit reported to date with dopaminergic cell transplantation has been using allogenic human fetal ventral mesencephalic tissue transplantation 9-12). Transplantation of fetal dopaminergic cells in PD has the advantage of potentially providing synaptic replenishment of dopamine that are capable of regulation of dopamine output and thus theoreticaly avoids or ameliorates the complications from chronic intermittent levodopa therapy. Disadvantages include supply shortage, storage problems, and potential contamination. Moreover, such studies are expensive and raise ethical concerns (13-18).

There have been numerous attempts to find alternative tissues to overcome these problems. Human retinal pigment epithelial (hRPE) cells represent an alternative to human fetal tissue transplants in PD patients. These cells can be obtained easily in large quantities, secretes levodopa and possibly dopamine, survive well after transplantation when attached to biocompatible microcarriers, and do not require systemic immunosuppression. Human RPE cells are found in the inner layer of the neural retina located between the photoreceptors and the choriocapillaris (19,20). They are the epithelial cells that form tight junctions and are considered to play an important role in maintaining the blood-retinal barrier. In addition, hRPE cells may have nutritive, phagocytic, and trophic functions. Human RPE cells produce levodopa, the precursor to dopamine, via the enzyme tyrosinase as a precursor to the formation of their characteristic brown-black eumelanin pigment (21,22). These cells are also reported to secrete dopamine and express D_2 receptors (23-25). They contain vesicles, which have the vesicular monoamine transporter (VMAT) sensitive to reserpine/TBZ blockade, but do not appear to have the dopamine transporter (DAT). Unlike fetal mesencephalic tissue, hRPE cells do not differentiate to form axons or make synaptic connections with the host after transplantation (26). The expression of the D_2 type of dopaminergic receptors on their cell surface probably serves as autoreceptors to regulate the dopaminergic function of hRPE cells. Human RPE cells have been reported to secrete a number of growth factors (27): platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), transforming growth factor (TGF), vascular endothelia growth factor (VEGF), nitric oxide, pigment epithelial-derived factor (PEDF) and Fas-ligand (28,29). Human RPE cells are readily isolated from eyes obtained from eye banks and can easily be grown in culture (30). They can be grown and expanded in tissue culture, stored for prolonged periods of time and extensively tested prior to transplantation. Cells derived from a single donor eye could potentially treat several hundred patients. Thus, hRPE cells appear to be a dopaminergic cell type that might serve as readily available, transplantable source of dopamine and its precursors, as well as potentially therapeutic growth factors.

There are many factors that contribute to ocular immune privilege (31). RPE cells express CD95L and release TGF-beta, both of which may create local immunosuppression (32-36). However, fetal RPE cells do express MHC I and many minor histocompatibility antigens (37,38). Culturing RPE cells can induce MHC class II expression (39,40). Allogeneic neonatal RPE cells can sensitize recipient host if grafted into nonimmune-privileged sites but not if grafted into immune-privileged sites (32). Thus, allogenic hRPE cells appear to be a suitable dopaminergic cell source for transplantation in advanced PD patients and systemic immunosuppression may not be necessary. Placing them on microcarriers can increase their survival and immune-privilege after transplantation. Microcarriers made of gelatin and other materials have traditionally been employed in cell culture *in vitro* to enhance the cell viability. Human RPE cells are anchorage dependent cells and undergo apoptosis *in vivo* or *in vitro* in the absence of a support matrix (41). Similarly, cells passively attached to biocompatible microcarriers and transplanted into the brain of rodents and non-human primates display prolonged and enhanced survival *in vivo*, even in the absence of immunosuppression (23,42-44). This is known as cell coated microcarrier (CCM™) technology and appears broadly applicable in terms of cell type and microcarrier composition. It may represent a solution to enhancing cell survival and is the technological basis for hRPE cells attached to gelatin microcarriers or Spheramine® (45-47).

The preclinical basis for this clinical trial was an initial rodent study followed by a blinded placebo controlled primate study (26,48,49). Sixteen monkeys were enrolled after attaining a stable right MPTP- induced hemiparkinsonian (HP) state for 3 months. Each animal was assessed for their responsiveness to optimal doses of oral levodopa/carbidopa and randomized into 4 equally balanced groups to receive either low dose Spheramine® (approximately 12,000 cells/target), high dose Spheramine®. (approximately 60,000 cells/target), microcarriers alone or needle sham surgery. All animals were operated using a high-resolution 3-D MRI guided stereotactic transplantation technique into the left striatum (2 caudate targets and 3 putamen targets). Blinded behavioral assessments at 3 months (N=12) showed a significant (p=0.01, Fishers' exact test) mean improvement of 56% in monkey UPDRS scores in the Spheramine® treated animals compared to 16% improvement in the control animals (microcarriers alone and needle sham). The high dose of Spheramine® produced a robust improvement (50-60% from baseline, p < 0.05) in hemiparkinsonian monkeys that remained constant throughout the duration of the 12-month study that was statistically significant different from surgical controls (p<0.05). The low dose group Spheramine® and the microcarrier group did not show a statistically significant improvement from baseline nor were they statistically different from the sham surgical control group at any time point. Histological examination of the brain revealed cells consistent with hRPE cells attached to beads at the implantation sites (50). Minimal inflammatory response was seen in and around the injection tracts. In another

study, ^{11}C-raclopride positron emission tomography (PET) imaging 1 month after transplantation into bilaterally lesioned MPTP monkeys revealed decreased binding in the area that was precisely co-registered to the location of implantation of Spheramine®suggesting enhanced dopamine levels (51). The results from this blinded and placebo controlled study indicated that xenotransplanted human RPE-GM improved parkinsonian behavior and was well tolerated in non-immunosuppressed MPTP treated monkeys for up to 12 months.

These preliminary results indicated that stereotactic intrastriatal hRPE-GM transplantation might be a potentially useful therapy for advanced PD. In addition, primate toxicology studies demonstrated no adverse effect with injections of over 450,000 cells and no migration of cells (50). Based on these very promising preclinical data, the FDA approved a pilot clinical trial of intrastriatal transplantation of Spheramine®in patients with advanced PD. We are reporting the six-month results of this open label study.

3. MATERIAL AND METHODS

3.1. Spheramine® production

Spheramine® is produced by attaching hRPE cells to 100-micron crosslinked porcine gelatin microcarriers. The human RPE cells, which constitute the active component of Spheramine®, are isolated from postmortem human eye tissue. Tissue from which the cells are isolated is acquired from donors tested and determined to be free from hepatitis B and C, toxoplasma, Herpes simplex I and II, HIV 1 and 2, HTLV I and II, Treponema pallidum, Chlamydia trachomatis, and cytomegalovirus. Human RPE cells isolated from the tissue are expanded under good manufacturing practice (GMP) conditions and tested for sterility, mycoplasma, endotoxin, adventitious viruses, bovine viruses, HIV I and II, HTLV I and II, HHV-6, hepatitis A, B and C, cytomegalovirus, and Epstein-Barr virus, and are also examined by transmission electron microscopy for presence of any viral particles. The crosslinked gelatin microcarriers, the excipient component of Spheramine®, are prepared under GMP conditions from certified porcine gelatin and subjected to a variety of tests for purity. Prior to use in manufacturing Spheramine®, they are subjected to steam sterilization.

3.2. Method of Dose Delivery

To determine the correct method to deliver an optimum dose of Spheramine® gelatin microcarriers were prepared as follows. The dry microcarriers were swollen and hydrated in calcium and magnesium free phosphate buffer solution (2.5 ml PBS per 50 mg dry microcarriers) for at least 1 hour at room temperature. Without removing the PBS, the microcarriers were sterilized by autoclaving (121ºC, 15 min., 15 psi). The microcarriers were allowed to settle by gravity. The PBS was removed by suction, and the same amount of fresh PBS added. The suspension was mixed, the microcarriers allowed to settle, and the PBS removed. The microcarriers were washed twice more with PBS containing a minimum of 10% fetal bovine serum, and

finally with Hank's balanced salt solution (HBSS). A slurry of microcarriers was prepared by allowing them to settle in sterile siliconized 1.7 ml polypropylene vials (Fisher Scientific) and removing of the supernatant fluid. Twenty-five microliters (25 μl) of microcarrier slurry was loaded into a sterile 21 gauge stainless steel needles (9.65 inches long) attached to a sterile Hamilton syringe, 250 μl, back fill filled with HBSS. A 50 μl "dose" was delivered into the well of an 8 well polystyrene chamber slide (Fisher Scientific) using a delivery speed of 15 seconds per dose. A second 50 μl was delivered to an adjacent well to clear out any remaining slurry in the needle. The procedure was repeated ten times, and the amount delivered and residual amount were estimated. Using the pulsed method, the ten doses were repeated. Two pulses were used for each 25 μl of delivered sample. The pulses were less than 2 seconds each. Identical test doses were injected into fresh calf brains obtained from a slaughterhouse. Residual amounts were determined as above and delivered amounts determined by direct examination of the needle tracts.

3.3. Implantation Procedures

A clinical study sponsored by Titan Pharmaceuticals Inc. was initiated after IRB approval at Emory University School of Medicine. After completion of an appropriate informed consent, six Hoehn and Yahr stage III-IV Parkinson's patients with advanced PD (Table 1), were enrolled in an open label, pilot clinical trial to evaluate the safety and effectiveness of Spheramine® (52-54). The mean age was 52.2 years. The mean disease duration was 10.2 years with mean Hoehn and Yahr state 3.75 in the off stage. Following a stabilization period of about three months under optimal PD medication, each patient received baseline CAPIT assessment and underwent unilateral MRI-guided stereotactic surgery placing over 65,000 cells into 5 tracts in the post-commissural putamen contralateral to the patient's worst effected side (55).

A Functional CRW stereotactic frame in conjunction with a "grid array" for implantation into the putamen that was custom manufactured by Radionics (Tyco Healthcare Group LP, Ma) was used for this procedure. Local anesthesia was used prior to affixing a frame to the patient's skull, and then a localizer was attached to the head frame. With the localizer in place, the patient had MR imaging with a fast spin-echo inversion-recovery sequence to optimize the gray to white matter differences or border distinctions (56). Scans were rectilinear to the frame and from the external ear canals to the top of the skull. From these fiducial markers stereotactic coordinates are generated which targeted the post-commissural putamen. The initial locations for targets 1 to 5 were determined on axial images at the level of the anterior and posterior commissars (AC – PC level). Targets 1 and 2 were parallel and posterior to the anterior commeasure at the widest part of the post-commissural putamen (Figure 1 and 2). They were 5mm apart and equal distance from the edge of the putamen (the distance from the edge varied with each patient depending on the width of the putamen at that plane). Targets 3 to 5 were 5mm apart (Figure 3 and 4), posterior to target 1 and angled to place them in the center of the putamen (3 to 6 degrees off the

Table 1. Patient Summary Demographics

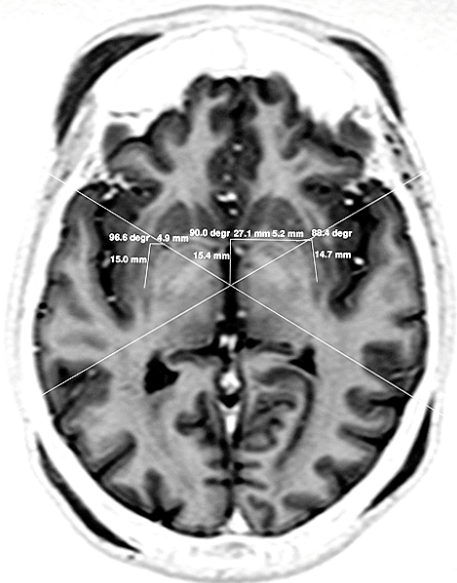| Patient Characteristic	Mean	Range
Age	52.2 years	47 – 56
Gender	3 male, 3 female	-
Duration of PD Symptoms	10.2 years	6 – 12
Baseline Hoehn & Yahr "OFF" Score	3.75	3.0 – 4.0
Baseline Hoehn & Yahr "ON" Score	2.5	2.0 – 3.0
Baseline UPDRS Total Score	118	103 – 139
Baseline UPDRS Motor "OFF" Score	52	43 - 63

Figure 1. An inversion recovery axial MR image at the AC-PC level demonstrates the initial calculations of x, y and z for the left anterior target (#1). The right targeting plan is also shown. Implantation of the putamen is accomplished by creating an inverted "L" shape with the short limb across the widest segment of the post-commissural putamen and the long limb down the center of the post-commissural putamen. The tracts angle from anterior to posterior and from medial to lateral.
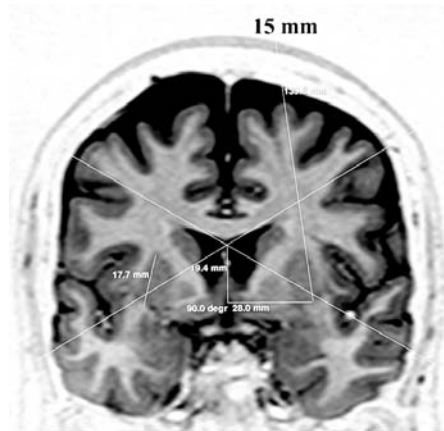

Figure 2. An inversion recovery coronal MR image with the axial anterior targets is used to recalculate the target coordinates and to maximize the excursion through the putamen from a point 15mm from midline (burr hole location). Arcs and ring angles are determine on the basis of the final target coordinates. The putamenal heights are also estimated (as on the right) to determine deposit heights.

perpendicular to the line from target 1 to 2 resulting in an inverted L shape). These initial coordinates were then plotted on coronal images and the final coordinates and angles calculated so that the trajectory would go through a point on the skull 15 mm off midline and bisect the putamen for tracts 3 through 5 and ensuring parallel tracts in the widest part of the post-commissural putamen for tracts 1 and 2. The target coordinates were recalculated to the bottom of the putamen at each site. The heights of the trajectories through the putamen for each tract were determined (Figure 2).

In the operating room under general anesthetic and in the supine position, a coronal incision was made in the scalp anterior to the coronal suture to allow access to the skull at the proposed entry site. A burr hole was made in the calvarium, .5mm anterior to the coronal suture and 1.5 cm from midline. The dura was opened sharply and removed with a bipolar coagulator to allow safe and smooth penetration by the probes. Fibrin glue was used to seal the CSF space. The patient was then placed in the semi-seated position. Five entries into the putamen with 9.65 cm long smooth tipped needles gradually tapered to 21 gauge (Popper and Sons, NY). The putamen sites were approached through the ipsilateral burr hole at a ring angle of 60-80 degrees anterior and an arc angle of 5-12 degrees on the contralateral side. The needle passes in the putamen were spaced 5 mm apart, to evenly distribute the Spheramine® over the post-commissural region. Coordinates for target 1 was set up on the phantom and the depths for targets 1-3 confirmed. Targets 1-3 were set at right angles 5 mm apart and target 1 at the lateral anterior edge of the burr hole so that all three could be placed without moving the frame. A guide tube with a sylet was gently introduced 10 mm above the top of the putamen. A solid needle identical in shape to the tapered implantation needle was introduced to target in order to create a cavity in the tract. After one minute, it was removed and the injection needle inserted, thus, preventing coring of tissue by the injection needle. Fifty microliters of the suspension was injected incrementally by initially going to the deepest target site then withdrawing half the height of the putamen for the first injection site. For each tract, there was a wait of one minute after insertion of the needle and prior to injection of cells in the deepest target site to allow elastic recoil of the brain. After five minutes the needle is moved so that the second injection was made at the top of putamen. The two deposits were made approximately 5 to 10 mm apart over a linear tract length of 10 to 20 mm as determined by the MRI. Injection of the 25 μl was in a quick burst (<1 sec). After the final injection of cells into each tract, there was a wait of 5 minutes to allow for the injected Spheramine® suspension to equilibrate so that there would be no movement of beads along the needle tract as the needle was removed from the brain. Withdrawal was slow with frequent brief stops to prevent any vacuum effect. The result is a column of cells extending from the deepest point, back along the needle track to the dorsal edge of the putamen. The residual fluid was delivered into an 8 well polystyrene chamber slide (Fisher Scientific) to clear out any remaining slurry in the needle and the amount delivered and residual amount were
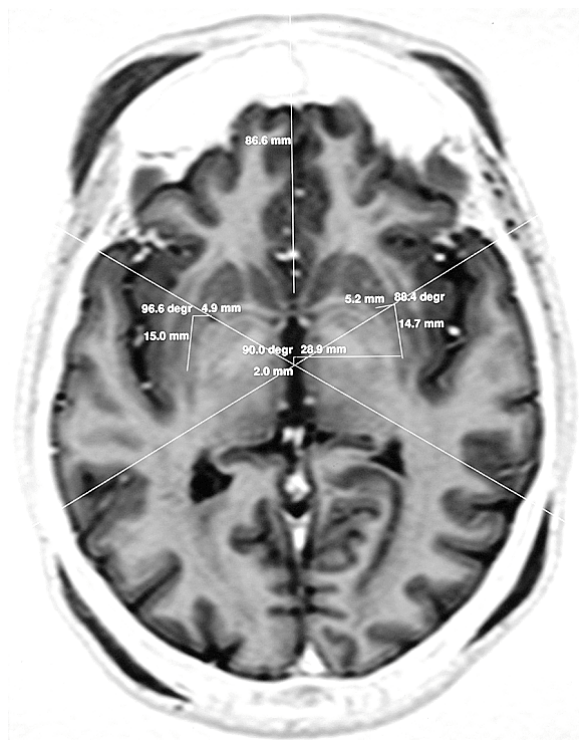
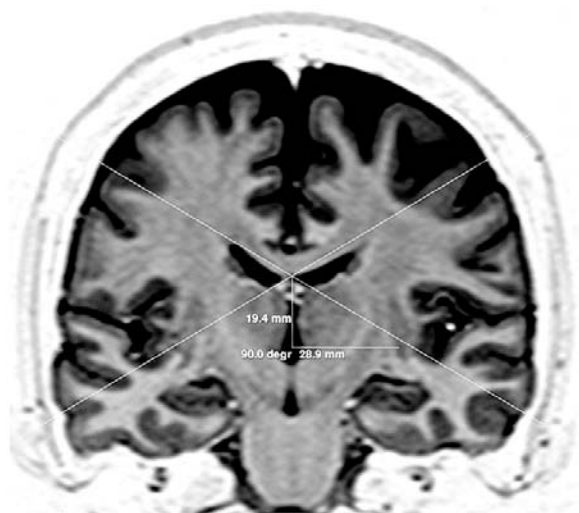**Figure 3.** The same axial MR image as Figure 1 is used to calculate the left posterior target (#5).

Figure 4. A coronal MR image at the level for target #5 is used to recalculate the left posterior target coordinates, angles and estimate of putamenal height as in Figure 2.

estimated. The phantom was then set for target 5 and the depths for targets 4 and 5 were confirmed. The angles were then set so the two targets were centered in the burr hole and 5 mm apart. The guide tube and needles were introduced as above. Fifty microliter volumes of Spheramine® suspension were implanted at each of the posterior putamen target sites in the same manner as above. Thus, a total of 250 μl (approximately 325, 000 cells based

on estimates from cell counts of digested Spheramine® samples) was injected.

At the end of the procedure, methyl methacrylate was used to cover the burr hole. The galeal was approximated with 3-0 Vicryl, in a simple interrupted inverted pattern, the incisions were closed with a running 3-0-nylon suture, a sterile dressing was applied, and the stereotaxic frame was removed. The patient is taken to the recovery room and then transferred to a hospital room. The patient was allowed to eat and resume normal activities on the first post-operative day. The patient was discharged to home generally within three days, after a post-surgical MRI was performed.

3.4. Statistical Analysis

Clinical characteristics and time tests at 6 months post-operatively were compared to baseline. The X^2 test or Fisher's exact test was used to determine the significance of differences for categorical variables, and the Student's t test or Wilcoxon two-sample test was used for continuous variables (57). All tests were two-tailed and each reported p value corresponded to a single comparison. A p value less than 0.05 was considered significant.

4. RESULTS

The preclinical injections using slurry of gelatin microcarriers, the pulsed method of delivery was superior to a steady delivery using the syringe and needle apparatus outlined for surgery. The slow gradual injection technique so successfully used with fetal cell suspensions was consistently less successful in completely delivering the microcarriers *in vitro* (Table 2). These results were confirmed by performing the same types of injections into tracts established in fresh calf brains followed by visual inspection of the tracts and evaluation of residuals in the needles. In reviewing the residual fluid in the needles after the injection into the six patients, only one needle had a tract residual of Spheramine®.

Assessment of the safety and tolerability of Spheramine®, the primary objectives of this pilot study, consisted of a number of parameters including periodic MRI evaluation, elicited adverse events, vital signs, standard hematology, clotting profile, serum chemistry, urinalysis and neuropsychological evaluation. Although some patients are currently over 12 months postoperatively, only six month follow-up data will be presented here. All patients had monthly safety assessments and post-implant CAPIT evaluations at 1, 3 and 6 months. Improvement in the primary outcome measure, the Unified Parkinson's Disease Rating Scale motor score (UPDRS-M) in the practically defined off state (off all anti-parkinsonian medication for at least 12 hours), was observed in all patients (Table 3). At 1, 3 and 6 months post-operatively, the mean UPDRS-M off score (n=6) improved 28%, 35% and 34% respectively (Figure 5), from a pre-operative baseline. The mean UPDRS-M off score at 6 months post-implant was significantly improved (p<0.001). Improvements started at 1 to 3 months and have continued in most cases (Figure 6).

Table 2. In Vitro LRPE-GM Testing

	Steady Delivery		Pulsed Delivery	
Trial Number	Amount Delivered	Residual Amount	Amount Delivered	Residual Amount
1	0	+++++	+++++	0
2	++	+++	+++++	0
3	+	++++	+++++	0
4	++	+++	+++++	0
5	++++	+	+++++	0
6	+	++++	+++++	0
7	++	+++	++++	+
8	+++	++	+++++	0
9	+++	++	+++++	0
10	++	+++	+++++	0
Average	2	3	4.9	0.1

Rating: Full Dose +++++; Partial Dose ++++ to +; No Dose 0. Using slurry of gelatin microcarriers, the pulsed method of delivery was superior to a steady delivery using the syringe and needle apparatus outlined for surgery. These results were confirmed by performing the same types of injections into tracts established in fresh calf brains followed by visual inspection of the tracts and evaluation of residuals in the needles.
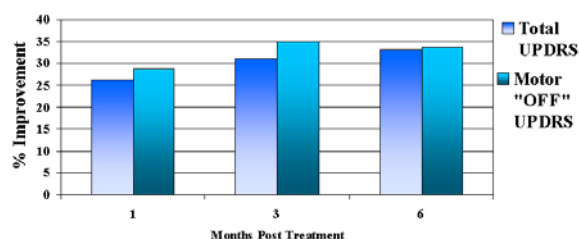

Figure 5. Graph of the mean percentage of improvement over time for UPDRS total motor scores (secondary outcome measure), and motor UPDRS (primary outcome measure) in the off state.
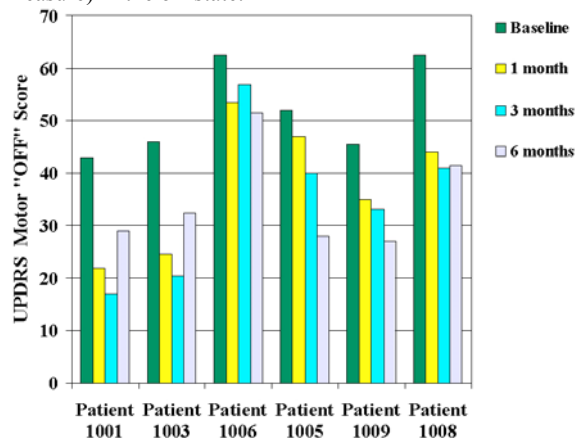

Figure 6. This graph illustrates the individual patient motor off UPDRS scores over time.

Improvements in secondary outcome measures include the total UPDRS (33%) (Figure 5 and Figure 7), Timed Motor Tests (on, 14%; off 23%), PDQ39 QOL (30%), and Schwab and England Physician Rated ADL (on, 11%; off 30%). All were significantly improved (p <0.05 or better). Bilateral improvement was observed in motor symptoms, with the greater effect seen contralateral to the implants. Moderate to marked reductions (37-53%) in time spent in the "off" state are currently seen in half the patients. "On" time without dyskinesias was increased in all patients and moderate to marked reductions (37-53%) in time spent in the "off" state are currently seen in five of six the patients. Three of six patients currently have lower Dyskinesia Rating Scale scores than at baseline, while the scores of the other three are unchanged from baseline values. No "off-state" dyskinesias have been observed. Post-op MRI scans revealed accurate placement of tracts in all patients (Fig.8). All patients tolerated surgery well and no major adverse events occurred. A small intraoperative hemorrhage lateral to third implant tract was detected in one patient (Figure 9); no significant mass effect was observed, and no neurological deficit was noted, either immediately or subsequently. Follow-up MR imaging at three and six months was performed on all patients, the images were consistent with a normal healing process and tolerability of the implant product.

5. DISCUSSION

The results of this clinical study suggest that intrastriatal transplantation of hRPE cells attached to gelatin microcarriers is a potential therapeutic treatment for advanced PD. Assessment of the safety and tolerability of Spheramine®, the primary objectives of this pilot study, demonstrated all patients tolerated surgery well and no major adverse events occurred. Follow-up MR imaging is consistent with a normal healing process and tolerability of the implanted product. All six patients have improvement in the primary outcome measure, the UPDRS-M score in the practically defined off state. Those patients out to 12 months post-implant, the mean UPDRS-M (off) score continued to improve. Improvements in the secondary measures were also marked. Studies of the injection technique demonstrated the need for a rapid injection so that the Spheramine® would move with the vehicle. This same technique was used in the *in vivo* primate studies and the histological studies confirmed both a high level of delivery and good cell survival.

The gold standard for neural transplantation is fetal tissue. In an attempt to compare fetal ventral mesencephalic (FVM) tissue transplantation with other surgical procedures used in the treatment of advanced PD,

Spheramine®

Table 3. Clinical Response to Spheramine®

	Patient 1001	Patient 1003	Patient 1006	Patient 1005	Patient 1009	Patient 1008
Individual Patients Total UPDRS Scores Over Time						
Baseline	102.5	113	130.5	106.5	116.5	139
1month	55	72	109.5	95.5	81	114.5
3 months	54	60.5	107	91	68.5	113.5
6 months	74	79.5	93.5	60.5	61	108.5
Individual Patients UPDRS Motor "OFF" Score Over Time (PRIMARY OUTCOME MEASURE)						
	Patient 1001	Patient 1003	Patient 1006	Patient 1005	Patient 1009	Patient 1008
Baseline	43	46	62.5	52	45.5	62.5
1 month	22	24.5	53.5	47	35	44
3 months	17	20.5	57	40	33	41
6 months	29	32.5	51.5	28	27	40.5

Mean Percentage Improvement Over Time UPDRS Total Score and Motor "OFF" Score

	1 month	3 months	6 months
Total UPDRS	26.2	31	33.1
Motor "OFF" UPDRS	28.7	34.9	33.6
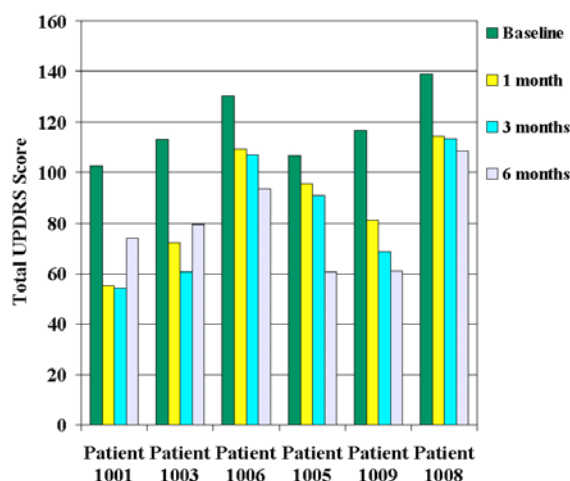
Figure 7. This graph illustrates the individual patient total off UPDRS scores over time.
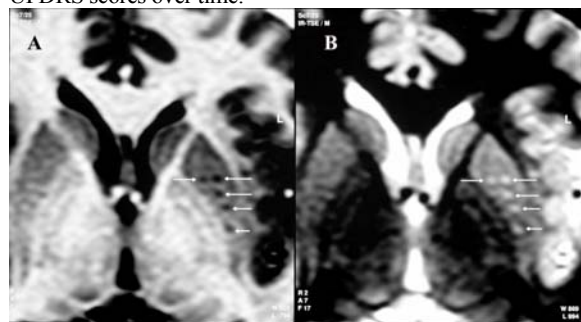
Figure 8. This pair of post-operative inversion recovery MR images demonstrates 5 tracts 5mm apart in the post-commissural putamen in the real (left) and modulus (right) modes.

nineteen research papers published between 1991-1998 were rated by a working group of experts (58). This group concluded, "Human fetal transplantation into the striatum in PD appears to be an encouraging procedure when performed on severely advanced PD patients. It is promising, because its efficacy appears to be good in the published open table reports, and its associated morbidity and mortality are low". In order to obtain objective efficacy and safety data, the National Institute of Health (NIH) funded two multicenter placebo controlled prospective double blind studies. The purpose of these studies was to objectively determine whether striatal FVM transplantation provides clinically meaningful benefit to PD patients. The design of each study included a "sham" surgery arm in which one half of enrolled patients were randomized to undergo an imitation procedure involving general anesthesia, cranial burr holes and identical post operative care.

Data from the first NIH funded placebo controlled collaborative clinical trial involving team of investigators from the Columbia University in New York and the University of Colorado in Denver is published (59,60). In this study 40 PD patients were randomized to receive either FVM implants or sham surgery. Results at one year showed significant but modest motor improvements as determined by "off" UPDRS scores, in patients younger than age 60, with the majority of improvement in rigidity and bradykinesia. Older patients did no show significant improvement. Acute significant adverse events were statistically significantly higher in fetal transplanted patients, but not related to the procedure. One patient died due to accidental injury in a storm. Autopsy of the brain in this patient showed survival of ~40,000
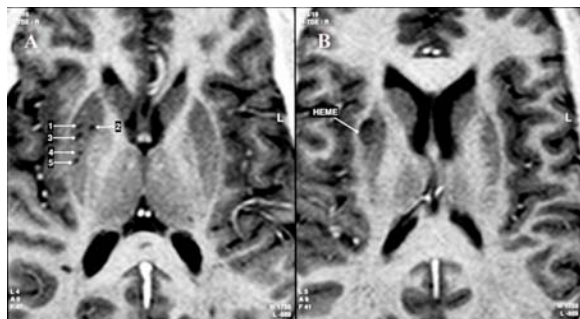
Figure 9. On the left, a post-operative inversion recovery axial MR image demonstrates accurate placement of the 5 trajectories in the inverted "L" shape. On the right, a small asymptomatic hemorrhage (Heme) is seen above and lateral to the putamen in the same patient.

transplanted dopaminergic cells. The most surprising finding of the study however, were the late emergence of unexpected, disabling "off" period dyskinesias in 15% of the transplanted patients (61). These dyskinesias occur in patients with positive PET scans indicated positive growth of the graft *in vivo*. The mechanism mediating these runaway dyskinesias is believed to be the non-homogeneous fiber innervations derived from fetal nigral grafts produced hyperdopaminergic "hot spots" that cause excessive local release of dopamine. These patients have shown arm and leg dyskinesias that interfere with activities of daily living and walking, as well as facial dystonia. In one patient, symptoms were severe enough to warrant the placement of a feeding tube for nutritional support. In three patients, deep brain stimulation has given symptomatic relief (62).

This NIH study was the first prospective randomized double blind placebo controlled trial for any neurosurgical therapy for PD. The results from this very important study provide valuable objective information on the clinical benefits of fetal transplantation and raises some troubling questions. First, this study objectively proved that striatal allogenic fetal transplantation in PD patients <60 years of age improves motor function particularly for bradykinesia and rigidity. These findings validate the results from previously published open label human and the preclinical animal studies, which led up to these clinical trials. Secondly, the prominent placebo effect on the global rating score of the "sham" surgery patients raises the question whether all surgical interventions for PD should be scrutinized using randomized prospective placebo controlled clinical trials. A second NIH funded placebo controlled collaborative clinical trial involving team of investigators from the University of South Florida, Mount Sinai Medical Center, University of British Columbia and Rush Presbyterian St. Luke's medical center is not published but preliminary presentations of the data suggest no effective improvement in patients regardless of age, but improvement related to the degree of levodopa responsiveness (Olanow personal communication). The study design was different in design and paralleled that utilized in the successful animal models. Like the first study there was the late emergence of unexpected,

disabling "off" period dyskinesias in the transplanted patients.

It is unclear whether transplanted neurons can completely replace the dopamine synapses in the PD patients in order to restore the "basal ganglia thalamocortical pathways" to its normal state. If the gold standard is not demonstrating dramatic improvement in clinical studies, why would Spheramine® be expected to produce improvements and why might it not produce dyskinesia? Unlike fetal tissue, hRPE cells do not differentiate to form axons or make synaptic connections with the host after transplantation. This might suggest a disadvantage, but in fact it may be a distinct advantage. The hRPE cells are probably acting as a dopaminergic pump and do not hyperinnervate the host. Multiple clinical studies have demonstrated that a continuous supply of dopamine or its precursors can decrease the motor fluctuations (4,6,7). The inability of the advanced PD patient to store and regulate the presynaptic and postsynaptic dopamine is believed to be responsible for motor fluctuations and dyskinesias (63-66). The hRPE cells may serve to replace this storage function and the expression of the D_2 type of dopaminergic receptors on the hRPE cell surface may serve as autoreceptors to regulate the dopaminergic function of hRPE cells. Although dopamine does not diffuse far in the extracellular fluid of normal tissue, it does disperse farther in denervated tissue. Moreover, its precursors will spread even farther (67). The decrease in raclopride binding in the preclinical non-human primate PET studies suggests this degree of diffusion is sufficient to prevent receptor supersensitivity, thus, improving symptoms and adverse side effects of therapy (51). The improvements in the clinical exam and the lack of new dyskinesia also suggest that the patient's dopamine function is better regulated after Spheramine implantation.

In summary, despite the failures of fetal grafts to produce the degree of desired improvement by specific techniques, cell transplantation continues to be promising experimental therapeutic modalities for PD. Advances in the scientific and technical aspects of cell transplantation are needed to continue to refine this form of therapy and possibly improve its efficacy. Recently completed clinical trials of fetal tissue transplantation for PD have shown that the efficacy of surgical interventions for PD must be objectively assessed. The effects of cell transplantation on levodopa induced dyskinesia remains unresolved with mixed results from different studies. The open label Spheramine® transplantation data is promising and appears to be of most benefit to PD patients for bradykinesia, rigidity and "on/off" fluctuations without the induced "off" dyskinesias. This type of "replacement therapy" of a deficient neurotransmitter or its precursor in the parkinsonian brain may be sufficient for "normalization" of the dopamine function in the putamen and stabilize long term recovery of symptoms. The ability to harvest large numbers of hRPE cell from non-fetal sources, culture them under good manufacturing practices to provide a sterile and standardized product, and implant them on an elective basis are major advantages. Nevertheless, results of small open label transplant studies in PD are suspect since the results

of the NIH sponsored fetal transplantation studies. A prospective randomized double blind placebo controlled clinical trial of intrastriatal transplantation of Spheramine® in advanced PD patients is underway to evaluate the possible benefits in a rigorous manner.

6. ACKNOWLEDGEMENT

7. REFERENCES

1. Hornykiewicz, O. & Kish, S.J. Biochemical pathophysiology of Parkinson's disease. *Adv Neurol* 45, 19-34 (1986)

2. Wichmann, T., DeLong, M.R. & Vitek, J.L. Pathophysiological considerations in basal ganglia surgery: role of the basal ganglia in hypokinetic and hyperkinetic movement disorders. *Prog Neurol Surg*, 31-57 (2000)

3. Jankovic, J. & Marsden, C.D. *Therapeutic strategies in Parkinson's disease*, 191-220 (Williams & Williams, Baltimore, 1998)

4. Chase, T.N., Engber, T.M. & Mouradian, M.M. Palliative and prophylactic benefits of continuously administered dopaminomimetics in Parkinson's disease. *Neurology* 44, S15-8 (1994)

5. Marsden, C.D. Problems with long-term levodopa therapy for Parkinson's disease. *Clin Neuropharmacol* 17 Suppl 2, S32-44 (1994)

6. Chase, T.N., Juncos, J., Serrati, C., Fabbrini, G. & Bruno, G. Fluctuation in response to chronic levodopa therapy: pathogenetic and therapeutic considerations. *Adv Neurol* 45, 477-80 (1987)

7. Fabbrini, G., Juncos, J., Mouradian, M.M., Serrati, C. & Chase, T.N. Levodopa pharmacokinetic mechanisms and motor fluctuations in Parkinson's disease. *Ann Neurol* 21, 370-6 (1987)

8. Backlund, E.O., Granberg, P.O., Hamberger, B., Knutsson, E., Martensson, A., Sedvall, G., Seiger, A. & Olson, L. Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. *J Neurosurg* 62, 169-73 (1985)

9. Freed, C.R., Breeze, R.E., Rosenberg, N.L., Schneck, S.A., Kriek, E., Qi, J.X., Lone, T., Zhang, Y.B., Snyder, J.A. & Wells, T.H. Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N Engl J Med* 327, 1549-55 (1992)

10. Hauser, R.A., Freeman, T.B., Snow, B.J., Nauert, M., Gauger, L., Kordower, J.H. & Olanow, C.W. Long-term evaluation of bilateral fetal nigral transplantation in Parkinson disease. *Arch Neurol* 56, 179-87 (1999)

11. Lindvall, O. Update on fetal transplantation: the Swedish experience. *Mov Disord* 13 Suppl 1, 83-7 (1998)

12. Widner, H., Tetrud, J., Rehncrona, S., Snow, B., Brundin, P., Gustavii, B., Bjorklund, A., Lindvall, O. & Langston, J.W. Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *N Engl J Med* 327, 1556-63 (1992)

13. Burris, J.F. Placebo surgery in trials of therapy for Parkinson's disease. *N Engl J Med* 342, 353; author reply 354-5 (2000)

14. Freed, C.R., Breeze, R.E. & Fahn, S. Placebo surgery in trials of therapy for Parkinson's disease. *N Engl J Med* 342, 353-4; author reply 354-5 (2000)

15. Freeman, T.B., Vawter, D.E., Leaverton, P.E., Godbold, J.H., Hauser, R.A., Goetz, C.G. & Olanow, C.W. Use of placebo surgery in controlled trials of a cellular-based therapy for Parkinson's disease. *N Engl J Med* 341, 988-92 (1999)

16. Horisberger, J.D. Placebo surgery in trials of therapy for Parkinson's disease. *N Engl J Med* 342, 354-5 (2000)

17. Slattery, J. Placebo surgery in trials of therapy for Parkinson's disease. *N Engl J Med* 342, 354; author reply 355 (2000)

18. Weiner, W.J. Placebo surgery in trials of therapy for Parkinson's disease. *N Engl J Med* 342, 353; author reply 354-5 (2000)

19. Marmor, M.F. & Wolfensberger, T.J. *The retinal pigments epithelium: function and disease.*, (Oxford University Press, New York, 1998)

20. Schraermeyer, U. & Heimann, K. Current understanding on the role of retinal pigment epithelium and its pigmentation. *Pigment Cell Res* 12, 219-36 (1999)

21. Pawelek, J.M. & Korner, A.M. The biosynthesis of mammalian melanin. *Am Sci* 70, 136-45 (1982)

22. Boulton, M. Melanin and the retinal pigment epithelium. in *The Retinal Pigment Epithelium: Function and Disease.* (ed. Marmor, M.F., Wolfensberger, T.J., eds.) 68-65 (Oxford University Press, New York, 1988)

23. Cherksey, B.D. Microcarrier preadhesion enhances long-term survival of adult cells implanted into the mammalian brain. *Exp Neurol*, S18 (1994)

24. Marchionini, D.M., Subramanian, T., Burnette, B., Miller, G.W., Iuvone, P.M., Potter, E.M. & Cornfeldt, M.L. Dopaminergic properties of retinal pigmented epithelial cells attached to microcarriers (RPE-M) transplanted into Parkinsonian animals. *Amer Soc Neural Trans Repair Abstracts*, A-05 (1999)

25. Burnside, B. & Bost-Usinger, L. The retinal pigment epithelial cytoskeleton. in *The Retinal Pigment Epithelium: Function and Disease* (ed. Marmor, M.F., Wolfensberger, T.J., eds.) 459-477 (Oxford University Press, New York, 1998)

26. Subramanian, T., Marchionini, D., Potter, E.M. & Cornfeldt, M.L. Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response. *Cell Transplant* 11, 207-14 (2002)

27. Campochiaro, P.A. Growth factors in the retinal pigment epithelium and retina. in *The Retinal Pigment Epithelium: Function and Disease* (ed. Marmor, M.F., Wolfensberger, T.J., eds.) 459-477 (Oxford University Press, New York, 1998)

28. Tombran-Tink, J., Chader, G.G. & Johnson, L.V. PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. *Exp Eye Res* 53, 411-4 (1991)

29. Jorgensen, A., Wiencke, A.K., la Cour, M., Kaestel, C.G., Madsen, H.O., Hamann, S., Lui, G.M., Scherfig, E.,

Prause, J.U., Svejgaard, A., Odum, N., Nissen, M.H. & Ropke, C. Human retinal pigment epithelial cell-induced apoptosis in activated T cells. *Invest Ophthalmol Vis Sci* 39, 1590-9 (1998)

30. Pfeffer, B. Improved methodology for cell culture of human and monkey retinal pigment epitheliaum. *Prg Retina Res* 10, 251-291 (1991)

31. Wenkel, H. & Streilein, J.W. Analysis of immune deviation elicited by antigens injected into the subretinal space. *Invest Ophthalmol Vis Sci* 39, 1823-34 (1998)

32. Wenkel, H. & Streilein, J.W. Evidence that retinal pigment epithelium functions as an immune-privileged tissue. *Invest Ophthalmol Vis Sci* 41, 3467-73 (2000)

33. Liversidge, J. & Forrester, J.V. Regulation of immune responses by the retinal pigment epithelium. in *The Retinal Pigment Epithelium. Function and Disease.* (ed. Marmor, M.F., Wolfensberger, T.J., eds.) 511-27. (Oxford University Press, New York, 1998)

34. Tanihara, H., Yoshida, M., Matsumoto, M. & Yoshimura, N. Identification of transforming growth factor-beta expressed in cultured human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 34, 413-9 (1993)

35. Holtkamp, G.M., de Vos, A.F., Kijlstra, A. & Peek, R. Expression of multiple forms of IL-1 receptor antagonist (IL-1ra) by human retinal pigment epithelial cells: identification of a new IL-1ra exon. *Eur J Immunol* 29, 215-24 (1999)

36. Campochiaro, P.A. Cytokine production by retinal pigmented epithelial cells. *Int Rev Cytol* 146, 75-82 (1993)

37. Dutt, K., Waldrep, J.C., Kaplan, H.J., Del Monte, M., Semple, E. & Verly, G. In vitro phenotypic and functional characterization of human pigment epithelial cell lines. *Curr Eye Res* 8, 435-40 (1989)

38. Rezai, K.A., Semnani, R.T., Patel, S.C., Ernest, J.T. & van Seventer, G.A. The immunogenic potential of human fetal retinal pigment epithelium and its relation to transplantation. *Invest Ophthalmol Vis Sci* 38, 2662-71 (1997)

39. Percopo, C.M., Hooks, J.J., Shinohara, T., Caspi, R. & Detrick, B. Cytokine-mediated activation of a neuronal retinal resident cell provokes antigen presentation. *J Immunol* 145, 4101-7 (1990)

40. Liversidge, J., Sewell, H.F., Thomson, A.W. & Forrester, J.V. Lymphokine-induced MHC class II antigen expression on cultured retinal pigment epithelial cells and the influence of cyclosporin A. *Immunology* 63, 313-7 (1988)

41. Tezel, T.H. & Del Priore, L.V. Reattachment to a substrate prevents apoptosis of human retinal pigment epithelium. *Graefes Arch Clin Exp Ophthalmol* 235, 41-7 (1997)

42. Cherksey, B.D. Method of increasing viability of cells which are administered to the brain or spinal cord. (U.S. Patent, 1997)

43. Cherksey, B.D. Method for transplanting cells into the brain and therapeutic uses therefor. (U.S. Patent, 1998)

44. Cherksey, B.D. Method for transplanting cells into the brain and therapeutic uses therefor. (U.S. Patent, 2000)

45. Borlongan, C.V., Saporta, S. & Sanberg, P.R. Intrastriatal transplantation of rat adrenal chromaffin cells seeded on microcarrier beads promote long-term functional recovery in hemiparkinsonian rats. *Exp Neurol* 151, 203-14 (1998)

46. Cherksey, B.D., Sapirstein, V.S. & Geraci, A.L. Adrenal chromaffin cells on microcarriers exhibit enhanced long-term functional effects when implanted into the mammalian brain. *Neuroscience* 75, 657-64 (1996)

47. Saporta, S., Borlongan, C., Moore, J., Mejia-Millan, E., Jones, S.L., Bonness, P., Randall, T.S., Allen, R.C., Freeman, T.B. & Sanberg, P.R. Microcarrier enhanced survival of human and rat fetal ventral mesencephalon cells implanted in the rat striatum. *Cell Transplant* 6, 579-84 (1997)

48. Potter, B.M., Kidwell, W. & Cornfeldt, M. Functional effects of intrastriatal hRPE grafts in hemiparkinsonian rats is enhanced by adhering to microcarriers. in *Soc for Neurosci Abstracts* (27th Annual Meeting: 778.10, 1997)

49. Subramanian, T., Bakay, R.A.E. & Cornfeldt, M.E., et al. Blinded placebo-controlled trial to assess the effects of striatal transplantation of human retinal pigmented epithelial cells attached to microcarriers (hRPE-M) in parkinsonian monkeys. *Parkinsonism Rel Dis* 5, S111 (1999)

50. Watts, R.L., Raiser, C.D., Stover, N.P., Cornfeldt, M.L., Schweikert, A.W., Allen, R.C., Subramanian, T., Doudet, D., Honey, C.R. & Bakay, R.A.E. Stereotaxic intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson's disease. *J Neural Transm.* 65 (Suppl.), 215-227 (2003)

51. Doudet, D., Honey, C., Schweikert, A.W. & Cornfeldt, M.L. PET imaging of implante human retinal pigment epithelial (hRPE) cells on gelatin microcarriers (Spheramine) in the MPTP-induced primate model of Parkinson's disease. in *Abs. Internat Neural Transpl Repair Meeting* (Denver, CO, 2002)

52. Watts, R.L., Raiser, C.D., Stover, N.P., Cornfeldt, M.L., Schweikert, A.W., Subramanian, T. & Bakay, R.A. Stereotaxic intrastriatal implantation of retinal pigment epithelial cells attached to microcarriers in advanced parkinson disease (PD) patients: a pilot study. *Neurology* 56 (Suppl. 3), P04.102 (2001)

53. Watts, R.L., Raiser, C.D., Stover, N.P., Cornfeldt, M.L., Schweikert, A.W., Allen, R.C., Subramanian, T. & Bakay, R.A. Stereotaxic intrastriatal implantation of retinal pigment epithelial cells attached to microcarriers in advanced Parkinson's disease (PD) patients: a pilot study in six patients. *Parkinsonism Rel Dis* 7(Suppl.), S87, P-TU-305 (2001)

54. Watts, R.L., Freeman, T.B., Hauser, R.A., Bakay, R.A., Ellias, S.A., Stoessl, A.J., Eidelberg, D. & Fink, J.S. A double-blind, randomized, controlled, multicenter clinical trial of the safety and efficacy of stereotaxic intrastriatal implantation of fetal porcine ventral mesencephalic tissue (Neurocell-PD™) versus imitation surgery in patients with Parkinson's disease (PD) *Parkinsonism Rel Dis 7* (Suppl.), S87, P-TU-304 (2001)

55. Langston, J.W., Widner, H., Goetz, C.G., Brooks, D., Fahn, S., Freeman, T. & Watts, R. Core assessment program for intracerebral transplantations (CAPIT) *Mov Disord* 7, 2-13 (1992)

56. Reich, C.A., Hudgins, P.A., Sheppard, S.K., Starr, P.A. & Bakay, R.A.E. A High-Resolution Fast Spin-Echo Inversion-Recovery Sequence for Pre-Operative

Localization of the Internal Globus Pallidus. *Am J Neuroradiol* 21, 928-31 (1997)

57. Fisher, L.D. & Bell, G.V. *Biostatistics: a methodology for the health sciences*, (Wiley, New York, 1993)

58. Hallett, M. & Litvan, I. Evaluation of surgery for Parkinson's disease: a report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. The Task Force on Surgery for Parkinson's Disease. *Neurology* 53, 1910-1921 (1999)

59. Fahn, S., Greene, P.E., Tsai, W.Y., Eidelberg, D., Winfield, H. & Dillon, S. Double-blind controlled trial of human embryonic dopaminergic tissue transplants in advanced parkinson's disease; clinical outcomes. *Neurology* (5), A405 (1999)

60. Fahn, S. Double-blind controlled trial of embryonic dopaminergic tissue transplants in advanced parkinson's disease. in *6th International Congress of Parkinson's Disease and Movement Disorders* (Barcelona, Spain, 2000)

61. Freed, C.R., Greene, P.E., Breeze, R.E., Tsai, W.Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J.Q., Eidelberg, D. & Fahn, S. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 344, 710-9 (2001)

62. Bakay, R.A.E. Is transplantation to treat Parkinson's disease dead? *Neurosurgery* 49, 576-80 (2001)

63. Fabbrini, G., Mouradian, M.M., Juncos, J.L., Schlegel, J., Mohr, E. & Chase, T.N. Motor fluctuations in Parkinson's disease: central pathophysiological mechanisms, Part I. *Ann Neurol* 24, 366-71 (1988)

64. Colosimo, C., Merello, M., Hughes, A.J., Sieradzan, K. & Lees, A.J. Motor response to acute dopaminergic challenge with apomorphine and levodopa in Parkinson's disease: implications for the pathogenesis of the on-off phenomenon. *J Neurol Neurosurg Psychiatry* 61, 634-7 (1996)

65. Leenders, K.L., Poewe, W.H. & Palmer, A.J. Inhibition of (18F) flurodopa uptake into human brain by amino acids demonstrated by positron emission tomography. *Ann Neurol* 20, 258-262 (1986)

66. Obeso, J.A., Rodriguez-Oroz, M.C., Rodriguez, M., DeLong, M.R. & Olanow, C.W. Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. *Ann Neurol* 47(4 Suppl 1), S22-32; discussion S32-4 (2000)

67. Hornykiewicz, O. Dopamine and Parkinson's disease. A personal view of the past, the present, and the future. *Adv Neurol* 86, 1-11 (2001)

Send correspondence to: Roy A.E. Bakay, M.D., Professor and Vice-Chairman, Department of Neurosurgery, Rush-Presbyterian St. Luke's Hospital, Chicago, IL, 1725 W. Harrison St., Ste. 970, Chicago, IL 60611, Tel: 312-942-1682, Fax:312-942-2176, E-mail: rbakay@cinn.org