EXPEDITED PUBLICATION

A Double-blind Controlled Trial of Bilateral Fetal Nigral Transplantation in Parkinson's Disease

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Thirty-four patients with advanced Parkinson's disease participated in a prospective 24-month double-blind, placebo-controlled trial of fetal nigral transplantation. Patients were randomized to receive bilateral transplantation with one or four donors per side or a placebo procedure. The primary end point was change between baseline and final visits in motor component of the Unified Parkinson's Disease Rating Scale in the practically defined off state. There was no significant overall treatment effect ($p = 0.244$). Patients in the placebo and one-donor groups deteriorated by 9.4 ± 4.25 and 3.5 ± 4.23 points, respectively, whereas those in the four-donor group improved by 0.72 ± 4.05 points. Pairwise comparisons were not significant, although the four-donor versus placebo groups yielded a p value of 0.096. Stratification based on disease severity showed a treatment effect in milder patients ($p = 0.006$). Striatal fluorodopa uptake was significantly increased after transplantation in both groups and robust survival of dopamine neurons was observed at postmortem examination. Fifty-six percent of transplanted patients developed dyskinesia that persisted after overnight withdrawal of dopaminergic medication ("off"-medication dyskinesia). Fetal nigral transplantation currently cannot be recommended as a therapy for PD based on these results.

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a relatively selective loss of nigrostriatal dopaminergic neurons and a reduction in striatal dopamine. Current treatment primarily relies on a dopamine replacement strategy and is effective, particularly in the early stages of the disease. However, chronic dopaminergic therapy is limited by motor complications, the development of unresponsive features that do not respond to dopaminergic therapies, and disease progression.[1] Consequently, most patients eventually develop disability that cannot be controlled with standard medical therapies. This has spurred a search for alternate treatments that might improve long-term outcome.

Transplantation of fetal nigral dopamine neurons is a rational approach to the treatment of PD because (1) there is a relatively selective loss of nigrostriatal dopaminergic neurons; (2) nigral dopaminergic neurons primarily modulate striatal function and provide tonic stimulation of target receptors; (3) downstream basal ganglia neurons are relatively preserved; and (4) dopaminergic therapies provide clinical benefits.[2] Preclinical studies have demonstrated that implanted fetal nigral mesencephalic dopaminergic neurons can survive, manufacture dopamine, exhibit normal electrical firing patterns, reinnervate the striatum, form normal-appearing connections with host neurons, and improve motor functions in parkinsonian rodents and primates.[3–8] In advanced PD patients, uncontrolled open-label trials of fetal nigral transplantation have been reported to provide clinically meaningful improvement in most,[9–15] but not all,[16] studies. We also noted significant clinical benefits after fetal nigral transplantation coupled with an increase in striatal fluorodopa uptake on positron emission tomography (PET).[17,18] Postmortem studies demonstrated survival of approximately 100,000 im-

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For a list of contributors to this study, see the Appendix on page 413.

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planted dopamine neurons per side with organotypic reinnervation of the striatum.[19,20] However, open-label studies are subject to being influenced by placebo response and physician bias.[21] Indeed, an earlier double-blind, placebo-controlled trial failed to show significant clinical benefit after fetal nigral transplantation in a quality-of-life measure, the primary outcome variable.[22] In this study, transplantation was associated with modest improvement in more traditional motor outcomes, particularly in patients younger than 60 years, but approximately 15% developed a disabling form of dyskinesia that persisted for days or even weeks after cessation of L-dopa.[23,24] The role of transplantation as a treatment for PD thus has not been established. We now report the results of the second double-blind, placebo-controlled trial of fetal nigral transplantation in PD.

Patients and Methods

Study Design

The study was performed as a prospective, 2-year, multidose, placebo-controlled, double-blind trial of fetal nigral transplantation in patients with advanced PD.

Patients

PD patients aged 30 to 75 years were eligible to participate in the study. Inclusion criteria included at least two cardinal features of parkinsonism (tremor, rigidity, bradykinesia), a good response to L-dopa, stable doses of antiparkinsonian medications, and motor complications that could not be controlled with pharmacological therapy. Exclusion criteria included atypical parkinsonism, major psychiatric illness, dementia that precluded giving informed consent, clinically meaningful medical or laboratory abnormalities, concurrent use of neuroleptic drugs, and previous intracranial neurosurgery. The protocol was reviewed and approved by the institutional review board of each participating center as well as the performance and safety monitoring board of the National Institutes of Health. After providing written informed consent, patients underwent laboratory screening and were excluded from further participation if they had evidence of infection with human immunodeficiency virus, hepatitis, or syphilis.

Study Sites

Patients were enrolled and evaluated at the Movement Disorder Centers in New York (Mount Sinai) and Chicago (Rush). All surgical procedures were performed in Tampa, Florida. PET studies were performed in Vancouver, Canada.

Randomization

Patients who met entry criteria and underwent baseline evaluations were assigned to one of three treatment groups by using a computer-generated simple randomization scheme with no blocking: (1) bilateral fetal nigral transplantation into the postcommissural putamen utilizing one donor per side, (2) bilateral fetal nigral transplantation into the postcommissural putamen utilizing four donors per side, or (3) bilateral placebo procedures. The neurosurgeon was the only member of the study team who was aware of the specific treatment of any patient. All other study investigators were blinded to both treatment group and specific treatment intervention.

Transplantation Protocol and Surgical Procedure

All surgical procedures were performed by the same neurosurgeon (T.B.F.). The details of the transplant protocol and the rationale for choosing transplant variables have been described previously in detail.[2,17] In brief, fetal tissue was obtained from women undergoing elective abortions in accordance with federal, state, and local laws. Informed consent for the use of the cadaver fetus was sought only after the patient had signed surgical consent for abortion. There was no monetary or other inducement, and donation of embryonic tissue was made without restriction as to the recipient or whether the tissue would be used in human or laboratory research. Maternal donors and fetal tissue were screened for transmittable infectious agents.[25] Solid mesencephalic grafts derived from donor embryos aged 6 to 9 weeks after conception were used in this study and stored in cool hibernation medium at 8°C for no more than 2 days before transplantation.[26] Surgery was performed using propofol induction followed by laryngeal mask airway and general inhalation anesthesia. Patients receiving a transplantation procedure had tissue from either one or four donors per side grafted stereotactically into the postcommissural putamen bilaterally in two staged procedures separated by approximately 1 week. Eight needle tracts were used per side, and four tissue deposits were placed into each tract so that graft deposits were separated by no more than 5mm in three dimensions. Implanted tissue was diluted such that the same volume of material was injected into each deposit site for patients in both transplant groups. Broad-spectrum antibiotics were provided perioperatively and discontinued if culture results were negative; a full course of antibiotics was used if there was evidence of infection. Patients randomized to the placebo group were treated in an identical manner except that they received partial burr holes that did not penetrate the inner table of the skull, needles were not inserted into the brain, and no tissue was implanted. All patients were treated with cyclosporine 6mg/kg/day for 2 weeks before the first procedure. The dose was reduced to 2mg/kg/day 2 weeks after the second procedure and was discontinued at 6 months. Renal function was monitored during this time. Postoperatively, antiparkinsonian medications were reinstituted at preoperative doses and manipulated only in the case of inadequate symptom control or adverse events. Dosage adjustments were performed by a blinded treating investigator.

Evaluations

Clinical evaluations were performed at baseline and at 1, 3, 6, 9, 12, 15, 18, 21, and 24 months after transplantation. All evaluations were performed by a separate blinded evaluator who played no other role in the study. Evaluations included the Unified Parkinson's Disease Rating Scale (UPDAS)[27] performed in the practically defined "off" state (approximately 12 hours after the last evening dose of medication) and in the best "on" state (peak response after administration of

morning antiparkinsonian medication).[28] The percentage of time during the waking day that patients experienced poor mobility ("off") or good mobility ("on") with or without dyskinesia was calculated based on home diaries completed by the patients or their caregivers at half-hour intervals during the week before each evaluation. Patients and caregivers were trained in the completion of these diaries. Dyskinesias were assessed at the end of the study by a second blinded rater based on videotapes obtained at each visit in the practically defined off and best on states and presented to the rater in randomized order. Scores were calculated using a modified abnormal involuntary movement scale (range, 0–28).[29] Striatal fluorodopa uptake on PET was calculated at baseline and at yearly intervals after surgery by using standard techniques that have been reported previously.[30] L-Dopa dose equivalents were calculated based on the following formula: mg L-dopa in Sinemet + (0.8 × mg L-dopa in Sinemet CR) + (100 × mg pergolide) + (100 × mg pramipexole) + (30 × mg ropinirole).

Outcome Measures and Statistical Analysis

The primary outcome measure in the study was the change between baseline and final visit in UPDRS motor score (range, 0–108) in the practically defined "off" state. Secondary end points included home diary measure of percentage of "on" time without dyskinesia and individual components of the UPDRS. The main outcome variable for the PET study was the change from baseline to final visit in putamenal fluorodopa uptake using the striatal to occipital ratio calculated 60 to 90 minutes after tracer injection. Statistical analysis to assess differences among treatment groups was performed using an analysis of covariance adjusted for baseline score and an F-test with two degrees of freedom.[31] p values for between group comparisons were adjusted for multiple comparisons using the Tukey–Kramer method. The Spearman correlation coefficient was used to assess correlations between continuous variables. Differences in baseline demographics were assessed using a one-way analysis of variance. The Kruskal–Wallis test was used to test for differences between groups with nonnormal data. An intention-to-treat analysis was performed using last visit carried forward to impute for missing data. All means are shown ± standard error except where otherwise indicated.

The study was funded by the National Institutes of Health. None of the investigators have a financial interest in the results of this study.

Results

Thirty-four patients gave informed consent, were randomized, and underwent bilateral operative procedures. Baseline demographics are provided in Table 1. Thirty-one patients completed the study; two died during the course of the study from unrelated causes (one from myocardial infarction and one from drowning 15 and 18 months after surgery, respectively), and one withdrew consent after 1 month.

There was no overall treatment effect for the primary end point ($p = 0.244$). The mean adjusted change from baseline to final visit in UPDRS motor score during practically defined "off" was 9.4 ± 4.25, 3.5 ± 4.23, and −0.72 ± 4.05 for the placebo, one-donor, and four-donor groups, respectively (increased scores represent worsening and negative scores represent improvement). Pairwise comparisons were also not significant, although comparison of the four-donor versus placebo groups yielded a p value of 0.096. The mean UPDRS motor scores at each visit for patients in each of the three treatment groups are illustrated in Figure 1. No significant treatment effect was observed in patients younger than 60 years (data not shown). However, stratification based on baseline median UPDRS motor "off" score demonstrated a treatment effect in patients with less severe motor scores (UPDRS ≤49;

Table 1. Baseline Demographics (Mean ± SD)

Patient Demographics	Placebo	One Donor	Four Donors	Total Group
Number	11	11	12	34
Male sex	6	9	9	24
Mean age at time of surgery (yr)	58.0 ± 9.5	57.5 ± 9.0	60.0 ± 7.2	58.5 ± 8.4
Mean age at diagnosis (yr)	43.8 ± 11.5	46.9 ± 10.8	51.8 ± 5.9	47.6 ± 9.9
Mean UPDRS motor score[a]—off period[b]	51.5 ± 12.5	47.9 ± 14.6	48.6 ± 10.2	49.3 ± 12.2
Mean UPDRS motor score[a]—on period[c]	23.3 ± 9.5	20.5 ± 10.6	19.7 ± 12.6	21.1 ± 10.8
Mean UPDRS ADL score[d]—off period[b]	28.9 ± 8.1	26.6 ± 8.2	25.8 ± 8.0	27.1 ± 7.9
Mean UPDRS ADL score[d]—on period[c]	5.8 ± 4.9	11.5 ± 5.2	11.3 ± 7.6	9.6 ± 6.5
Off time in waking day (± SD)[e] (%)	49.2 ± 9.1	36.9 ± 7.5	45.2 ± 10.8	43.8 ± 10.4
On time without dyskinesia (± SD) (%)	38.8 ± 16.3	40.9 ± 23.9	36.8 ± 20.5	38.8 ± 19.9
Mean L-dopa equivalents per day (mg ± SD)	1,399.1 ± 1198	1,257.4 ± 475	1,427.7 ± 529	1,363.3 ± 776.2

[a]The motor score has a range of 0 to 108 with higher levels indicating greater severity.
[b]Off-period evaluations were performed when patients had been withdrawn overnight from antiparkinsonian medications for approximately 12 hours.
[c]On-period evaluations were performed during maximal clinical benefit approximately 1 to 2 hours after the first morning dose of antiparkinsonian medication; $p < 0.05$.
[d]Range 0–48.

SD = standard deviation; UPDRS = Unified Parkinson Disease Rating Scale; ADL = Activities of Daily Living.

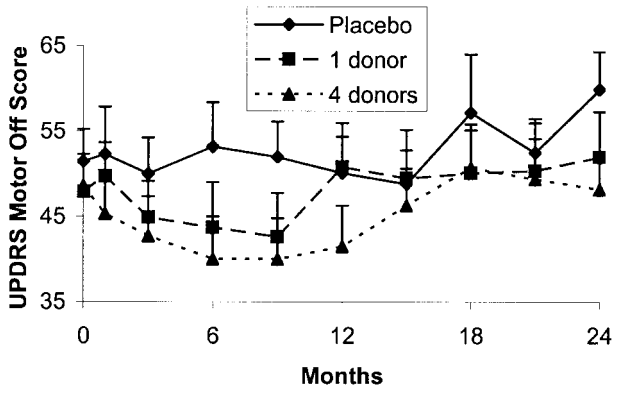

Fig 1. Mean (± SE) Unified Parkinson's Disease Rating Scale (UPDRS) motor score in the practically defined "off" state at each visit for patients in each of the three treatment groups. Note that patients in the one- and four-donor transplant groups were improved in comparison with placebo-treated patients after 6 and 9 months of treatment ($p < 0.05$*), but deteriorated thereafter. Note that this degree of improvement corresponds to the magnitude of improvement observed in open-label studies[9,15,17] and that deterioration coincides with the timing of the withdrawal of cyclosporine.*

$p = 0.006$). Patients with less severe disease who received transplantation with four donors per side (n = 6) improved by an adjusted mean score of 1.5 ± 4.2, whereas those in the placebo group (n = 6) deteriorated by 21.4 ± 4.3 ($p = 0.005$). Patients in this stratum who received transplantation with one donor per side (n = 6) deteriorated by 7.3 ± 4.3 and were not significantly different from patients in the placebo group ($p = 0.093$) or the four-donor group ($p = 0.346$). Mean scores at each visit for patients in these subgroups are illustrated in Figure 2.

No significant difference between treatment groups was noted for the major secondary end point (change from baseline in percentage of "on" time without dyskinesia) or for other predetermined secondary end points. The unadjusted change score ± standard error between baseline and final visit for primary and secondary end points is provided in Table 2. The change in L-dopa dose equivalents from baseline to 2 years similarly did not differ significantly among the treatment groups.

Transplantation was associated with a significant increase in fluorodopa uptake on PET in both the left and right putamen ($p < 0.001$ for each; see Table 2 and Fig 3). In comparison with placebo, the change from baseline in putamenal fluorodopa uptake was significantly greater in both the one-donor ($p = 0.01$) and four-donor ($p < 0.001$) groups on both left and right sides. Most improvement occurred by 12 months, with only minimal additional increase at 24 months. No change from baseline was detected in the non-grafted caudate nucleus in any of the treatment groups.

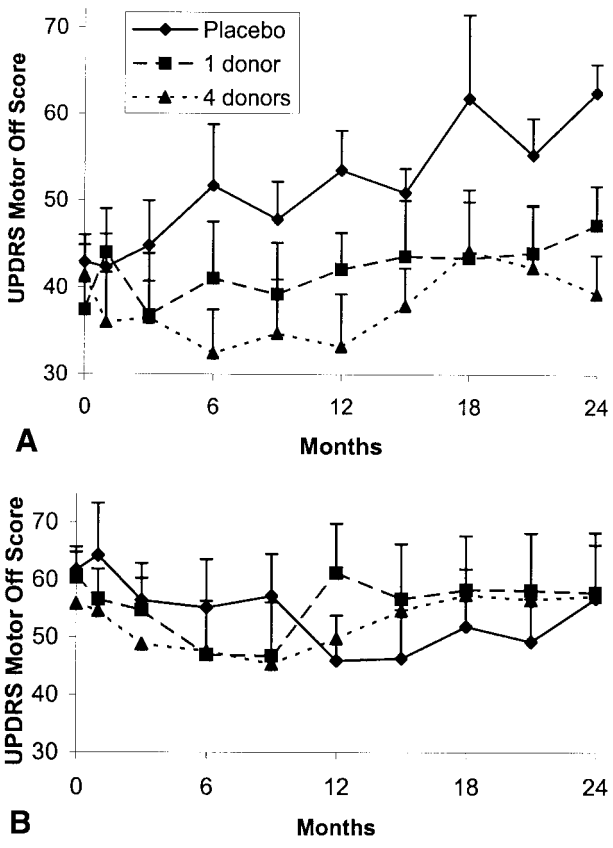

Fig 2. Mean (± SE) Unified Parkinson's Disease Rating Scale (UPDRS) motor scores in the practically defined "off" state at each visit are provided for patients with (a) less severe disease (baseline UPDRS score ≤49) and (b) more severe disease (baseline UPDRS score >49). Note that transplantation was associated with an overall treatment effect ($p < 0.006$*) in patients with less severe disease despite the relatively small sample size, and that patients in the four-donor group were significantly improved in comparison with placebo (*$p < 0.005$*). No significant change between treatment groups was noted in patients with more severe disease.*

Autopsies were performed on two patients who died during the study (drowning, myocardial infarction) and three other who died during 29 months of follow-up after completing the study, each from causes unrelated to study procedures (respiratory failure, cancer of the lung, and pneumonia at 43, 47, and 53 months after surgery). Two were in the four-donor group, two in the one-donor group, and one in the placebo group. Representative tyrosine hydroxylase (TH) histopathological studies are provided in Figure 4a. In the four-donor group, there were high numbers of healthy-appearing dopamine neurons with robust cell survival in each graft deposit (a total of 70,000–120,000 per side), and organotypic seamless reinnervation of the striatum similar to what we have described previously.[19,20] In the one-donor group, grafts deposits were smaller with more concentrated cytoarchitecture and there was survival of

Table 2. Unadjusted Change Scores from Baseline to 24 Months (mean ± SE)[a]

				p Values			
	Placebo	One Donor	Four Donors	Overall	One Donor vs Placebo	Four Donors vs Placebo	One Donor vs Four Donors
UPDRS scores[b]							
UPDRS motor off (range 0–108)	8.4 ± 5.5	4.1 ± 4.8	−0.42 ± 2.8	0.244	0.334	0.096	0.479
UPDRS ADL off (range 0–52)	0.8 ± 3.2	1.5 ± 2.1	0.0 ± 2.3	0.770	0.992	0.780	0.843
UPDRS total off (range 0–160)	9.2 ± 7.9	5.5 ± 6.3	−0.4 ± 3.3	0.314	0.717	0.282	0.729
UPDRS motor on (range 0–108)	6.7 ± 4.5	14.9 ± 4.6	14.4 ± 4.8	0.481	0.515	0.573	0.993
UPDRS ADL on (range 0–52)	9.2 ± 2.0	7.3 ± 1.9	7.2 ± 1.4	0.582	0.643	0.609	0.999
UPDRS total on (range 0–160)	15.9 ± 6.1	22.2 ± 5.7	21.5 ± 5.4	0.700	0.725	0.763	0.996
Tremor off (range 0–20)	1.1 ± 0.8	−0.7 ± 0.8	−1.3 ± 0.7	0.074	0.241	0.068	0.798
Tremor on (range 0–20)	−0.3 ± 0.2	0.27 ± 0.3	0.3 ± 0.2	0.464	0.544	0.495	0.998
Rigidity off (0–20)	1.2 ± 1.2	0.4 ± 1.2	−0.2 ± 1.1	0.630	0.862	0.602	0.900
Rigidity on (0–20)	1.4 ± 0.9	2.5 ± 1.4	2.5 ± 1.2	0.807	0.856	0.819	0.998
Bradykinesia off (range 0–4)	0.1 ± 0.3	−0.1 ± 0.2	−0.4 ± 0.2	0.398	0.716	0.366	0.843
Bradykinesia on (range 0–4)	0.3 ± 0.3	0.8 ± 0.3	1.1 ± 0.3	0.312	0.582	0.289	0.860
Postural stability off (range 0–4)	0.1 ± 0.5	0.8 ± 0.3	0.2 ± 0.3	0.436	0.405	0.751	0.834
Postural stability on (range 0–4)	0.1 ± 0.3	1.0 ± 0.4	1.5 ± 0.3	0.017	0.160	0.013	0.484
Home diary							
Percentage on time without dyskinesia	−8.7 ± 6.8	−5.6 ± 6.9	−4.5 ± 9.7	0.932	0.939	0.947	0.999
Percentage off time	0.8 ± 5.6	7.8 ± 5.7	−0.9 ± 8.1	0.676	0.789	0.985	0.662
Percentage on time with dyskinesia	7.9 ± 7.5	−2.2 ± 6.3	5.4 ± 7.6	0.851	0.915	0.990	0.845
Striatal fluorodopa uptake–PET (striatal: occipital ratio)[c]							
L putamen	−0.0145 ± 0.0181	0.2045 ± 0.0492	0.3600 ± 0.0567	<0.001	0.007	<0.001	0.057
R putamen	0.0109 ± 0.0193	0.2209 ± 0.0437	0.3175 ± 0.0674	<0.001	0.013	<0.001	0.375
L caudate	−0.0664 ± 0.0414	−0.0073 ± 0.0683	0.0792 ± 0.0483	0.113	0.491	0.094	0.590
R caudate	0.0600 ± 0.0482	0.0845 ± 0.0368	0.1183 ± 0.0240	0.215	0.578	0.188	0.724
L-Dopa dose equivalents	6.2 ± 134.4	−248.4 ± 164.1	−161.3 ± 104.2	0.333	0.304	0.651	0.796

[a]All means presented in this table are unadjusted. Note that means presented in text for the primary end point are adjusted for baseline values and thus differ from those presented in this table. All *p* values were calculated using an analysis of covariance with adjustment for baseline value. In addition, *p* values for secondary end points were adjusted with the Tukey–Kramer method for multiple comparisons.
[b]Increase represents deterioration; decrease represents improvement.
[c]Increase represents improvement; decrease represents worsening.

SE = standard error; UPDRS = Unified Parkinson's Disease Rating Scale; PET = positron emission tomography; L = left; R = right.

approximately 30,000 cells per side, although there was still uninterrupted reinnervation of the striatum between graft deposits. Virtually no TH immunostaining was present in nongrafted regions of transplant patients, nor in the striatum of the placebo patient. CD45 immunostaining, a marker of activated microglia and immune reactivity, was increased in the grafted striatum compared with controls, and staining was particularly prominent in and around graft deposits (see Fig 4b).

Adverse events occurred more frequently in patients who received a transplantation procedure, but there were no serious perioperative complication (Table 3).

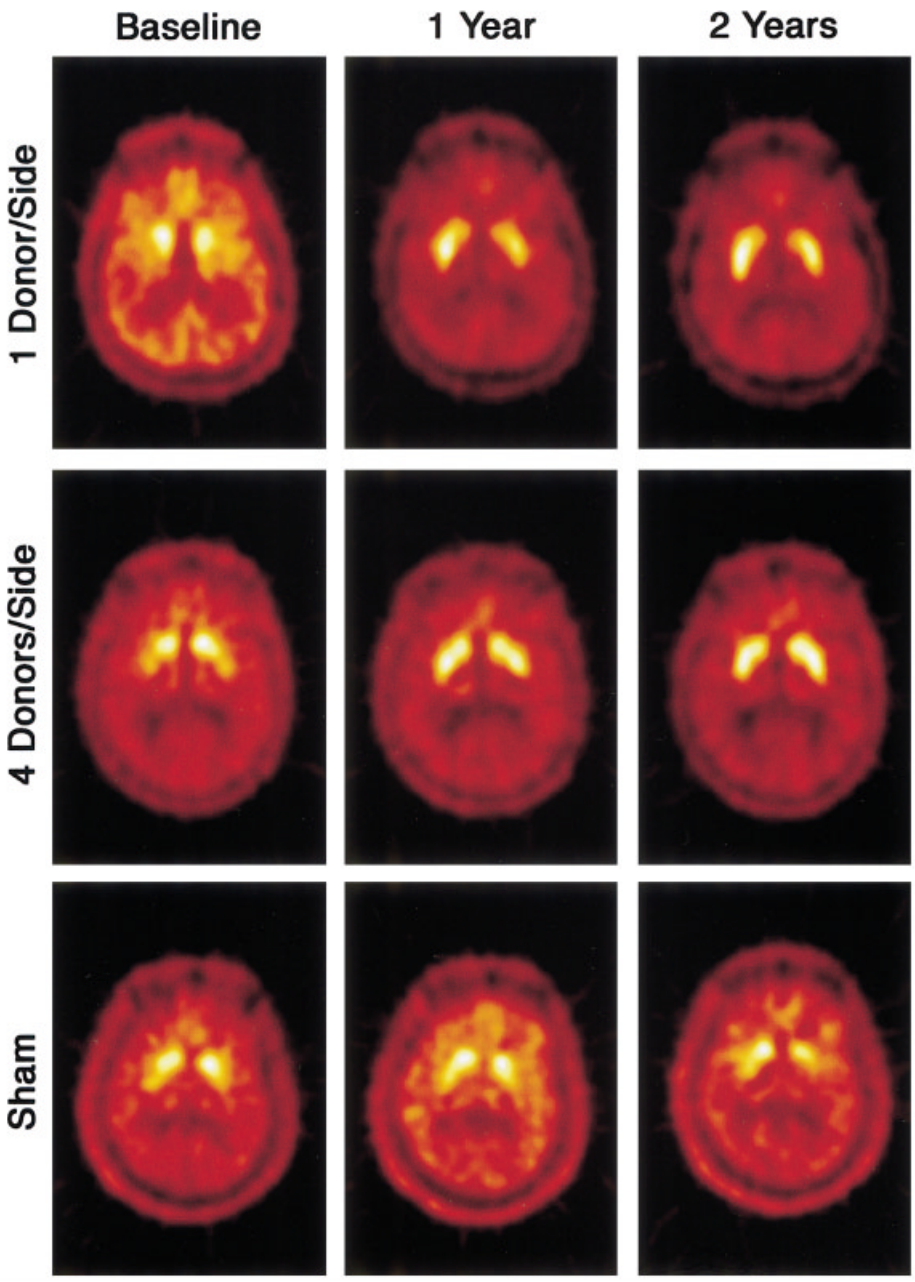

Fig 3. Representative positron emission tomography images of striatal fluorodopa uptake at baseline, 1 year, and 2 years in patients receiving transplantation with one donor per side (a), four donors per side (b), or a placebo procedure (c). Note the increase from baseline in striatal fluorodopa uptake in transplanted patients, whereas striatal fluorodopa uptake is decreased in comparison with baseline in the placebo patient, presumably reflecting disease progression.

Cyclosporine was well tolerated and did not have to be prematurely withdrawn from any patient. Dyskinesia was the most common and most serious adverse event. Analysis of videotapes presented in random order to a blinded rater noted that all but one patient had peak-dose dyskinesia during best "on" periods at baseline, and the dyskinesia score did not significantly worsen over the course of the study ($p = 0.277$; see Table 3). In contrast, no patient had dyskinesia during practically defined "off" periods at baseline, but 13 of 23 transplanted patients (56.5%) developed dyskinesia in the practically defined "off" state (off-medication dyskinesia) during the course of the study, whereas this complication was not seen in any patient in the placebo group. Off-medication dyskinesia tended to develop 6 to 12 months after transplantation and consisted of stereotypic, rhythmic movements of one or both lower extremities. In three patients, the off-

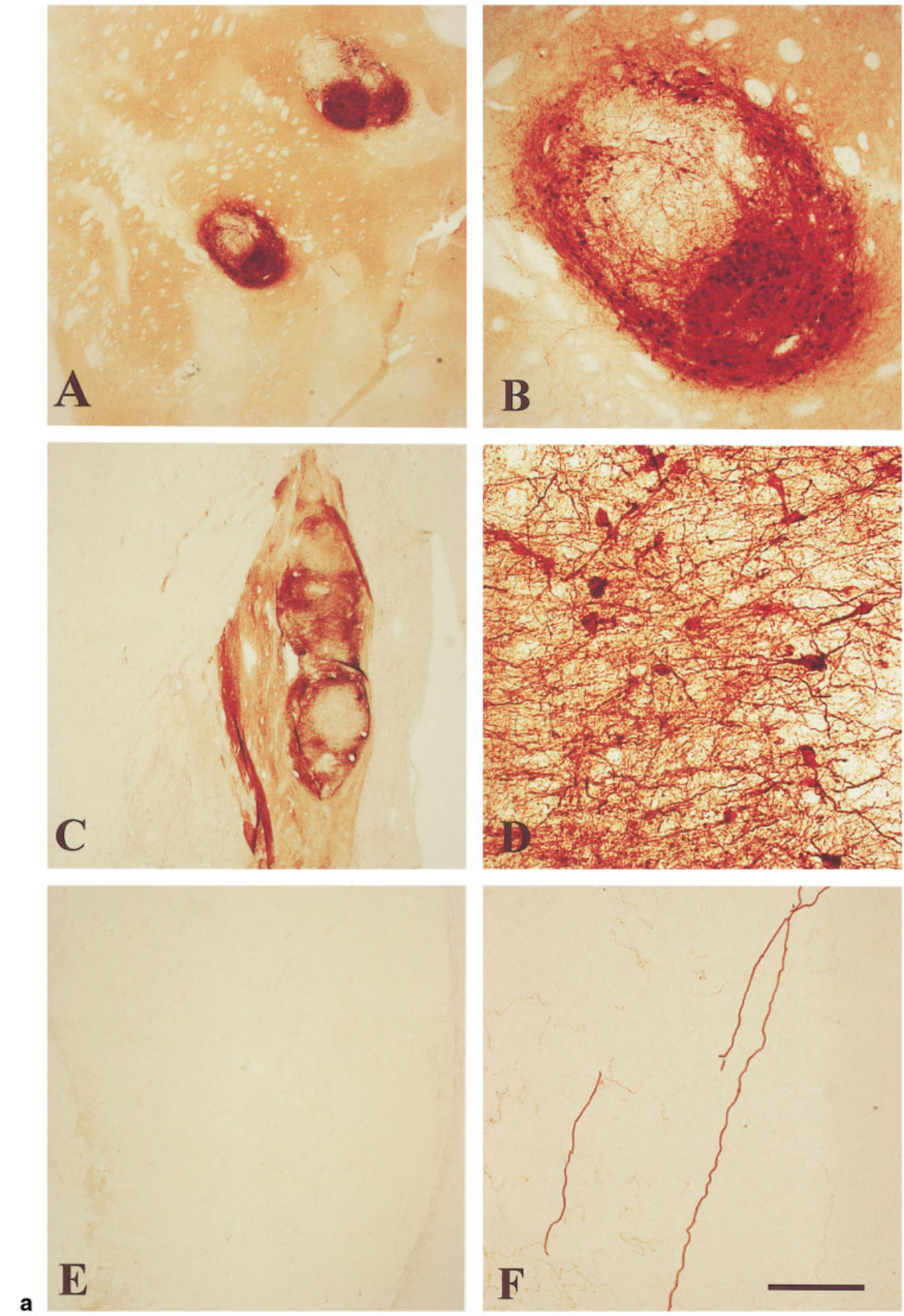

Fig 4. Figure and legend continue.

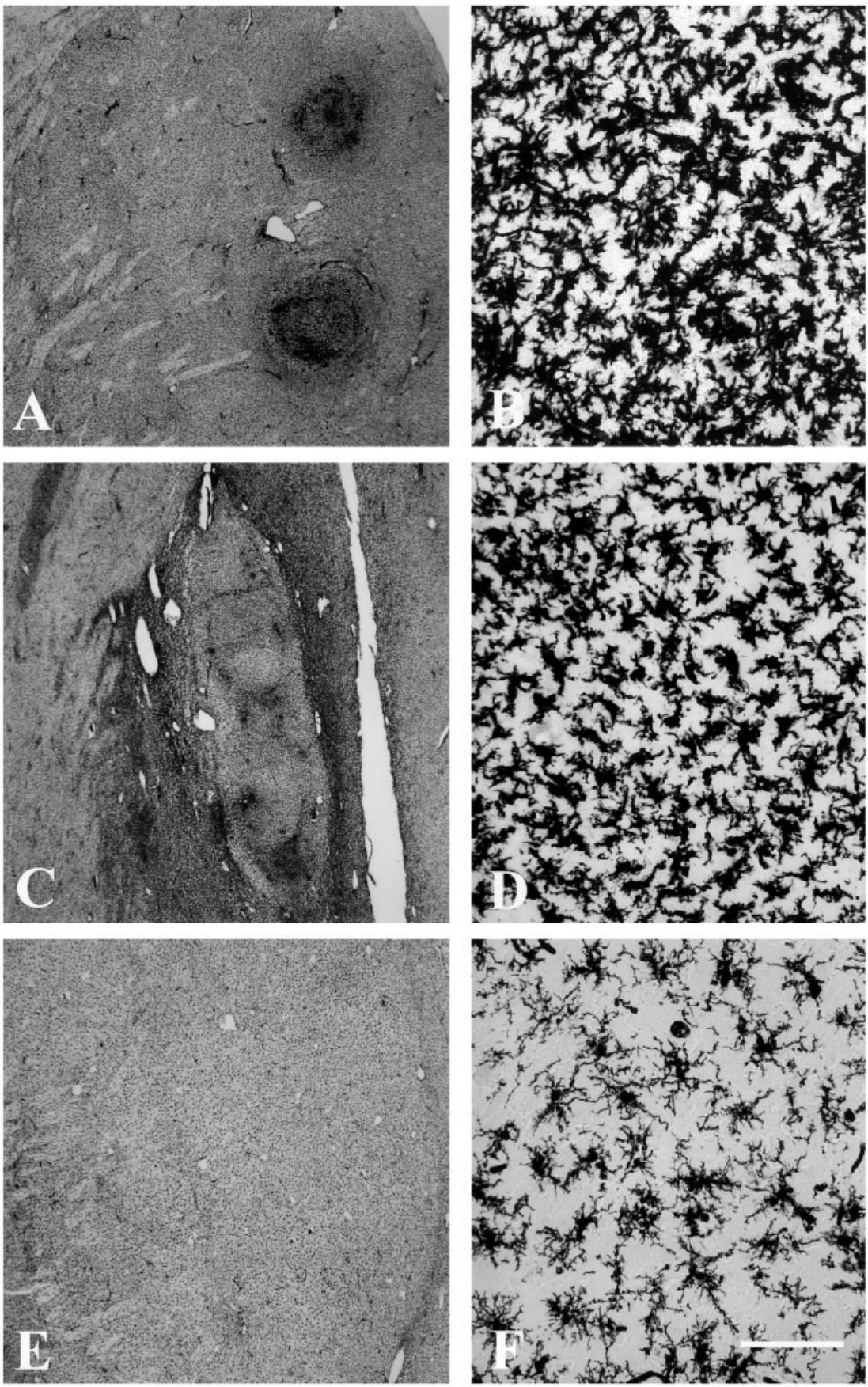

Figure 4 (continued)

medication dyskinesia was disabling, necessitating a surgical intervention when the study was completed. The change from baseline to final visit in off-medication dyskinesia score was significantly greater than placebo in both the one- and four-donors groups ($p < 0.01$), but there was no difference between the two transplant groups ($p = 0.862$). No correlation was detected between off-medication dyskinesia scores and on-medication dyskinesia score, UPDRS motor score, L-dopa dose equivalents, or striatal fluorodopa uptake.

Discussion

We demonstrate in a prospective, randomized, double-blind placebo-controlled trial that fetal nigral transplantation using one or four donors per side does not significantly improve the motor features of PD. Although there was a positive trend in transplanted patients, and those receiving four donors per side just failed to show significant improvement versus placebo ($p = 0.096$), the study failed to meet its primary or secondary clinical end points. Furthermore, more than half of transplanted patients experienced off-medication dyskinesia, a side effect that had not been reported before the institution of transplant procedures.[32] PET studies demonstrated significant increases in striatal fluorodopa uptake in transplanted regions, with more pronounced changes in the four donor group. Autopsy studies demonstrated robust graft survival with normal-appearing reinnervation of the striatum, again with more profound effects in the four-donor group. Thus, fetal nigral transplantation failed to provide significant clinical benefits despite autopsy and PET evidence of survival of high numbers of implanted cells.

We did observe a significant transplant benefit in the subset of patients with less severe motor scores at baseline, although this reflects failure of transplanted patients to deteriorate rather than improvement in parkinsonian features (see Fig 1). We also noted that transplanted patients with four donors per side were improved at 6 and 9 months in comparison with baseline ($p < 0.05$), comparable with what has been reported in open-label trials,[9,15,17] and deteriorated thereafter. It is interesting that this coincides with the timing of the discontinuation of cyclosporine, raising the possibility that some degree of graft rejection may have occurred and prevented continuing benefit. Indeed, there was increased CD45 staining in and around graft deposits in transplanted patients (Fig 3b), consistent with an immune reaction and similar to what we have previously described.[33] Furthermore, increased FD uptake on PET was primarily detected at 1 year, with very little change thereafter. It thus is possible that immune rejection after discontinuation of cyclosporine limited the benefit that could have been obtained with fetal nigral transplantation.

Our results are similar to those reported in the other double-blind placebo-controlled trial of human fetal transplantation in PD,[22] even though we used a protocol that used higher numbers of implanted cells, more even distribution of deposits in target regions, immunosuppression for 6 months, and 2 years of follow-up. Both studies failed to meet their primary end point despite increased striatal fluorodopa uptake on PET and evidence of surviving transplanted neurons at postmortem. They reported motor benefits in a subpopulation of patients younger than 60 years. We did not detect selective benefits in younger patients but noted significant improvement in those with milder disease at baseline. It will be interesting to determine if younger patients in the first study also had milder disease severity. Both studies observed potentially disabling off-medication dyskinesia in transplanted patients. Ma and colleagues report increased fluorodopa uptake in transplanted patients who experienced off-medication dyskinesia particularly in local regions of the striatum, and suggested that they are related to a regional imbalance in dopamine release.[24] We found no regional or global differences in striatal fluorodopa uptake between transplanted patients who did or did not develop off-medication dyskinesia at either baseline or at 24 months. Furthermore, there was no difference in the frequency or severity of off-medication dyskinesia in patients receiving transplantation with one or four donors per side, arguing against this hypothesis. Hagell and colleagues suggested that off-medication

Fig 4. (a) Representative low- and high-power tyrosine hydroxylase-immunostained sections through the striatum of patients receiving transplantation with one donor per side (A, B), four donors per side (C, D), and placebo (E, F). Note that graft deposits in the four donor group are cylindrical in appearance and that fibers stream from the graft to provide continuous innervation of the surrounding striatum. Approximately 100,000 tyrosine hydroxylase (TH)–positive cells were counted per side. In contrast, in the one donor group, deposits were smaller with more concentrated cytoarchitecture and only 30,000 surviving cells per side. Transplantation in either group was associated with continuous and seamless innervation of the striatum. Virtually no TH staining was noted in the placebo group. (b) Low- and high-power photomicrographs of CD45 immunostaining, a measure of activated microglia and immune reactivity, in the one donor (A, B), four donors (C, D), and placebo (E, F) groups. Note that CD45 staining is increased in the one and four donor groups compared with placebo and that staining is particularly increased in and around the region of the graft deposit (see A vs C). These findings are consistent with some degree of immune rejection having occurred in transplanted groups and may account for the clinical deterioration that occurred following withdrawal of immunosuppression (see Fig 1). Scale bars in A, C, E = 4mm; B, D, F = 500μm.

Table 3. Adverse Events Associated with Transplantation

Event Details	Placebo (N = 11)	One Donor/Side (N = 11)	Four Donors/Side (N = 12)
Self-reported adverse events (>2 reports)			
Dyskinesia	2	7	6
Insomnia	2	6	5
Depression	6	4	2
Constipation	5	4	2
Hypertension	4	3	3
Increased blood urea nitrogen	2	5	2
Confusion	0	4[a]	5[a]
Hallucinations	2	2	5
Nausea	0	6	2
Headache	2	4	0
Bone fracture	2	2	2
Diarrhea	2	1	2
Urinary incontinence	0	2	2
Falls	1	1	2
Tingling skin	1	3	0
Visual hallucination	0	2	1
Increased creatinine	1	2	0
Weight loss	0	2	1
Dehydration	0	1	2
Urinary frequency	0	1	2
Infection	0	1	2
Incisional pain	2	1	0
Indigestion	2	1	0
Dysphagia	2	1	0
Leg edema	1	1	1
Mean number adverse events per patient	9.9	13.5	9.5
Adverse event rate/patient day	0.39	0.57	0.66
Video-based assessment of dyskinesia			
On medication dyskinesia			
No. of patients at baseline	11	10	12
No. of patients at final visit	11	11	12
Mean score at baseline (0–28)	6.4 ± 5.3	7.4 ± 5.0	5.1 ± 3.9
Mean score at final visit (0–28)	8.9 ± 5.7	11.2 ± 6.0	6.2 ± 4.1
Off-medication dyskinesia			
No. of patients at baseline	0	0	0
No. of patients at final visit	0	7	6
Mean score at baseline (0–28)	0	0	0
Mean score at final visit (0–28)	0 ± 0	3.2 ± 3.7[b]	2.7 ± 3.0[b]

[a] $p < 0.05$ vs placebo group.
[b] $p < 0.01$ vs baseline value and vs placebo group.

dyskinesias are related to on-medication dyskinesia and maintaining cells in culture for 4 weeks.[33] We did not culture cells, stored them for no more than 48 hours, and found no correlation between on- and off-medication dyskinesia scores. Current evidence suggests that dyskinesias are related to pulsatile stimulation of dopamine receptors.[34] This theory would predict that a transplant-mediated increase in striatal dopamine terminals should ameliorate dyskinesia by making dopamine available to receptors in a more continuous manner.[35] Indeed, we observed reduced dyskinesia in our open-label trial,[18] and transplantation was reported to reduce dyskinesia in a rodent model.[35] It is possible that graft variability, nonuniform striatal reinnervation, partial immune rejection, and altered synapse formation in our patients could have caused regional inhomogeneities with pulsatile stimulation leading to off-medication dyskinesia. We think it is more likely that

off-medication dyskinesia represent a variant of diphasic dyskinesia due to incomplete or aberrant reinnervation of the striatum. Diphasic dyskinesias typically occur at the onset and end of a L-dopa dose and reflect relatively lower rather than higher striatal dopamine concentrations. The off-medication dyskinesia seen in our patients were similar in appearance to diphasic dyskinesias and also resembled the dyskinesia that occurs with falling dopamine concentrations after stopping continuous L-dopa infusions. Indeed, diphasic dyskinesia can be associated with simultaneous parkinsonism in other body parts and frequently are improved with increased doses of L-dopa. Although there was an increase in striatal FD uptake on PET in our patients, levels were still below the lower limits of normal. Off-medication dyskinesia after transplantation thus may reflect partial, but inadequate, graft survival that is sufficient to produce, store, and release low levels of dopamine for a prolonged period of time after a dose of L-dopa, but not sufficient to induce an antiparkinsonian response. Although this interpretation is speculative, it has important implications. It suggests that transplant protocols that lead to an increased number of surviving dopamine neurons and terminals might provide both an enhanced clinical response and a decreased likelihood of developing off-medication dyskinesia. Stem cell therapies represent a potential source of unlimited numbers of dopamine neurons and might offer such a solution. Laboratory studies to test the safety and efficacy of these approaches are clearly warranted before proceeding with further human trials.

This study illustrates the importance of performing placebo-controlled double-blind trials for assessing new treatments[21] and demonstrates the feasibility of using this type of study design even with surgical therapies. This study did not confirm the clinical benefits reported in open-label trials. Furthermore, unanticipated and potentially disabling off-medication dyskinesias developed in greater than 50% of patients. We cannot therefore recommend fetal nigral transplantation as a therapy for PD at this time. It is possible, however, that enhanced benefits can be obtained in patients with milder disease, with transplantation of higher numbers of cells, and with more prolonged immunosuppression. These studies have important implications for trials of stem cells and other cell-based therapies.

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Appendix

The following individuals contributed to this study: Barry Snow, MD, Jean Michel Gracies, MD, PhD, Myrna Schear, MD, Glen Stebbins, PhD, Thomas J. Ruth, PhD, Debbie Cote, RN, Marie Thiel, RN, Reina Benabou, MD, Jeana Jaglin, RN, Deborah Scott, RN, Barbara Legg, RN, Jess McKenzie, RN, Teresa Dobko, BSc, Jairo Munñoz, BSc, and Erin Moshier, MS.

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