

## Treating Parkinson Disease with Adult Stem Cells

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### Abstract

Parkinson disease affects ~2% of all people 70 years of age and older. People with Parkinson disease exhibit excessive shaking (tremors) at rest, loss of mental function, loss of involuntary function, and psychiatric problems. A proposed experimental cure for Parkinson disease is the transplantation of healthy nerve cells into the brain. It has been proposed that these nerve cells be taken from either aborted fetuses or derived from embryonic stem cells. Due to ethical and moral issues that proposal will probably not become a reality. Endogenous adult totipotent stem cells and adult pluripotent stem cells are very similar to embryonic stem cells. These primitive adult stem cells will form neurons, glia, skin, muscle, fat, cartilage, bone, blood vessels, blood cells, liver cells and pancreas cells under the appropriate inductive conditions. The current report proposes the use of adult totipotent stem cells for the treatment of Parkinson disease. As a test of this proposal, adult totipotent stem cells were utilized in a bedside clinical autologous phase-0 efficacy trial in adult humans with Parkinson disease. The results from this study suggested an efficacious response utilizing adult totipotent stem cells as a treatment modality for Parkinson disease.

**Keywords:** Parkinson disease; Adult; Stem cells; Totipotent; Pluripotent; Rat; Human

### Introduction

Parkinson's disease is a neurodegenerative condition that tends to present late in life. This condition is characterized by the presence of bradykinesia, a resting tremor, and rigidity. Various degrees of cognitive, autonomic, and psychiatric abnormalities may also be present [1,2]. Parkinson disease affects millions of humans. It is a common neurodegenerative disease with a lifetime incidence of 2.5% and a prevalence of at least 2% in individuals over 70 years of age [1]. This disease afflicts primarily the dopaminergic neurons, which have their cell bodies located in the *substantia nigra pars compacta* (SNpc). These neurons send axons to the caudate and putamen (collectively known as the *corpus striatum*). The progressive loss of these cells results in the gradual decrease over time of striatal dopamine levels, which in turn produces a decrease in striatal output to the thalamus. These alterations result in a decrease in cortical motor output. This decrease can account for some of the observed motor symptoms, especially bradykinesia and rigidity, but other features such as a resting tremor probably have a largely non-dopaminergic component [3].

Patients can be effectively treated with drugs that target the dopaminergic nigro-striatal pathway, but over time the efficacy of these medications is limited by the development of profound motor fluctuations and dyskinesias [1]. At this stage of the disease other therapies are often required, including deep brain stimulation. However, all these treatments are only symptomatic and do little to halt or reverse the progression of the disease [1]. Therapies that actually cure patients of Parkinson disease are still not available, but cell based therapies offer exciting possibilities [1,4]. Neural transplantation as a treatment modality for Parkinson disease is based on a well-defined biological mechanism: recovery of function following the restoration of dopaminergic transmission in the *corpus striatum*. Lindvall [4] proposed that four different cellular sources could be used to form dopaminergic neurons for neural transplantation for Parkinson disease: (a) embryonic stem cells from a fertilized egg; (b) neural stem cells from an embryonic brain; (c) neural stem cells from an adult brain; or (d) stem cells from other tissues. The crucial issue is whether

the transplanted cells would form functional dopaminergic neurons, regardless of the source of the stem cells [4].

In a previous animal study we chose to examine the effects of transplanting a Lac-Z genomically labeled naïve pluripotent stem cell clone [5] derived from skeletal muscle into the brains of adult rats that had been lesioned with 6-hydroxydopamine [6,7]. In the following clinical phase-0 efficacy trial we chose to examine the effects of infusing autologous totipotent and pluripotent stem cells [8] into the brains of individuals with Parkinson disease. The science behind our bench-top animal study and bedside clinical study is multi-fold. Young et al. [9] reported the isolation and single cell cloning of adult-derived pluripotent stem cells from the connective tissue stroma of multiple organs in animals and humans. They demonstrated that a clonal population of adult-derived pluripotent cells was capable of objectively forming 63 of the 220+ possible cells of the body, including multiple types of neurons, oligodendrocytes, astrocytes and capillaries. Young and Black [8] reported the isolation, single cell cloning, and characterization of adult-derived totipotent stem cells from the connective tissue stroma of multiple organs in animals and humans. They demonstrated that a clonal population of these stem cells was capable of objectively forming 66 of the 220+ possible cells of the body, including multiple types of neurons, oligodendrocytes, astrocytes, capillaries and spermatogonia. Unfortunately, at the time these studies were performed we only had assays to detect 66 of the 200+ possible cells in the body. Therefore, the limited number of cell types analyzed was due to the paucity of objective assays rather than the absolute number of differentiated cell types that these stem cells would form. When injected into an animal, the totipotent

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stem cells would home to damaged tissue sites and only replace the damaged tissues. These studies occurred in rodent models of induced myocardial infarction and induced Parkinson disease [8-10]. Young et al. also demonstrated that the single cell clonal populations of pluripotent stem cells and totipotent stem cells would maintain a normal karyotype after multiple cell doublings [7,8,11] and could increase these stem cells circulating in the peripheral blood by trauma [12], moderate exercise [13,14] and ingestion of a cyanobacter [13,14].

Protein	60-70%
Fat	20-30%
Minerals	3-9%
Lipids	2-8%
Pigments	1-4%
Moisture	3-7%
Chlorophyll	0.55%
Calories	2600 kcal
<b>Essential Fatty Acids</b>	
Alpha-Linoleic Acid (Omega 3)	29.50 mg
Gamma-Linolenic Acid (Omega 6)	6.00 mg
<b>Vitamins</b>	
Provitamin A Beta Carotene	2000 IU
Thiamin (B1)	4.70 µg
Riboflavin (B2)	57.30 µg
Niacin (B3)	0.16 mg
Pantothenic Acid (B5)	6.80 µg
Pyridoxine (B6)	11.10 µg
Cobalamin (B12)	8.00 µg
Inositol	46.50 µg
Vitamin C (Ascorbic Acid )	6.70 mg
Vitamin D	160.00 µg
Vitamin E	1.70 IU
Vitamin K	45.52 µg
Biotin	0.30 µg
Folic Acid	1.00 µg
Choline	2.30 µg
<b>Minerals</b>	
Boron	0.15 mg
Calcium	14.00 mg
Chloride	0.47 mg
Chromium	0.53 µg
Cobalt	2.00 µg
Copper	4.30 µg
Fluoride	38.00 µg
Germanium	0.27 µg
Iodine	0.53 µg
Iron	350.70 µg
Magnesium	2.20 mg
Manganese	32.00 µg
Molybdenum	3.30 µg
Nickel	5.30 µg
Potassium	12.00 µg
Phosphorus	5.20 µg
Selenium	0.67 µg
Silicon	186.50 µg
Sodium	2.70 mg
Tin	0.47 µg
Titanium	46.60 µg
Vanadium	2.70 µg
Zinc	18.70 µg

Table 1: Composition of 1 gram of RTAFA,

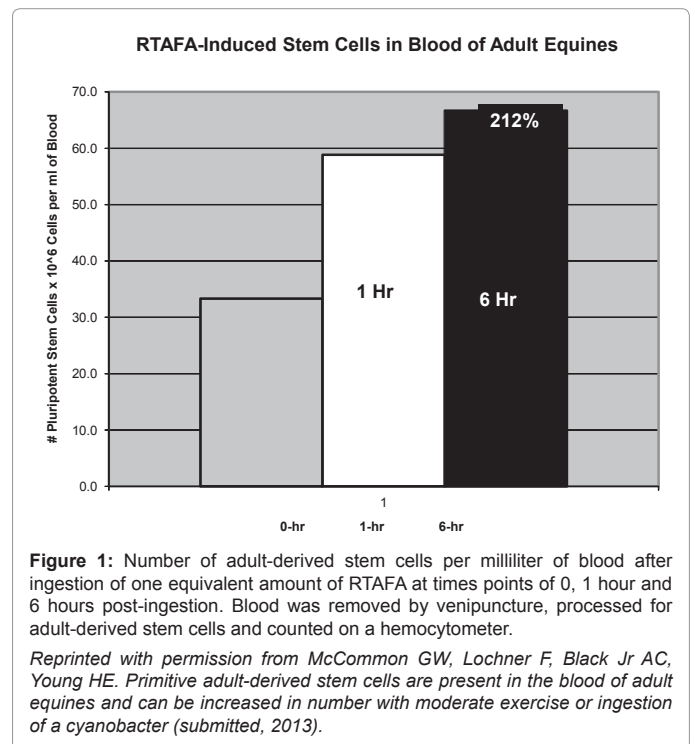


Figure 1: Number of adult-derived stem cells per milliliter of blood after ingestion of one equivalent amount of RTAFA at times points of 0, 1 hour and 6 hours post-ingestion. Blood was removed by venipuncture, processed for adult-derived stem cells and counted on a hemocytometer.

Reprinted with permission from McCommon GW, Lochner F, Black Jr AC, Young HE. Primitive adult-derived stem cells are present in the blood of adult equines and can be increased in number with moderate exercise or ingestion of a cyanobacter (submitted, 2013).

The cyanobacter RTAFA (Table 1) (Regeneration Technologies, Macon, GA), was examined in equines with respect to its ability to stimulate the endogenous production of primitive adult stem cells. The results suggested that RTAFA stimulated the proliferation and reverse-diapedesis of excess adult stem cells into the peripheral vasculature where the adult stem cells could be easily harvested, isolated and counted (Figure 1) [13,14].

To date, we have been able to stimulate an increase in quantity of adult totipotent stem cells and adult pluripotent stem cells in an individual's vasculature using RTAFA, thus making the individual their own bioreactor for generating excess adult stem cells for harvest and subsequent use. The autologous adult totipotent stem cells and autologous adult pluripotent stem cells were harvested via venipuncture and the primitive stem cells separated from the blood elements. The primitive stem cells were rinsed to remove serum proteins and pristine autologous adult totipotent and adult pluripotent stem cells were transfused in a safe and efficient manner within a two day period [14,15]. The current phase-0 efficacy study was intended to verify these results using a targeted number of subjects with objective assays.

Finally, we note that ultimately there may be better methods for the introduction of stem cells to bypass the blood-brain barrier to efficiently improve motor outcomes. We have one non-invasive technique and two invasive techniques to allow stem cells entrance into the sub-arachnoid cisterns of the central nervous system. The first technique is intra-nasal infusion [16] of the primitive totipotent stem cells into the superior nasal cavity, where they travel between the olfactory epithelial cells, along the olfactory processes, through the cribiform plate, and travel along the olfactory nerves to enter the subarachnoid cisterns of the brain without traversing the blood-brain barrier [15].

The two invasive procedures involve either intrathecal injection (reverse spinal tap) or stereotactic injections. Intrathecal injections

allow the primitive stem cells to physically bypass the blood-brain barrier, migrate into the subarachnoid spaces of the spinal cord and traverse to the appropriate damaged neuronal sites. Unfortunately, this technique creates scar tissue at the site(s) of injection. Stereotactic injection is direct injection of primitive stem cells into the lesion site, after removing portions of the scalp and boring holes in the cranium [17]. The stereotactic injection procedure also physically bypasses the blood-brain barrier, but is considered major surgery and performed under general anesthesia. For the clinical study reported herein we chose the least invasive and most tolerated technique yet available to us, intra-nasal infusion [16] of adult totipotent stem cells.

## Materials and Methods

The use of humans in this study complied with the guidelines of The Medical Center of Central Georgia Investigational Review Board (MCCG-IRB). These guidelines reflect the criteria for humane human care of the National Research Council prepared by the Institute of Human Resources and published by the National Institutes of Health.

### Study objectives

The overall objective of this study was to mobilize autologous adult totipotent and pluripotent stem cells into the blood stream in situ at sufficient levels to provide a continual source of autologous adult stem cells for cell, tissue, and organ-associated Parkinson repair. We used a Parkinson disease (PD) population. We targeted first the motor changes in these patients, as well as assessed the overall improvement of cognition, affect, function, adjustment, and caregiver burden.

### Criteria to assess Parkinson subjects for inclusion

1. Subjects meeting Queen's Square Criteria for Parkinson disease.
2. No signs of more extensive neurodegeneration indicating atypical Parkinsonism.
3. A positive response to levodopa or dopamine agonist.
4. Subjects aged 60-85 years.
5. Subjects must have completed at least the 9<sup>th</sup> grade and be fluent in English.
6. Psychotropic medications will be allowed if the subject has been on a stable dose for at least one month.
7. Benzodiazepines will be allowed if taken during the day prior to 6:00 pm and not taken as a sleep aid.
8. Parkinson disease subjects will not currently be experiencing dementia (DSM-IV criteria).
9. Presence of a caregiver.
10. MMSE 20 or greater.

### Criteria to assess Parkinson subjects for exclusion

1. Subjects taking Coumadin (Warfarin). (There is approximately 23 micrograms of vitamin-K per capsule of AFA (Table 1) that has the potential to interfere with the anti-coagulation action of Coumadin. Therefore, we will leave the decision to exclude the subject from the trial in the hands of the Subject's own physician).
2. Subjects with severe hepatic impairment.
3. Subjects with severe COPD.
4. Subjects exceedingly frail based on multiple systems criteria (as

determined by Dr. Robinson).

5. Subjects with galactorrhea.
  6. Subjects with prolactin sensitive tumors.
  7. Parkinsonism due to Parkinson's-plus diagnoses or to medication.
  8. Subjects with a communicable disease, i.e., HIV, Hepatitis, etc.
  9. Subjects having deep brain stimulation.
- Number of subjects

Subjects who met inclusion/exclusion criteria were admitted. Based on other studies with PD patients in this area with sleep [18] we expect 10% dropout. We enrolled 10 participants and their caregivers.

### Study design

This is a Phase "0" Clinical Intervention Trial with adult totipotent and pluripotent stem cells—First in Parkinson disease patients. Baseline ratings on outcome measures will serve as control values. At time zero (Pre-Screen), before start of ingestion of RTAFA to make themselves their own sterile bioreactors for the propagation and reverse diapadesis of autologous totipotent stem cells and autologous pluripotent stem cells, 10 out of 10 subjects were given a code number (#'s from 1–10), and screened for age, gender, marital status, and education (Table 2). The ten volunteers were then scored for CIBC, UPD-total, Hoehn-Yahr, ESS-Total, FAQ-Total, and BDI-Total, as well as cognition and caregiver burden (Tables 4-13) (see references below). At the end of three months of ingestion of RTAFA a second set of tests was performed. Two test subjects, #'s 4 and 8, dropped out of the study before the second set of testing was performed. This left 8 participants in the study.

Participant #s	1	2	3	4	5	6	7	8	9	10
Gender	M	M	M	F	M	M	F	M	F	M
Age	68	65	73	49	80	74	72	58	84	50
Education*	5	2	2	4	4	4	2	3	4	5
Marital_Status**	5	1	1	4	1	1	4	4	3	1

\*5=college, 4=AA degree, 3=some college, 2=HS, 1=< HS

\*\*5=widowed, 4=cohabitating, 3=divorced, 2=single, 1=married

Table 2: Parkinson Data From Screening Participants.

Time	N	Mean	Std. Deviation
Pre-Screen	10	4.00	.000
Pre	8	3.75	1.309
Post	8	4.25	1.164
Postpost	7	3.86	1.819

Table 3: Overall ratings: Repeated CIBC Ratings.

Time	N	Mean	Std. Deviation
Pre	8	2.063	.623
Post	8	2.500	.267
Postpost	8	2.375	.354

Table 4: PD-Specific Variables: Repeated Hoehn-Yahr Scores.

Time	N	Mean	Std. Deviation
Pre	8	71.250	9.910
Post	8	67.500	11.650
Postpost	7	68.571	18.644

Table 5: PD-Specific Variables: Repeated Schwab-England Scale of Daily Living Scores.

Time	N	Mean	Std. Deviation
Pre	8	37.750	13.615
Post	8	41.625	12.580
Postpost	7	39.143	18.452

Table 6: PD-Specific Variables: Repeated UPDRS Total Scores.

Time	N	Mean	Std. Deviation
Pre-Screen	10	5.700	8.394
Pre	7	6.286	8.655
Post	7	4.429	4.541
Postpost	6	8.000	8.832

Table 7: PD-Specific variables: Repeated FAQ Total Scores.

Time	N	Mean	Std. Deviation
Pre-Screen	10	10.000	4.922
Pre	8	8.750	5.625
Post	8	7.625	5.097
Postpost	7	7.429	6.294

Table 8: Sleep: Repeated Epworth Sleepiness Scale (Total Scores).

Time	N	Mean	Std. Deviation
Pre-Screen	10	8.600	7.516
Pre	6	6.667	5.317
Post	7	10.571	8.121
Postpost	4	9.500	14.708

Table 9: Depression: Repeated Beck Depression Inventory Total Scores.

Time	N	Mean	Std. Deviation
Pre-Screen	8	76.250	46.145
Pre	8	80.875	47.885
Post	8	86.125	54.104
Postpost	7	88.429	60.931

Table 10: Cognition: Repeated Trails A.

Time	N	Mean	Std Deviation
Pre-Screen	8	144.625	43.684
Pre	7	152.857	35.456
Post	8	152.625	33.406
Postpost	7	151.571	36.669

Table 11: Cognition: Repeated Trails B.

The participants then underwent intra-nasal and intravenous infusion of autologous totipotent stem cells and pluripotent stem cells. This was accomplished by withdrawing 400 milliliters of whole blood and placing the blood into EDTA-containing 10-ml hemovac tubes (BD Sciences). Subsequent processing of the blood was performed using universal precautions. The tubes were inverted several times to mix the whole blood with the EDTA and then the tubes were placed upright into a tube holder and placed into a 4°C refrigerator for 48 hours. During this time period the blood elements (red blood cells, white blood cells and platelets) separated from the serum and totipotent stem cells and pluripotent stem cells by gravity. After 48 hours the hemovac tubes containing the separated cellular elements were sprayed with 70% alcohol and placed within a UV/alcohol-sterilized Class-II HEPA-filtered Biosafety cabinet. The serum was removed from the tubes and placed into sterile 15-ml polypropylene tubes (Falcon). The hemovac tubes were sprayed with 70% alcohol and placed into biohazard bags for disposal. The serum was then processed for stem cell separation. The 15-ml tubes were spun at 500 rcf (relative

Time	N	Mean	Std. Deviation
Pre-Screen	6	24.167	15.968
Pre	5	19.800	14.618
Post	4	20.750	20.006
Postpost	4	21.250	21.093

Table 12: Caregiver Burden: Repeated Zarit Burden Scale Total Scores.

	ESCs or iPSCs	ATSCs <sup>1</sup> & APSCs <sup>2</sup>
Proliferation	Unlimited	Unlimited
Cell Types	All Somatic	All Somatic
Naïve <i>in vitro</i> <sup>3</sup>	Spontaneous Differentiation <sup>4</sup>	Quiescent
Naïve <i>in vivo</i>	Teratomas	Repair or Quiescence
Implant <i>in vivo</i>	Differentiated cells	Naïve cells
HLA <sup>5</sup> Mismatch	Allergenic	Allergenic
HLA Match	Therapeutic Cloning	Autologous

<sup>1</sup>ATSCs: Adult Totipotent Stem Cells

<sup>2</sup>APSCs: Adult Pluripotent Stem Cells

<sup>3</sup>Naïve *in vitro*, cells are placed in a medium void of any inductive inhibitory factors, i.e., Leukemia Inhibitory Factor (LIF), ESGRO or Anti-Differentiation Factor (ADF) [Young, 2004].

<sup>4</sup>Spontaneous differentiation into embryonic or fetal parts representing all three primary germ layer lineages.

<sup>5</sup>HLA: Human Leucocyte Antigen, i.e., HLA CLASS-I, HLA-DR-CLASS-II, antigens used by the body to distinguish self (autologous) from non-self (allogeneic).

Table 13: Stem Cell Characteristics for Transplantation.

centrifugal force) to pellet the larger pluripotent germ layer lineage stem cells. After removal from the centrifuge the outside of the tubes were sprayed with 70% alcohol and placed inside the Class-II Biosafety hood. The supernatant was decanted to a second set of sterile 15-ml tubes and the tubes centrifuged at 4,000 rcf to pellet the remaining totipotent stem cells and pluripotent stem cells. The supernatant was removed and discarded. The cell pellets were reconstituted in 1-ml volumes of sterile saline and pooled, using sterile technique. The tubes containing the suspended cells were spun a second time at 4,000 rcf to pellet the stem cells, washed with 1-ml volume of sterile saline, 7 tubes pooled at each wash step, and 7 ml of sterile saline added to each tube, using sterile technique. At each pooling the cells were spun at 4,000 rcf to pellet the totipotent and smaller pluripotent stem cells and separate the stem cells from their serum. These multiple wash/pooling procedures were performed to wash away any residual serum proteins adhering to the stem cells.

The participants were prepared for intra-nasal infusion of totipotent stem cells and pluripotent stem cells as follows. Each participant was asked to clean out their nasal passages by inhaling volumes of sterile 0.65% saline solution (Ocean) and then blowing nasal contents into sink. This was repeated 3-4 times to insure loss of sticky mucus from the inside of the nasal passages. The participant was then placed in the supine position with their head lower than their body (modified Trendelenberg position), with their nostrils pointing upward. Stem cells suspended in sterile saline were dropped (via 1-cc syringe barrel) into each nostril onto the olfactory epithelium. After administering the stem cells the participant was asked to remain in that position for 5 minutes to insure the deposition of the stem cells on the olfactory mucosa with migration through the mucosa, along the olfactory processes, through the cribriform plate, to the olfactory bulb, and posteriorly along the olfactory nerves to gain entrance past the blood-brain barrier and to the subarachnoid cisterns of the brain and spinal cord. After five minutes each participant was helped to the sitting position and allowed to remain in that position for 30 minutes to adjust for vertical equilibrium.

Test subjects were then assessed at regular intervals. This occurred at baseline, and three months post baseline (prior to the intra-nasal infusion of autologous adult totipotent and pluripotent stem cells) (“pre”), and at 2 weeks (“post”) and four months post procedure (“post-post”).

### Parkinson assessment criteria

Participants were evaluated in the following ways. We evaluated participants at the above mentioned intervals. We also assessed caregivers at three month intervals. The areas that are targeted include the following Parkinson Assessment Criteria:

- (1) Motor → UPDRS-III [19];
- (2) Cognition → Trail Making Part A and B [20];
- (3) Affect → Beck Depression Scale-II (BDI-II) [21];
- (4) Function → Functional Assessment Questionnaire (FAQ) [22],

Schwab and England disability scale [23] and Hoehn-Yahr Scale [24];

- (5) Sleep: Epworth Sleepiness Scale (ESS) [23];

(6) Overall clinical improvement with the CIBIC-Plus (Clinician’s Interview-Based

Impression of Change Plus Caregiver Input); and

- (7) Caregiver: Zarit Burden Scale [25].

Prior to study entrance, each patient underwent; 1) chart review for medication regimen, medical conditions, and laboratory values, 2) physical examination and 3) diagnostic dementia evaluations completed by the investigators. We also applied a Mini Mental State Exam (MMSE) [26] at entry to assure level of possible dementia and competence. Scores must be 20 or greater to assure levels of mild dementia or better, assuring at a minimum ability to fill out questionnaires. Informed consent was obtained from all willing participants using a Medical Center of Central Georgia Institutional Review Board approved consent form. In the event of guardianship, consent was obtained from the guardian as well.

The study population involved subjects with PD diagnosed by Queen Square criteria. All subjects were taking levodopa or dopamine agonists, or both. They may be on other medications, including cholinesterase inhibitors. All eligible candidates had a Modified Hoehn-Yahr Staging 2 (bilateral disease) to 4 (Severe Disability), as well as an MMSE of 20 or greater. There were subjects with medical co-morbidities as well as other complications (e.g., psychiatric history, living situations, poor life habits, etc.).

The use and amount of RTAFA capsules is more involved. A standard dosing regimen as described by Regeneration Technologies was followed unless contraindicated by changes in the PD participant’s evaluation criteria. At baseline all participants were measured without taking the compound. After initial measurements were taken the subjects were started on one capsule containing 500 mg of RTAFA. Only three RTAFA capsules were taken throughout the duration of the study. In addition, at baseline (0 months) and at intervals discussed above, the participants and their caregivers were evaluated.

Subjects were evaluated for clinical improvement from baseline. We also measured burden of caregivers by the Zarit Burden Scale [25]. We note too that we were attentive to safety issues. We were especially attentive to PT, PTT and INR, issues related to coagulation.

Descriptive statistics are provided. The primary target was the UPDRS-III over the time period of seven months as the end point. Caregiver responses were also part of this secondary analysis.

### Results

There were 10 subjects at the start of the trial and two dropped out. The average age was 67, education was just above high school and there were 7 males and 3 females (Table 2). The average MMSE was 26.8, normal. We honed in on the salient variables for PD, for cognition, for depression, for sleep, and for adjustment (Tables 3-12). Overall, the participants showed a variable pattern. Regarding overall ratings in all areas (CIBIC), the subjects went from a standard baseline of 4.0 to 3.8, a slight improvement (Table 3). This scale rates the person on mental issues, behavior, and functioning. The ratings then went from moderately ill to mildly ill.

Regarding PD-specific problems, we applied the Hoehn-Yahr (Table 4), the Schwab England (Table 5) and the overall PD rating of the UPDRS (Table 6). Each of these was measured at pre, post and post-post only. All three showed little change. In effect, the level of PD symptoms and staging remained similar throughout the study period of 7 months. We had a measure for overall adjustment, FAQ (Table 7). Throughout the entire time period of the study the subjects actually got slightly worse over time—6.2 to 8.0.

Next we considered affect and sleep. We used the BDI-II and ESS. From pre-pre to post-post these two variables showed minor changes. Sleep got better; depression got slightly worse. The numbers, however, were very similar over the course of the study. Sleep scores improved from 10 (insomnia problems) to 7.4 (normal area); depression scores remained below 10 (normal) (Tables 8 and 9).

We assessed cognition. We used the Trials A and B as markers of this area (Table 10 and 11). These are measures of speed of processing and executive functioning. In general, the scores were poor, 1-3 standard deviations lower than normal. Trails A got progressively worse over time; Trials B got worse at the last three measure periods. Cognition as measured by these markers did not improve.

Finally, we assessed caregiver ratings, Zarit Burden Scale (Table 12) [25]. Here subjects improved slightly over the study time frame. The initial rating was 24 and the last three were ~20. Caregivers saw the subjects as slightly better.

We present two figures with all the values for the individual patients. We chose a table of the UPDRS (Figure 2) and FAQ (Figure 3) as key markers for PD and adjustment. In sum, there was much

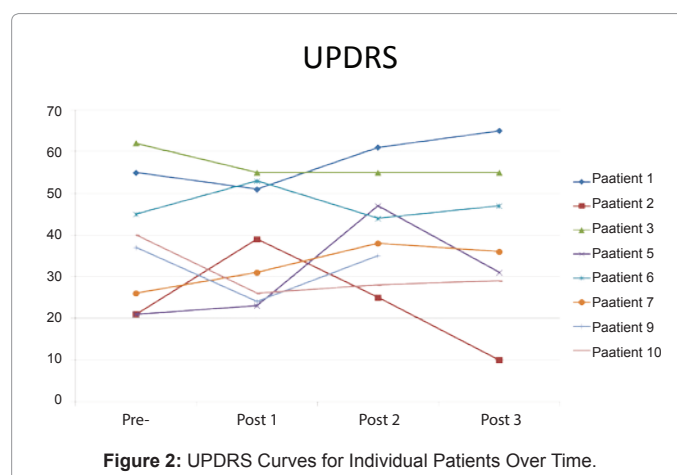


Figure 2: UPDRS Curves for Individual Patients Over Time.

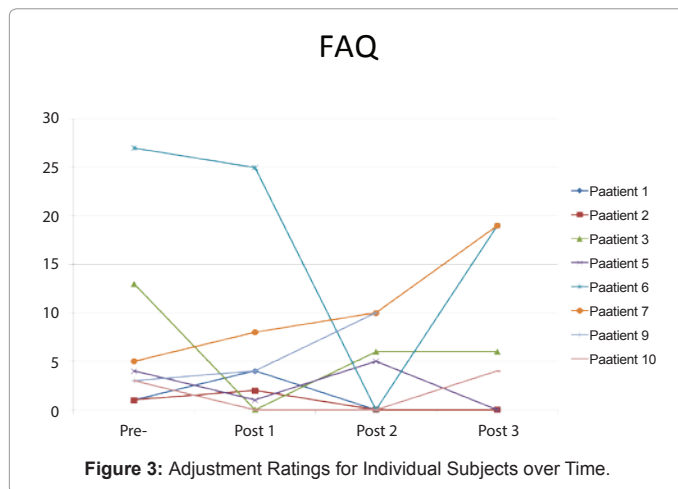


Figure 3: Adjustment Ratings for Individual Subjects over Time.

variability. But it can also be seen that, while some patients decreased in values (25%), there was considerable stability (50%) and some went up in values (25%).

## Discussion

Parkinson disease affects ~2% of all people 70 years of age and older. People with Parkinson disease exhibit excessive shaking (tremors) at rest, loss of mental function, loss of involuntary function, and psychiatric problems. A proposed experimental cure for Parkinson disease is the transplantation of healthy nerve cells into the brain [4]. It has been proposed that these nerve cells be taken from either aborted fetuses or derived from embryonic stem cells [4]. Due to ethical and moral issues, that proposal will probably not become a reality. Adult totipotent stem cells and adult pluripotent stem cells are very similar to embryonic stem cells in that they have the capability to form multiple cell types, i.e., neurons, interneurons, astrocytes, oligodendrocytes, keratinocytes, skeletal muscle, cardiac muscle, smooth muscle, unilocular fat cells, multilocular fat cells, hyaline cartilage, articular cartilage, growth plate cartilage, elastic cartilage, fibrocartilage, endochondral bone, intramembranous bone, endothelial cells, capillaries, arteries, veins, lymphatics, hematopoietic cells, gastrointestinal enterocytes, hepatocytes, oval cells, bile canalicular cells, biliary cells, pancreatic ductal cells, glucagon secreting  $\alpha$ -cells, insulin-secreting  $\beta$ -cells, somatostatin-secreting  $\delta$ -cells, etc., under the appropriate inductive conditions [7,10,14,17].

The current report proposed the use of adult totipotent stem cells and adult pluripotent stem cells for the treatment of Parkinson disease. Bench top and bedside model systems were examined. A Lac-Z transfected clonal population of adult pluripotent stem cells [9] was utilized in a bench-top 6-hydroxydopamine-induced niagral-lesioned midbrain allogeneic animal model [17]. Results from the animal study demonstrated replacement of dopaminergic neurons in the area of the 6-OHDA lesion as well as replacement of damaged neuronal cells, damaged neuronal supportive cells and damaged vascular structures caused by the needle injections. A second test of this proposal involved an autologous clinical phase-0 efficacy trial in adult humans with Parkinson disease. The results from the clinical study, utilizing the intra-nasal infusion of autologous adult totipotent stem cells, showed that some patient's outcome measures decreased in values (25%), there was considerable stability in some patient's outcome measures (50%) and some patient's outcome measures increased in values (25%). The results from both studies suggested an

efficacious response utilizing adult stem cells as a potential treatment modality for Parkinson disease.

While the goal of disease modification for Parkinson disease is reasonably clear, the task of determining whether a therapy is disease modifying is less clear. The underlying pathogenesis of Parkinson disease is not fully understood and, therefore, developing new disease modifying therapies remains difficult. The ultimate idea is to "neuroprotect" and, in so doing, to interfere with the underlying pathogenic mechanism of nigral cell death and/or rescue damaged but still viable cell neurons. The motor and non-motor symptoms of this disease presumably would be arrested and possibly reversed if stem cells were utilized.

From a broad perspective, the use of putative neuroprotective factors (with or without a known symptomatic effect) is critical in an understanding of Parkinson disease. In the recent past, with the possible exception of the ADAGIO trial [27], major Parkinson disease studies, e.g., ELLDOPA [28], DATATOP [29], and TEMPO [30] have failed to conclusively demonstrate a neuroprotective effect. Stem cells show the promise to be neuroprotective. In the current clinical phase-0 efficacy trial we accessed a group of PD patients at mid-level in the disease process. There was much variability and use of a stem cell model that can be improved upon. We believe now that we have the resources to conduct such a study, a carefully designed program to assess this, and have the flexibility to see its neuroprotective value. Results would be exceedingly informative and we could proceed to further clinical trials.

One promising avenue in the hunt for a remission in Parkinson disease symptoms does involve cell replacement therapy. Stem cells are a source for cell replacement therapy due to their ability to self-renew and their inherent plasticity that allows them to generate various types of cells from a single cell. There are two avenues of adult stem cell therapy that could be used for neurological diseases such as Parkinson disease, i.e., allogeneic stem cell therapy or autologous stem cell therapy. Either can be utilized currently if minimal manipulative procedures are used to derive the cells. Unfortunately, both have their advantages and disadvantages.

Allogeneic stem cells acquired from a donor(s) that do not carry mutations for serious and/or life-threatening familial diseases could replace cells carrying a genetic mutation causing the disease. However, unpublished studies from our lab suggest that matching genders as well as ABO groups and Rho-D positive or Rho-D negative must be taken into consideration when transplanting allogeneic stem cells. Our studies demonstrated that gender mismatch will cause long-term problems in the individual, i.e., male stem cells do not perform as expected when placed into an estrogen-progesterone-rich environment and female stem cells do not perform as expected when placed into a testosterone-rich environment. Similarly, matching of blood groups is essential for maintenance of donor cell longevity in the recipient. We would propose that since adult totipotent stem cells and adult pluripotent stem cells have the capability to make hematopoietic cells [9,10,14], that a few of these transplanted stem cells could find their way to the bone marrow of the recipient and form hematopoietic cells with cell surface markers of the donor. If the allogeneic cells were mismatched to the recipient, the recipient's immune system would likely recognize the differentiated cells as non-self and mount an inflammatory response against the 'perceived' invading cells. Antibody production by B-cells and plasma cells along with opsinization and phagocytosis of the invading cells by macrophages would potentially decrease the total number of donor cells available

for replacement and/or repair purposes. While this would be a definite problem in the systemic circulation, it may not necessarily be a problem for implanted cells within the central nervous system. The reasoning for this is that the central nervous system is protected by the blood-brain barrier and thus has been proposed as an immune-protected environment. However, there are macrophages within the central nervous system, e.g., microglial cells, with functions similar to their counterparts in the systemic circulation. Therefore, it is imperative that additional studies be performed to address these particular issues.

Autologous stem cells can also be used for transplantation therapies even if they carry mutations for serious and/or life-threatening familial diseases as long as the disease process is not activated until later in the cell's biological lifespan. The reason behind this is that true stem cells, i.e., totipotent stem cells, pluripotent stem cells and multipotent stem cells, have essentially unlimited proliferation potential, due to the presence of the telomerase enzyme [10]. However, once these stem cells commit to a particular tissue/cell lineage they lose the telomerase enzyme and assume all attributes of tissue-committed progenitor cells, including a defined biological lifespan of 8-10 population doublings for rodents [31] and 50-70 population doublings for humans [32]. We utilized uncommitted naïve autologous adult totipotent and pluripotent stem cells for the clinical therapy for Parkinson disease reported herein. These naïve autologous totipotent and pluripotent stem cells had yet to commit to a particular cell type and thus had a biological lifespan of zero [7,10,14]. Theoretically, newborn stem cells were transplanted into averaged 67 year old individuals with Parkinson disease, thereby giving the individuals an additional 67 years before Parkinson disease would appear in their transplanted cells. While this did not cure the disease it did give 75% of the individuals an additional time period before renewed onset of the symptoms. Autologous totipotent stem cells and autologous pluripotent stem cells have the potential to revolutionize the treatment of disease by targeting dysfunctional tissues and to repair damaged tissues without the use of immunosuppressive therapy, thereby making new treatments possible without significant adverse side effects [6-8,10,14,17].

The adult stem cell, a postnatal cell that has the ability for essentially unlimited population doublings and the ability to form any cell type below its lineage placement [8,14], is one of the key elements in regenerative medicine. Eleven species of mammals, including humans, possess their own endogenous highly plastic naïve adult stem cells, i.e., totipotent stem cells, pluripotent stem cells and multipotent stem cells [6-10,14,17,33,34]. Adult totipotent stem cells [8,14] and adult pluripotent stem cells [9,10,14], either allogeneic or autologous, are as highly plastic as embryonic stem cells [35] or iPS cells [36], but differ from them in two very crucial ways. Naïve adult totipotent stem cells and naïve adult pluripotent stem cells will not spontaneously differentiate in the absence of inductive inhibitory agents (such as leukemia inhibitory factor or anti-differentiation factor) in culture and they will not form teratomas (uncoupled embryonic development) when transplanted into an individual (Table 13) [6,8-10,14,17]. In addition, since adult totipotent stem cells and adult pluripotent stem cells [8-10,14], either allogeneic or autologous, are not derived from embryonic cells or aborted fetal tissues, they make excellent candidates for regenerative medicine as highly plastic transplantable cells that are ideal for the repair and restoration of a multitude of damaged tissues [5,6,9,10,14,17].

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