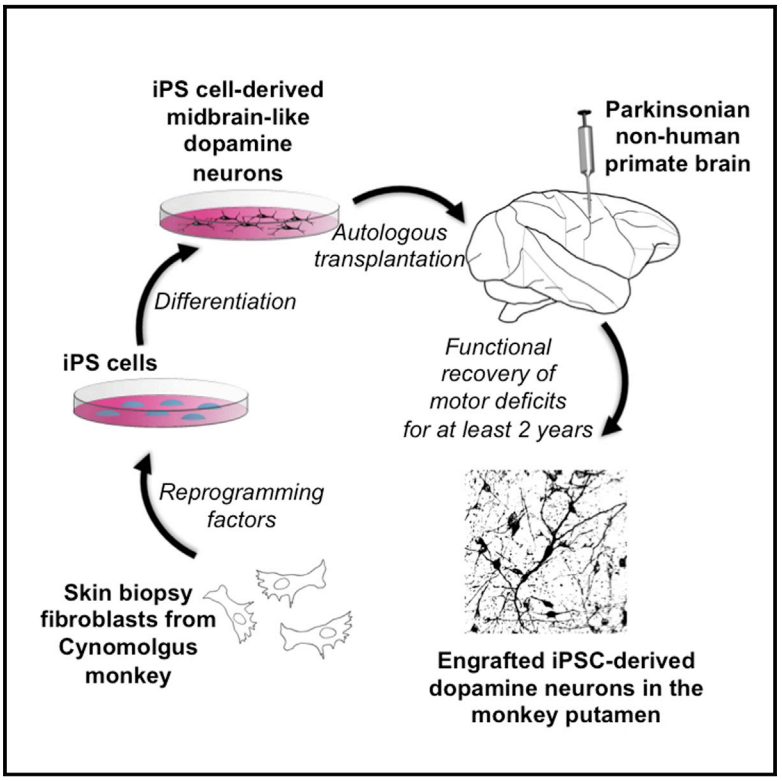
Brief Report

Cell Stem Cell

Successful Function of Autologous iPSC-Derived Dopamine Neurons following Transplantation in a Non-Human Primate Model of Parkinson's Disease

Graphical Abstract



Highlights

- A non-human primate model tests cell transplantation for PD therapy
- Autologous iPSC dopamine neurons can provide long-term functional recovery
- Transplanted cells survive for up to 2 years and reinnervate the host brain

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In Brief

A pre-clinical test of transplantation of autologous iPSC-derived dopamine neurons in a cynomolgus monkey model of Parkinson's disease provides proof of principle for long-term innervation and functional benefit without a requirement for immunosuppression.

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Brief Report

Successful Function of Autologous iPSC-Derived Dopamine Neurons following Transplantation in a Non-Human Primate Model of Parkinson's Disease

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SUMMARY

Autologous transplantation of patient-specific induced pluripotent stem cell (iPSC)-derived neurons is a potential clinical approach for treatment of neurological disease. Preclinical demonstration of long-term efficacy, feasibility, and safety of iPSC-derived dopamine neurons in non-human primate models will be an important step in clinical development of cell therapy. Here, we analyzed cynomolgus monkey (CM) iPSC-derived midbrain dopamine neurons for up to 2 years following autologous transplantation in a Parkinson's disease (PD) model. In one animal, with the most successful protocol, we found that unilateral engraftment of CM-iPSCs could provide a gradual onset of functional motor improvement contralateral to the side of dopamine neuron transplantation, and increased motor activity, without a need for immunosuppression. Postmortem analyses demonstrated robust survival of midbrain-like dopaminergic neurons and extensive outgrowth into the transplanted putamen. Our proof of concept findings support further development of autologous iPSC-derived cell transplantation for treatment of PD.

Cellular therapies offer an exciting opportunity to replace specific populations of cells in neurodegenerative diseases where symptoms are defined by the loss of a specific cell type, such as the degeneration of substantia nigra (SN) dopamine neurons in Parkinson's disease (PD). The use of induced pluripotent stem cell (iPSC)-derived neurons as an autologous cell source overcomes the current limitations posed by allogeneic donor cells in PD. Fetal ventral midbrain allografts can survive and function in the human PD brain for over 18 years (C.R. Freed et al., 2013, Soc. Neurosci., abstract; Hallett et al., 2014; Kefalopoulou et al., 2014; Mendez et al., 2005; Politis et al., 2010); however, such techniques will never become an easily accessible therapeutic option for patients due to the requirement of fetal donor tissue from elective abortions. Allografting in the brain also creates a greater immune reaction over time compared with isogeneic grafting (Duan et al., 1995; Morizane et al., 2013). The generation of midbrain-like dopamine neurons from patient-specific iPSCs and subsequent autologous transplantation is a rational long-term strategy for cell replacement in PD. Previous reports of autologous transplantation in a non-human primate PD model have demonstrated the advantage of autologous versus allogeneic grafts and shown dopamine neuron survival in the primate brain for up to 6 months to 1 year after transplantation (Emborg et al., 2013; Morizane et al., 2013; Sundberg et al., 2013). However, the long-term function, survival, and safety of iPSC-derived dopamine neurons following autologous transplantation in a non-human primate model of PD has not yet been established.

All studies were approved by the Harvard Medical School Institutional Animal Care and Use Committee (IACUC). To induce parkinsonism in cynomolgus monkeys (CMs), we administered systemic low-dose 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which resulted in a progressive and persistent reduction in global motor activity and a stable bilateral parkinsonian syndrome, including tremor, rigidity, bradykinesia, hypokinesia, posture/balance disturbances, and impairment in both gross and fine motor skills (Table S1) (Brownell et al., 1998a; Hantraye et al., 1992; Wüllner et al., 1994). All animals displayed a significant loss of dopamine transporters (DATs) in the putamen as measured by 11C-(2β-carbomethoxy-3β-(4-fluorophenyl) tropane) (^{11}C-CFT) binding potential, as previously described (Brownell et al., 1998a). In a successive series of studies, three MPTP-lesioned CMs (MF25-04, MF66-02, and MF27-04) received autologous transplantation of CM-iPSC-derived neural cells into the putamen in order to assess the function and survival of engrafted autologous iPSC-derived dopamine neurons. CM-iPSCs from MF25-04 were differentiated using the protocol of Cooper et al. (2010), and CM-iPSCs from MF27-04 and MF66-02 were differentiated using the protocol of Sundberg et al. (2013). No animals in this study received any immunosuppression for the duration of the study.

We recorded global daytime motor activity of MPTP-lesioned CMs that had received autologous transplantation of iPSC-derived neural cells, using an automated activity monitor (Figure 1A). At 6 months after transplantation, daytime activity counts in animal MF25-04 (autologous iPSC transplant) were

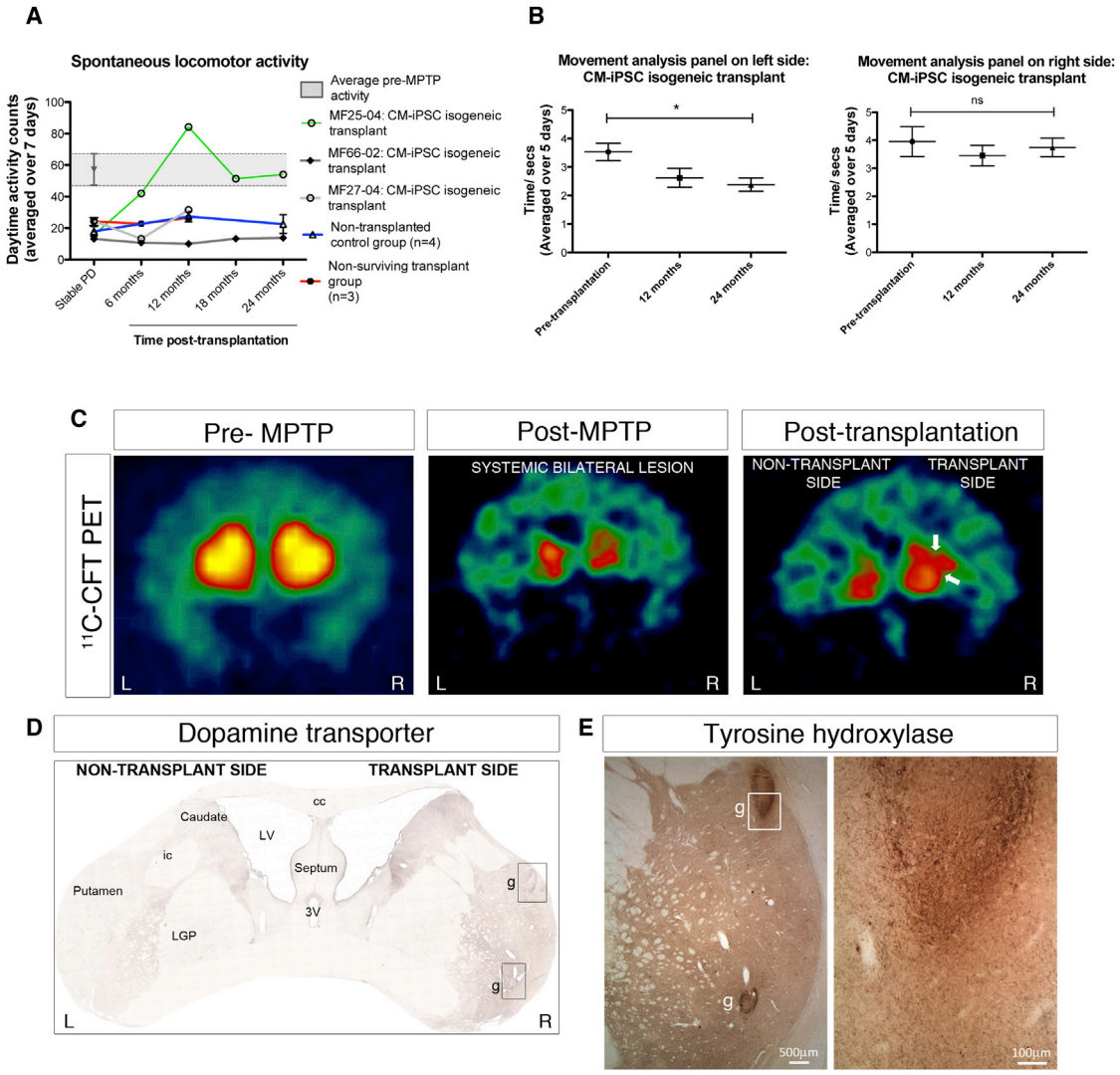

Figure 1. Functional Improvement of PD Motor Symptoms, Increased Dopamine Reuptake, and Reinnervation of the Transplanted Putamen after Autologous Transplantation of CM iPSC-Derived Dopamine Neurons

(A) Differentiated CM-iPSCs were transplanted unilaterally into the putamen of three CMs with stable, bilateral parkinsonism (MF25-04, MF66-02, MF27-04). The animals were followed for 1–2 years after transplantation. From 6 months after transplantation, functional improvement was observed in MF25-04, as determined by a sustained increase in global daytime (6 a.m. to 6 p.m.) activity. The non-transplanted control group and non-surviving transplant group represent the average data of n = 4 and n = 3 animals, respectively, and error bars show the SEM.

(B) Fine-motor skills in MF25-04 were assessed using a computerized reaching task MAP. At 2 years after transplantation, MAP performance in the left upper limb was significantly improved compared with pretransplantation values ($p < 0.05$, one-way ANOVA followed by Tukey's multiple comparison test). No change in performance was observed in the right upper limb. Data shown represent averages of five repeated tests (baseline), two repeated tests (1 year after transplantation), and three repeated tests (2 years after transplantation). Error bars represent the SEM.

(C) Functional analysis of dopamine reuptake in MF25-04 was measured by PET neuroimaging for ^{11}C-CFT, a marker of the DAT. Increased ^{11}C-CFT binding was observed in the transplanted putamen at 2 years after transplantation. White arrows indicate areas of hyperintense CFT PET signal.

(D) Low-power photomicrograph of DAT immunostaining in the transplanted (right, R) and non-transplanted (left, L) putamen in MF25-04 shows reinnervation of the transplanted side. Deposits of grafted dopamine neurons are indicated with boxes (g). IC, internal capsule; LGP, lateral globus pallidus; LV, lateral ventricle; 3V, third ventricle; cc, corpus callosum.

(E) Grafted dopamine neurons were also labeled using tyrosine hydroxylase (TH). The boxed area is shown at higher magnification in the right. Robust survival of dopamine neurons with outgrowth integration into the host putamen was observed.

increased by 146% compared with the stable parkinsonian pretransplantation activity in this animal. Over the subsequent 18 months of the study, motor activity in MF25-04 remained elevated and ranged from 178% to 292% above pretransplantation activity levels. At 2 years after transplantation, activity in this animal was 188% of stable MPTP baseline. The stable bilateral MPTP-lesion model used in this study provides an opportunity to assess asymmetry in movement functions following unilateral transplantation, using movement analysis panel (MAP) testing (Figure 1B). A progressive improvement in the use of left

(contralateral to the graft) upper limb motor function, compared with baseline values, was observed by 12 months after transplantation in MF25-04, and this reduction reached significance at 24 months after transplantation ($p < 0.05$, one-way ANOVA). Use of right upper limb motor function in MF25-04 was not significantly altered over the 24 months following the transplantation procedure. The severity of parkinsonian signs was also rated monthly using a parkinsonian rating scale (Table S1). We observed a reduction in the hypokinesia subsection of the parkinsonian rating scale in MF25-04, from a score of 2 prior to transplantation (maximum possible score is 3) to a score of 0 at 12 months after transplantation, and this remained stable at 0 until completion of the study (Table S1). Overall, the time course of functional recovery observed in MF25-04 was consistent with the developmental maturation, outgrowth, and connectivity of analogous fetal non-human primate dopamine neurons (Redmond et al., 2008; Tsui and Isacson, 2011). No marked changes in global motor activity, MAP test time, or hypokinesia were observed in animals MF27-04 and MF66-02 at 1–2 years after autologous transplantation of differentiated CM-iPSCs (Figure 1A; Table S1), suggestive of insufficient survival of engrafted CM-iPSC-derived midbrain dopamine neurons and reinnervation of the transplanted putamen (Grealish et al., 2010; Redmond et al., 2008). We also analyzed motor behavior in MPTP-lesioned CMs that received allogeneic transplantation with differentiated primate embryonic stem (ES) cells (Cyno-1) with no immunosuppression, as previously described (Sánchez-Pernaute et al., 2005), in which less than 50 surviving dopaminergic neurons were detected in the grafted putamen at postmortem (termed the ''non-surviving transplant'' group) (n = 3), and also in non-transplanted MPTP-lesioned CMs (n = 4) (Table S1). In the non-surviving transplant group, animals were followed for 12 months after the transplantation procedure before termination of the study; no significant changes in motor behavior were observed in these animals during this time (Figure 1A). Motor behavior was also not altered over a 2-year period in the non-transplant control group of parkinsonian animals (Figure 1A), consistent with the long-term functional stability of this bilateral MPTP non-human primate model (Brownell et al., 1998a; Hantraye et al., 1992; Wüllner et al., 1994).

Since functional improvement in parkinsonian motor symptoms was observed in the MF25-04 iPSC autologous transplant case, we performed ^{11}C-CFT-PET scanning to assess dopamine nerve terminals in the caudate and putamen at 2 years after transplantation of CM-iPSC-derived neural cells (Brownell et al., 1998a; Hantraye et al., 1992; Jenkins et al., 2004). At 2 years after transplantation, a marked increase in ^{11}C-CFT binding sites was observed in the right putamen compared with the non-transplanted left putamen, indicative of functional dopaminergic neurons on the transplanted side (Figure 1C).

Given the motor improvement and positive neuroimaging indicative of a functional graft in MF25-04, extensive postmortem examination of graft survival and morphology was performed at 2 years after transplantation. Macroscopic examination of the brain showed graft deposit sites within the putamen and normal striatal cytoarchitecture, with no displacement or compression of the host striatal parenchyma. Immunohistochemical labeling of DAT showed two distinct regions in the putamen containing clusters of DAT-immunoreactive (-ir) dopamine neurons (Figure 1D). Microscopic imaging of the entire transplanted and non-transplanted putamen showed markedly increased DAT labeling in the transplanted putamen compared with the non-transplant side, indicating robust reinnervation of the transplanted putamen from the engrafted CM-iPSC-derived dopaminergic neurons.

Stereological cell counts of TH-ir dopamine neurons showed the presence of 13,029 surviving transplanted dopaminergic neurons in the putamen. Engrafted TH-ir neurons (Figure 1E) were located predominantly around the periphery of the grafts and extended axons into the host putamen, similar to the pattern of A9-like dopamine neurons in rodent and human fetal VM transplant cases (Mendez et al., 2005; Vinuela et al., 2008). As a comparison, the number of surviving TH-ir dopamine neurons in the transplanted putamen of the CMs MF27-04 (Sundberg et al., 2013) and MF66-02 was also determined. These cases received autologous transplantation of iPSC-derived dopamine neurons, but in contrast to MF25-04, did not exhibit any functional improvement at 1–2 years after transplantation. A total of 8,551 and 7,938 TH-ir dopamine neurons was present in the transplanted putamen of MF27-04 and MF66-02, respectively. A comparison of outgrowth of TH-ir fibers into the putamen from the grafts of MF25-04, MF27-04, and MF66-02 showed far more extensive dopaminergic reinnervation in MF25-04 (Figures S2A–S2C), consistent with the functional recovery observed in this animal. These data indicate that improvement in motor function is achieved when an adequate number of transplanted midbrain dopamine neurons survive together with appropriate innervation of the transplanted putamen and that when there is not sufficient survival (less than 13,000 dopamine neurons) and poor dopamine axonal reinnervation of the putamen, CMs do not recover. Our data also show that in this series of iPSC transplantations, the Cooper et al. (2010) neuron differentiation protocol was the most successful. Dopamine neuron transplantation works at an adaptive level to provide a sufficient number of appropriate A9 dopaminergic neurons (and their synapses) in the striatum to initiate movement (function). In accord with this, we have also previously shown following fetal dopamine neuronal transplantation in a rat unilateral PD model (Brownell et al., 1998b) that behavioral recovery only occurs after a threshold of new striatal dopaminergic synapses is reached (75%–85% of the intact striatum). With the development of iPSC-derived midbrain-like dopamine neurons for clinical use in PD, it will be essential to take into consideration that survival of a minimal required dose of transplanted midbrain-like dopamine neurons (Grealish et al., 2014) and sufficient reinnervation of the denervated putamen is necessary for improvement of PD motor symptoms.

Colocalization of FOXA2/TH/βIII tubulin labeling confirmed the presence of midbrain-like dopamine neurons in the graft of MF25-04 (Figure 2A). A separate FOXA2/TH labeling was performed in parallel in each of the three CM-iPSC grafts (MF25-04, MF27-04, and MF66-02) and showed more frequent FOXA2/TH colabeled (midbrain-like) dopamine neurons in the graft of MF25-04 compared with the grafts in MF27-04 and MF66-02 (Figures S2D–S2F). A punctate expression of DAT along transplanted TH-ir cell bodies and processes confirmed the formation of mature synapses in MF25-04 (Figure 2B). For an additional measure of neuronal health, the localization of

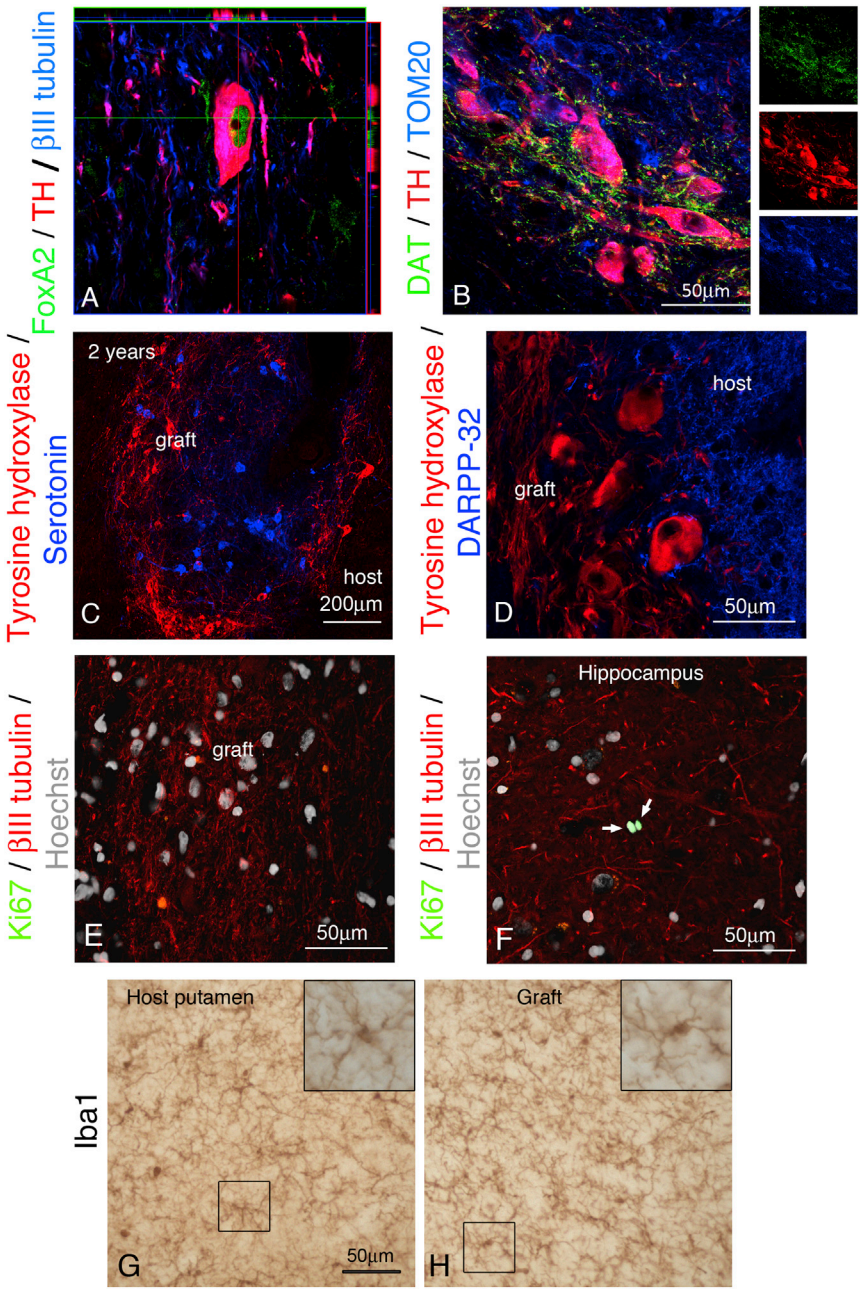

Figure 2. Phenotypes of Engrafted CM iPSC-Derived Neurons at 2 Years after Autologous Transplantation

Postmortem immunohistochemical analysis was used to further characterize the graft in MF25-04.

(A) Immunofluorescence staining confirmed that transplanted dopamine neurons were colabeled for FOXA2, TH, and βIII tubulin at 2 years after transplantation.

(B) Labeling for DAT, TH, and TOM20 (a mitochondrial outer membrane protein) showed a punctate expression of DAT along the fibers of transplanted dopamine neurons and a typical localization of mitochondria throughout the cell soma and neurites.

(C) Immunofluorescence staining for 5-HT demonstrated the presence and localization of serotonergic neurons within the graft.

(D) Labeling for DARPP-32, a marker of striatal GABAergic medium spiny neurons, shows robust labeling in the host putamen and occasional DARPP-32-ir fibers at the graft-host border.

(E and F) Ki-67 was used to determine whether proliferating cells were present in the CM-iPSC-derived neural cell graft from MF25-04. No Ki-67-immunoreactive cells were observed in the graft (E). As a positive control, several Ki-67-immunoreactive proliferating cells were observed in the hippocampus of the same animal (F) (identified with arrows).

(G and H) Histological analysis of microglia using Iba1 in the host putamen (G) and graft (H) shows typical resting microglia.

mitochondria within TH-ir neurons was assessed using the mitochondrial marker, translocase of outer mitochondrial membrane 20 (TOM20) (Hallett et al., 2014). A homogenous localization of TOM20-ir mitochondria throughout the cell soma and processes of transplanted neurons in MF25-04 was observed (Figure 2B), and there was no evidence of perinuclear accumulation or fragmentation of mitochondria, as has previously been reported during dopamine neuron stress or degeneration (Sterky et al., 2011).

Immunofluorescence labeling for 5HT (Figure 2C) was performed to examine serotonergic neurons contained in the CM-iPSC-derived transplant case, MF25-04. Serotonergic neurons were observed with a distribution and frequency similar to that previously reported for rodent and human fetal VM grafts (Mendez et al., 2008; Vinuela et al., 2008). Labeling for striatal medium spiny GABAergic neurons in the grafted putamen using DARPP-32 (Figure 2D) revealed robust DARPP-32 labeling in the host putamen perikarya and sparse DARPP-32-ir fibers at the graft-host border suggestive of some graft-host interaction as has been previously reported in fetal VM tissue (Doucet et al., 1989). Immunostaining with the proliferation marker, Ki-67, showed no positively labeled cells in the graft at 24 months after transplantation (Figures 2E and 2F). As a positive control, several Ki-67-labeled cells were observed in the dentate gyrus of the same animal (MF25-04). These data are in accord with our previous work using CM-iPSC-derived neural cell xenotransplantation in rats, where a low percentage of Ki-67-ir cells at 4 weeks after transplantation were observed, but no Ki-67-ir cells were found in mature grafts (16 weeks after transplantation) (Deleidi et al., 2011).

Histological analysis of microglial reactivity using immunohistochemistry for Iba1 revealed a local circumscribed increase in microglial cell density within the CM-iPSC-derived grafts (Figures 2G, 2H, S2H, and S2I). However, Iba1 labeling was not different to that observed in the needle tracts of the

"non-surviving" transplant group (Figure S2G), and there was no immune reaction in the host putamen surrounding the autologous iPSC grafts. A prior report indicates that compared with autologous transplantation, allogeneic iPSC-derived neural cells elicit greater microglial activation, as well as MHCII (class II major histocompatibility complex) microglial expression and infiltration of leukocytes at ~4 months after transplantation into the non-human primate brain (Morizane et al., 2013). The route of autologous transplantation, which requires no immunosuppression, may therefore be a more successful route for cell therapy than using cell sources that are not completely matched to the donor. Our work demonstrates in the successful case that dopamine synapse innervation is accompanied by the anticipated neurological improvement in parkinsonism. However, ~13,000 primate midbrain dopamine neurons were needed to reach the threshold of functional improvements. The next steps in this work require consistent protocols to provide equivalent or greater midbrain dopamine neuron survival, presumably by increasing the dose of midbrain dopamine neurons, and scale up toward potential future clinical trials.

The present findings provide the first proof of concept preclinical study to demonstrate that autologous iPSC-derived midbrain-like dopamine neurons can engraft and survive over an extended period of time (at least 2 years), and improve motor function, in a non-human primate model of PD. Such autologous iPSC-derived dopamine neurons can provide remarkable and complete reinnervation of the denervated putamen without any immunosuppression. We observed the most extensive dopaminergic axonal outgrowth reported in any study to date, including our own and others, after engraftment with iPSC-derived dopamine neurons in vivo (Emborg et al., 2013; Kikuchi et al., 2011; Sundberg et al., 2013). In addition, in the current study, we observed no graft overgrowth, tumor formation, or inflammatory reaction, which is consistent with our previous rodent studies using iPSC-derived neural cells (Hargus et al., 2010; Sundberg et al., 2013; Wernig et al., 2008).

A general concern about the use of autologous transplantation is whether underlying PD-associated genetic mutations present in transplanted neurons would increase the vulnerability of midbrain dopaminergic neurons to disease pathology. However, such vulnerabilities do not preclude cell function at a fairly optimal level for at least 50–60 years from birth in a typical patient with such genetic risk (which represents a minority of PD cases). In addition, given how transplanted fetal neurons remain healthy for many years in PD patients, despite ongoing disease processes in the host brain (Hallett et al., 2014), we would predict that even in cases with severe genetic risk, iPSC-derived neurons are a reasonable strategy.

In summary, our study clearly shows proof of concept for transplantation using iPSC-derived dopamine neurons in a preclinical context, and it is reasonable to infer that iPSC transplantation would provide clinical benefit and expected graft survival times similar to that observed with fetal transplantation (Barker et al., 2013; Hallett et al., 2014; Kefalopoulou et al., 2014). Our data showing neurologically relevant functional improvements with concomitant positive neuroimaging are essential for the continued development and clinical translation of cell therapy using iPSCs. Overall, there is a strong immunological, functional, and biological rationale for using midbrain dopamine neurons derived from iPSCs for cell replacement in PD in the future. Currently, it is essential to determine the optimal and safest dopamine neuron differentiation protocol to use, to evaluate the generation of different (non-neuronal) cell types, and to continue to refine the clinical protocols for generation of iPSC-derived midbrain dopamine neurons to be used for transplantation to PD patients.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2015.01.018.

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REFERENCES

Barker, R.A., Barrett, J., Mason, S.L., and Björklund, A. (2013). Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. Lancet Neurol. *12*, 84–91.

Brownell, A.-L., Jenkins, B.G., Elmaleh, D.R., Deacon, T.W., Spealman, R.D., and Isacson, O. (1998a). Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease. Nat. Med. *4*, 1308–1312.

Brownell, A.L., Livni, E., Galpern, W., and Isacson, O. (1998b). In vivo PET imaging in rat of dopamine terminals reveals functional neural transplants. Ann. Neurol. *43*, 387–390.

Cooper, O., Hargus, G., Deleidi, M., Blak, A., Osborn, T., Marlow, E., Lee, K., Levy, A., Perez-Torres, E., Yow, A., and Isacson, O. (2010). Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of SHH, FGF8a and specific regionalization by retinoic acid. Mol. Cell. Neurosci. *45*, 258–266.

Deleidi, M., Hargus, G., Hallett, P., Osborn, T., and Isacson, O. (2011). Development of histocompatible primate-induced pluripotent stem cells for neural transplantation. Stem Cells *29*, 1052–1063.

Doucet, G., Murata, Y., Brundin, P., Bosler, O., Mons, N., Geffard, M., Ouimet, C.C., and Björklund, A. (1989). Host afferents into intrastriatal transplants of fetal ventral mesencephalon. Exp. Neurol. *106*, 1–19.

Duan, W.M., Widner, H., and Brundin, P. (1995). Temporal pattern of host responses against intrastriatal grafts of syngeneic, allogeneic or xenogeneic embryonic neuronal tissue in rats. Exp. Brain Res. *104*, 227–242.

Emborg, M.E., Liu, Y., Xi, J., Zhang, X., Yin, Y., Lu, J., Joers, V., Swanson, C., Holden, J.E., and Zhang, S.C. (2013). Induced pluripotent stem cell-derived neural cells survive and mature in the nonhuman primate brain. Cell Rep. *3*, 646–650.

Grealish, S., Jönsson, M.E., Li, M., Kirik, D., Björklund, A., and Thompson, L.H. (2010). The A9 dopamine neuron component in grafts of ventral mesencephalon is an important determinant for recovery of motor function in a rat model of Parkinson's disease. Brain *133*, 482–495.

Grealish, S., Diguet, E., Kirkeby, A., Mattsson, B., Heuer, A., Bramoulle, Y., Van Camp, N., Perrier, A.L., Hantraye, P., Björklund, A., and Parmar, M. (2014). Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. Cell Stem Cell *15*, 653–665.

Hallett, P.J., Cooper, O., Sadi, D., Robertson, H., Mendez, I., and Isacson, O. (2014). Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. Cell Rep. *7*, 1755–1761.

Hantraye, P., Brownell, A.L., Elmaleh, D., Spealman, R.D., Wüllner, U., Brownell, G.L., Madras, B.K., and Isacson, O. (1992). Dopamine fiber detection by [11C]-CFT and PET in a primate model of parkinsonism. Neuroreport *3*, 265–268.

Hargus, G., Cooper, O., Deleidi, M., Levy, A., Lee, K., Marlow, E., Yow, A., Soldner, F., Hockemeyer, D., Hallett, P.J., et al. (2010). Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc. Natl. Acad. Sci. USA *107*, 15921–15926.

Jenkins, B.G., Sanchez-Pernaute, R., Brownell, A.L., Chen, Y.C., and Isacson, O. (2004). Mapping dopamine function in primates using pharmacologic magnetic resonance imaging. J. Neurosci. *24*, 9553–9560.

Kefalopoulou, Z., Politis, M., Piccini, P., Mencacci, N., Bhatia, K., Jahanshahi, M., Widner, H., Rehncrona, S., Brundin, P., Bjorklund, A., et al. (2014). Long-term clinical outcome of fetal cell transplantation for Parkinson disease: two case reports. JAMA Neurol. *71*, 83–87.

Kikuchi, T., Morizane, A., Doi, D., Onoe, H., Hayashi, T., Kawasaki, T., Saiki, H., Miyamoto, S., and Takahashi, J. (2011). Survival of human induced pluripotent stem cell-derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease. J. Parkinsons Dis. *1*, 395–412.

Mendez, I., Sanchez-Pernaute, R., Cooper, O., Viñuela, A., Ferrari, D., Björklund, L., Dagher, A., and Isacson, O. (2005). Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. Brain *128*, 1498–1510.

Mendez, I., Viñuela, A., Astradsson, A., Mukhida, K., Hallett, P., Robertson, H., Tierney, T., Holness, R., Dagher, A., Trojanowski, J.Q., and Isacson, O. (2008). Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years. Nat. Med. *14*, 507–509.

Morizane, A., Doi, D., Kikuchi, T., Okita, K., Hotta, A., Kawasaki, T., Hayashi, T., Onoe, H., Shiina, T., Yamanaka, S., and Takahashi, J. (2013). Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a non-human primate. Stem Cell Reports *1*, 283–292.

Politis, M., Wu, K., Loane, C., Quinn, N.P., Brooks, D.J., Rehncrona, S., Bjorklund, A., Lindvall, O., and Piccini, P. (2010). Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. Sci. Transl. Med. *2*, 38ra46.

Redmond, D.E., Jr., Vinuela, A., Kordower, J.H., and Isacson, O. (2008). Influence of cell preparation and target location on the behavioral recovery after striatal transplantation of fetal dopaminergic neurons in a primate model of Parkinson's disease. Neurobiol. Dis. *29*, 103–116.

Sánchez-Pernaute, R., Studer, L., Ferrari, D., Perrier, A., Lee, H., Viñuela, A., and Isacson, O. (2005). Long-term survival of dopamine neurons derived from parthenogenetic primate embryonic stem cells (cyno-1) after transplantation. Stem Cells *23*, 914–922.

Sterky, F.H., Lee, S., Wibom, R., Olson, L., and Larsson, N.G. (2011). Impaired mitochondrial transport and Parkin-independent degeneration of respiratory chain-deficient dopamine neurons in vivo. Proc. Natl. Acad. Sci. USA *108*, 12937–12942.

Sundberg, M., Bogetofte, H., Lawson, T., Jansson, J., Smith, G., Astradsson, A., Moore, M., Osborn, T., Cooper, O., Spealman, R., et al. (2013). Improved cell therapy protocols for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC-, and non-human primate iPSC-derived dopaminergic neurons. Stem Cells *31*, 1548–1562.

Tsui, A., and Isacson, O. (2011). Functions of the nigrostriatal dopaminergic synapse and the use of neurotransplantation in Parkinson's disease. J. Neurol. *258*, 1393–1405.

Vinuela, A., Hallett, P.J., Reske-Nielsen, C., Patterson, M., Sotnikova, T.D., Caron, M.G., Gainetdinov, R.R., and Isacson, O. (2008). Implanted reuptake-deficient or wild-type dopaminergic neurons improve ON L-dopa dyskinesias without OFF-dyskinesias in a rat model of Parkinson's disease. Brain *131*, 3361–3379.

Wernig, M., Zhao, J.P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Isacson, O., and Jaenisch, R. (2008). Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc. Natl. Acad. Sci. USA *105*, 5856–5861.

Wüllner, U., Pakzaban, P., Brownell, A.L., Hantraye, P., Burns, L., Shoup, T., Elmaleh, D., Petto, A.J., Spealman, R.D., Brownell, G.L., et al. (1994). Dopamine terminal loss and onset of motor symptoms in MPTP-treated monkeys: a positron emission tomography study with 11C-CFT. Exp. Neurol. *126*, 305–309.