Cell-based therapies for Parkinson disease —past insights and future potential

Roger A. Barker, Janelle Drouin-Ouellet and Malin Parmar

Abstract | Parkinson disease (PD) is characterized by loss of the A9 nigral neurons that provide dopaminergic innervation to the striatum. This discovery led to the successful instigation of dopaminergic drug treatments in the 1960s, although these drugs were soon recognized to lose some of their efficacy and generate their own adverse effects over time. Despite the fact that PD is now known to have extensive non-nigral pathology with a wide range of clinical features, dopaminergic drug therapies are still the mainstay of therapy, and work well for many years. Given the success of pharmacological dopamine replacement, pursuit of cell-based dopamine replacement strategies seemed to be the next logical step, and studies were initiated over 30 years ago to explore the possibility of dopaminergic cell transplantation. In this Review, we outline the history of this therapeutic approach to PD and highlight the lessons that we have learned *en route*. We discuss how the best clinical outcomes have been obtained with fetal ventral mesencephalic allografts, while acknowledging inconsistencies in the results owing to problems in trial design, patient selection, tissue preparation, and immunotherapy used post-grafting. We conclude by discussing the challenges of bringing the new generation of stem cell-derived dopamine cells to the clinic.

Barker, R. A. *et al. Nat. Rev. Neurol.* advance online publication 4 August 2015; doi:10.1038/nrneurol.2015.123

Introduction

Parkinson disease (PD) is a common neurodegenerative disorder of the CNS, the core pathology of which includes loss of dopaminergic nigral neurons and formation of α-synuclein-containing Lewy bodies.[1,2] Although the clinicopathological spectrum of PD is now recognized to be much broader, with many nonmotor features and extensive extranigral pathology,[3] the disease nevertheless responds well to dopaminergic agents in the early stages. Over time, however, these medications start to fail and produce their own adverse effects, such as dyskinesias and neuropsychiatric complications. These effects are attributable to the non-physiological delivery of dopamine, as well as to the off-target effects that will occur with any oral dopaminergic therapy.[4,5] Consequently, a need exists for a better, more physiological focal delivery system for dopamine. One such approach involves replacing the lost dopaminergic cells through neural grafting.

In this Review, we detail the origins of the dopaminergic cell transplantation approach for treating PD, focusing on the early preclinical work demonstrating the feasibility of such a strategy, before discussing the various clinical approaches that have been adopted over the years. This latter discussion highlights the importance of being able to critically appraise the strength of the preclinical evidence before trialling the treatment in patients, as many failed trials had limited data from the laboratory to support clinical adoption. The field of regenerative medicine should learn from these experiences, thereby avoiding some of the mistakes of the past as we enter a new era of stem cell-derived dopaminergic neuron transplants for PD.

1970s—the origins of neural grafting

The very first experiments involving transplantation of cells to the brain took place as long ago as 1890,[6] but the experiments that heralded the modern era of neural grafting for PD began in the 1970s in Sweden. Prompted by the emergence of new fluorescent staining techniques, this work was initially undertaken to study the development of catecholaminergic systems. The first experiments involved grafting of tissue into the immunologically privileged—and easily accessible—anterior chamber of the rat eye. This work by Olson and colleagues revealed an optimal gestational age for fetal ventral mesencephalic (fVM) dopaminergic cell survival and outgrowth, with evidence that the latter could be enhanced by co-grafting of suitable tissues and factors.[7–10] Although this work was invaluable, it was unable to address questions of brain repair and functionality.

Exploration of these questions became possible in the late 1970s following the development of the 6-hydroxydopamine (OHDA)-lesioned rat model of PD, which enabled selective and irreversible lesioning of the nigrostriatal pathway.[11–13] Although this model does not fully recapitulate PD, it is, nevertheless, extremely valuable in experiments that seek to investigate restoration of dopaminergic tone in the lesioned nigrostriatal pathway, and is a useful model by which to examine this aspect of PD.

John van Geest Centre for Brain Repair & Department of Neurology, Department of Clinical Neurosciences, University of Cambridge, Forvie Site, Cambridge CB2 0PY, UK (**R.A.B.**). Wallenberg Neuroscience Center, Division of Neurobiology and Lund Stem Cell Center, Lund University, BMC A11, S-221 84 Lund, Sweden (**J.D.-O.**, **M.P.**)

Correspondence to:
R.A.B.
rab46@cam.ac.uk

Competing interests
The authors declare no competing interests.

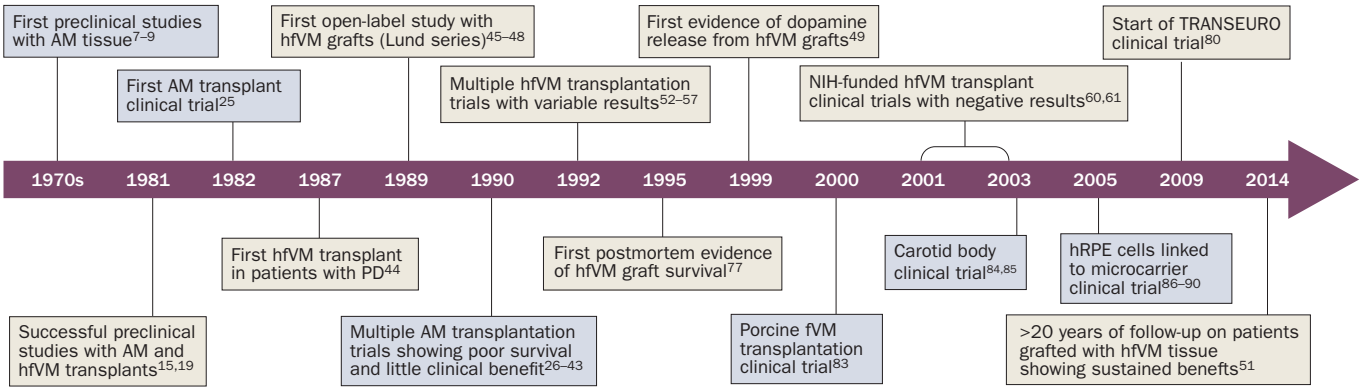

Figure 1 | Timeline of cell-based therapies for use in patients with PD. The key preclinical and clinical studies are highlighted. Trials of human fetal tissue transplants are shown in yellow boxes, and trials involving cells from other sources are shown in blue boxes. Abbreviations: AM, adrenal medullary; fVM, fetal ventral mesencephalic; GIDs, graft-induced dyskinesias; hfVM, human fVM; hRPE, human retinal pigmentary epithelial; PD, Parkinson disease.

The extent of the 6-OHDA lesion could easily be quantified according to the extent of rotation induced by drugs such as low-dose apomorphine or D-amphetamine,[14] and this approach was used to assess the functional efficacy of dopaminergic cell sources grafted into the dopamine-denervated striatum. These experiments were originally done using adrenal medullary[15] or fVM tissue, which was implanted as a solid graft either into the adjacent lateral ventricle or into preformed cavities within the striatum,[16–21] as techniques for making cell suspension grafts[22] had not yet been developed.

By the early 1980s, cell suspensions made from fVM tissue had been shown to survive, innervate the host striatum, receive afferent fibres, release dopamine and restore deficits in this model system (reviewed elsewhere[23]). The same could not be said for adrenal medullary tissue grafts, in which the number of surviving cells was low, with minimal evidence of fibre outgrowth and dopamine release[10] and only modest effects on drug-induced rotation.

On the basis of these preclinical data, one would predict that fVM transplants would fare better than adrenal medullary grafts in clinical trials. This prediction proved to be correct (see below), thereby reinforcing the point that dopaminergic cell grafts that cannot robustly survive and totally reverse drug-induced rotational behaviour in the 6-OHDA-lesioned rodent will not be successful in the clinical arena. This concept has subsequently been supported by experiments with many other cell types, including the Spheramine cell trials.[24]

Key points

- Dopaminergic drugs were established as an effective treatment for Parkinson disease (PD) in the 1960s, and are still the mainstay of therapy for this condition
- Experiments that heralded the modern era of neural grafting for PD began in the 1970s in Sweden
- Despite limited preclinical data, adrenal medullary transplantation was adopted by many groups during the 1980s, with largely disappointing results
- Human fetal ventral mesencephalic (fVM) allografts have been shown to survive and function for over 20 years in some patients
- The protocol for neural transplantation in patients with PD remains to be optimized
- Human fVM grafts are currently being revisited, and stem cell-based dopamine replacement therapies are close to clinical trials

1980s—adrenal medullary transplants

In 1982, two patients with PD in Lund, Sweden were grafted with adrenal medullary tissue placed into the caudate. This study was followed a few years later by putaminal grafting of adrenal medullary tissue in two further patients (Figures 1 and 2).[25] The rationale for this transplant approach was that the adrenal medulla produces catecholamines, including dopamine (albeit at very low levels). Therefore, any such transplant should increase the local concentration of dopamine, which could account for the preclinical efficacy of this approach (see above).[26] In these early grafted patients, however, the transplants had no major clinical benefits.

In 1987, the field changed dramatically when Madrazo *et al.* published a high-profile paper in the *New England Journal of Medicine*.[27] This study showed that solid grafts of adrenal medullary tissue placed into the head of the caudate, using an open neurosurgical approach, had major benefits in two patients with PD. Coupled to a positive editorial in the same issue of the journal,[28] this paper encouraged many groups to adopt this approach without critically appraising the original study and its conclusions.[29–38] As a result, a large number of patients were grafted, especially across the USA, even though the original preclinical data had shown only a modest effect. Nevertheless, this approach captured the imagination of the clinical community in an age before deep brain stimulation (DBS) was even discovered, and it was only when a registry was set up to gather together all the data, along with postmortem studies, that real concerns about its efficacy and safety began to emerge.[33,39–43] This analysis revealed not only that patients did not benefit to a significant and sustained extent, but also that many of the grafted patients had complications from the surgery, including postoperative psychiatric disturbances.

These findings, coupled to poor graft survival uncovered at postmortem (Table 1) eventually led to the

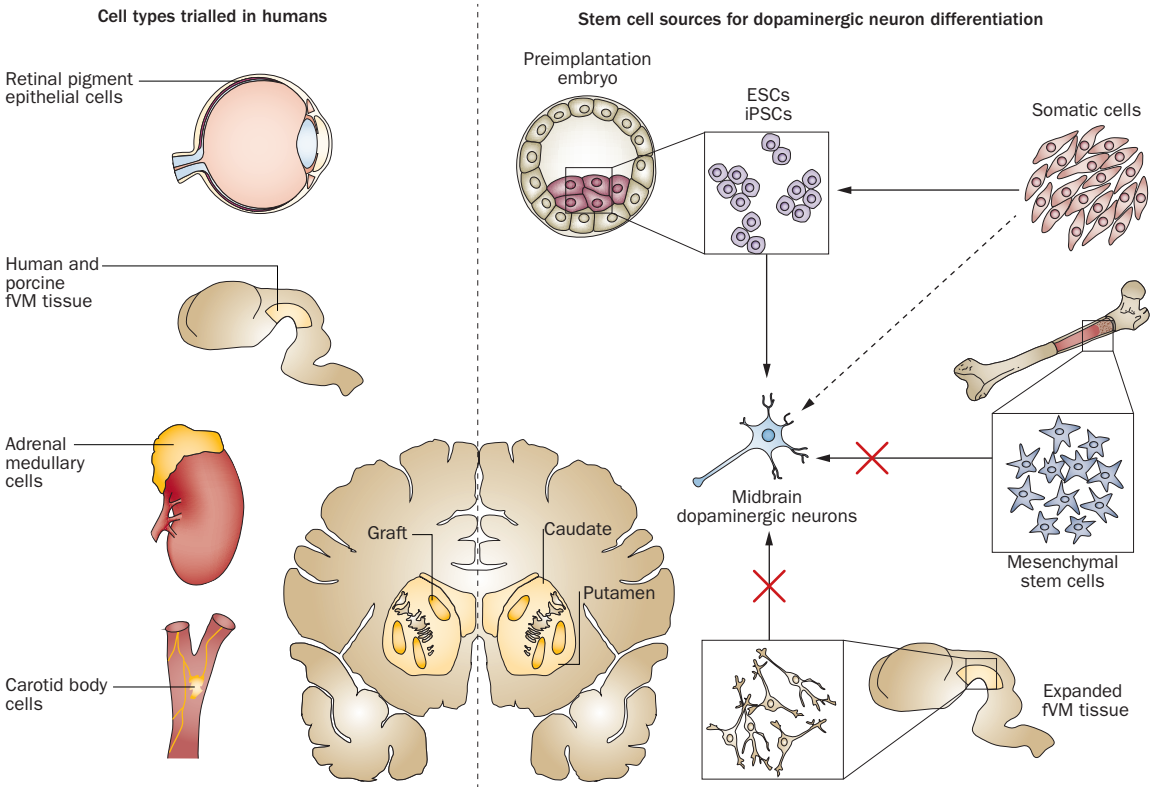

Figure 2 | Cells under consideration for use for grafting in PD. The left-hand side of the figure shows cell types that have been trialled in patients with PD, and the right-hand side shows sources of stem cells that have been used to generate midbrain dopaminergic neurons. Differentiated mesenchymal stem cells and expanded fVM tissue have not yet provided midbrain dopaminergic neurons, but this goal has been achieved with both ESCs and iPSCs. Direct reprogramming of fetal and adult somatic cells (dashed arrow) is also currently being explored. See also Box 1. Abbreviations: ESCs, embryonic stem cells; fVM, fetal ventral mesencephalic; iPSCs, induced pluripotent stem cells.

abandonment of this transplant approach, but not before many patients had been subjected to a therapy that was supported by very limited preclinical data.

1990s—the rise and fall of fVM grafts

In contrast to adrenal medullary grafts, preclinical reports of fVM transplantation were largely positive. Thus, conclusions from adrenal medullary tissue grafting in patients with PD cannot necessarily be applied to fVM tissue.

In 1987, the first fVM transplants in patients with PD were undertaken in Lund. The first two patients showed no improvement,[44] but the next two did improve, both clinically and on ^{18}F-dopa PET imaging.[45] Between these two pairs of operations, modifications were made to the amount of tissue grafted, the age of fetal tissue harvested, and the mode of delivery of the tissue, all of which probably accounted for the marked differences in clinical response.

These promising results paved the way for another 13 patients to be grafted in Lund over the 1990s in an iterative open-label fashion (Table 2).[46–48] These patients all received human fVM tissue prepared from between three and six fetuses (per side of the brain grafted), which was of gestational age 6–8 weeks, and was delivered either to the putamen or to both the caudate and putamen using an instrument specially designed by the neurosurgeon Stig Rehncrona. Immunosuppression consisting of cyclosporin A, azathioprine and steroids was given for at least 12 months after grafting. The results were variable, but overall the patients improved following transplantation. In the best cases, patients were able to come off their anti-PD medications altogether, and ^{18}F-dopa scanning provided evidence of restoration of normal dopamine signalling in the grafted striatum. In addition, these patients were shown to have grafts that released dopamine in a physiological fashion, with reactivation of the relevant cortical motor areas.[49,50] These patients have continued to be monitored, and in some instances the benefits of these grafts are still evident over 20 years later.[51]

This open-label study in Lund (which also involved patients from other European centres, such as London and Marburg) led to a number of other similar studies being undertaken at sites in Europe, the USA and Canada, with variable results.[52–55] In some case series, the results were modest, possibly owing to the age and amount of fVM tissue used, whereas others produced more-striking outcomes.[56,57]

In the USA, a number of similar studies were also producing encouraging results, despite a lack of federal funding support. In 1993, the newly elected President Bill Clinton allowed such funds to be made available, leading to two NIH-funded studies. In each study, the trial design was such that some patients would be grafted with

Table 1 | Cells other than human fVM tissue that have been trialled in Parkinson disease

Reference(s)	Type of trial	Method	Number of patients	General outcome	Number of surviving TH cells per side of brain at postmortem
Autologous adrenal medullary tissue					
Backlund *et al.* (1985)[25]	Phase I open-label	Unilateral, stereotaxic into caudate	2	Short-term improvement (days)	Not reported
Lindvall *et al.* (1987)[37]	Phase I open-label	Unilateral, stereotaxic into putamen	2	Temporary improvement (weeks)	Not reported
Madrazo *et al.* (1987)[27]	Phase I open-label	Lateral ventricle with partial implantation into caudate	2	Marked improvement	Necrotic adrenal medullary tissue;[137] necrotic adrenal medullary tissue, increased TH immunoreactivity in striatum[138]
Drucker-Colin *et al.* (1988)[30]	Phase I open-label	Lateral ventricle with partial implantation into caudate	11	Long-term improvement	Not reported
Jiao *et al.* (1988)[34]	Phase I open-label	Stereotaxic into caudate	4	Long-term improvement	Not reported
Goetz *et al.* (1989, 1990)[31,32] Olanow *et al.* (1990)[139]	Phase I open-label	Stereotaxic into caudate	19	Mixed, transient improvement, maximal at 6 months	Not reported
Kelly *et al.* (1989)[36]	Phase I open-label	Stereotaxic into caudate	8	Slight and variable improvement at 6 months	Not reported
Allen *et al.* (1989)[29]	Phase I open-label	Lateral ventricle with partial implantation into caudate	18	Slight improvement in younger patients at 12 months	Not reported
Jankovic *et al.* (1989)[33]	Phase I open-label	Lateral ventricle with partial implantation into caudate	3	Modest improvement	No viable element of the implant found, significant surrounding inflammatory response
hRPE cells (Spheramine)					
Watts *et al.* (2003)[89] Bakay *et al.* (2004)[86] Stover *et al.* (2005)[87]	Phase I open-label	Stereotaxic unilateral into putamen	6	Long-term improvement (41% UPDRS-motor score after 48 months)	Not reported
Gross *et al.* (2011)[90]	Phase II, randomized, double-blind with sham surgery	Stereotaxic bilateral into putamen	35 grafted with Spheramine, 36 received sham surgery	No treatment effect compared with sham-operated group (primary end point)	Very low hRPE cell count in graft, significant surrounding inflammatory response
Autologous carotid body cells					
Arjona *et al.* (2003)[84]	Phase I open-label	Stereotaxic bilateral into striatum	6*	Slight improvement, maximal at 6 months	Not reported
Minguez-Castellanos *et al.* (2007)[85]	Phase I–II, blinded	Stereotaxic bilateral into striatum	13*	Variable and modest improvement at 1 year	Not reported
Embryonic porcine ventral mesencephalic tissue					
Schumacher *et al.* (2000)[83]	Phase I open-label	Stereotaxic unilateral into striatum	12	Variable and modest improvement at 1 year	Very few porcine TH-positive cells found in a single case. Some surrounding inflammatory response

*These studies shared some of the same patients. Abbreviations: fVM, fetal ventral mesencephalic; hRPE, human retinal pigmentary epithelial; TH, tyrosine hydroxylase; UPDRS, Unified Parkinson's Disease Rating Scale.

fVM tissue while others would receive sham or imitation surgery with partial burr holes and no tissue engrafted. These double-blind, controlled trials were thought necessary to address whether the grafts were really efficacious or simply eliciting some sort of placebo effect, as had been seen previously with adrenal medullary transplants. Although this approach was laudable, some commentators at the time argued that such trials were premature given that the transplantation techniques had not been optimized.[58] The trials proceeded at the same time as DBS entered the clinic for the first time,[59] leading to comparisons of the relevant efficacy of these two approaches in the years that followed.

These two human fVM transplant trials (Table 2) enrolled patients with moderately advanced PD, who were grafted with varying amounts of human fVM tissue and exposed to differing degrees of post-transplantation immunosuppression.[60,61] In the first study, which used previously untested procedures, patients were grafted with relatively small amounts of fVM tissue delivered as a 'noodle' using a new transfrontal approach, and no immunosuppression was given post-grafting. Patients in the control arm were offered transplants after the primary end point of 12 months; thus, although 20 grafted patients were initially compared against 20 non-grafted patients, the blinding was lost after 1 year

Table 2 | The two NIH studies and the Lund open-label study using human fVM tissue to treat PD

Study feature	Freed *et al.* (2001)[60]	Olanow *et al.* (2003)[61]	Lund study[44,45,47–51,55]
Number of patients grafted	20 (plus 13 of the sham-grafted patients after 1 year of follow-up)	23 (11 with one fVM graft per side and 12 with four fVM grafts per side)	18 undertaken in four series of patients (including three patients with MPTP parkinsonism, one of which is not published)
Numbers with sham surgery	20 (reduced to seven after 1 year of follow-up)	11	None
Average age of cohort (years)	57 (range 34–75)	58.5 (range 30–75)	47.5 (range 37–68); the youngest is the unpublished third MPTP case operated in 1994
Disease duration (mean in years)	14	11	10.5
Number of fVM implants per side, and tissue age	Two: grafted as 'noodles' (7–8 weeks post-conception), stored for up to 28 days prior to grafting	One or four, solid pieces (6–9 weeks post-conception), stored up to 2 days at 8°C prior to grafting	Three to six implants per side as cell suspensions
Neurosurgical approach	Transfrontal, two tracts per side	Standard approach; eight needle tracts per side	Standard approach; increasing from three implants per side to eight
Immunotherapy given	None	Cyclosporin A only for 6 months	Triple immunotherapy for 12 months after last surgery—longest time anyone was on this therapy was 4 years
Primary end point	Subjective after 1 year No difference between groups (P=0.62)	UPDRS score in defined 'off' period at 2 years No difference when compared across all three groups (P=0.24)	No primary end point (iterative open-label study)
Change in UPDRS at time of primary end point in defined 'off' time	<60 years old: 59 to 40 >60 years old: 59 to 60	One implant: 48 to 51.5 Four implants: 49 to 48 Placebo: 51.5 to 61	For 10 patients with bilateral grafts at 10–24 months: 41 to 29
Proportion with graft-induced dyskinesias	15%	56.5%	100% (but only significant in three cases) Six of 14 patients had dyskinesias prior to surgery, which persisted after surgery; two of these individuals developed severe dyskinesias and one required deep brain stimulation Two MPTP cases had a marked reduction in levodopa-induced dyskinesias
Number of surviving tyrosine hydroxylase-positive cells per side of brain at postmortem	n=2 31,254 and 21,818	n=2 One fVM graft: 30,000 per side Four fVM grafts: 70,000–120,000 per side	n=4 Four to five fVM grafts per side: 12,100–29,500 Patient 4 is still being analysed
Subsequent follow-up	At 3 years, 19 patients in the original transplant group improved by 28% on their defined 'off' UPDRS score	None	Survivors still being followed up (six patients followed up for >18 years after grafting). Three off anti-PD medication, two on very small amounts, one MPTP case off all anti-PD medication

Abbreviations: fVM, fetal ventral mesencephalic; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson disease; UPDRS, Unified Parkinson's Disease Rating Scale.

as 13 of the sham-operated patients went on to receive an fVM graft.

In this trial, the results of which were published in 2001,[60] the patients in the transplantation group did not report feeling significantly better at 1 year—the primary end point. In addition, adverse effects, in the form of graft-induced dyskinesias (GIDs), were seen in 15% of those patients who were eventually grafted. The development of GIDs was discovered when participants, all of whom had levodopa-induced dyskinesias pre-grafting, continued to exhibit these involuntary movements in the absence of any dopaminergic medication. The GIDs were so severe in some cases that DBS was needed to ameliorate them.[62–64] Such problems had not previously been reported in the open-label studies, but further analysis revealed their presence in some patients.[65] The reason why some individuals developed GIDs was unknown, but one early theory implicated the non-homogeneous distribution of dopaminergic cells across the striatal complex, giving rise to hot spots of innervation.[66]

In the second NIH-funded trial, the results of which were reported 2 years later in 2003,[61] the design was such that patients received imitation surgery only, or a graft with tissue derived from either one or four fVMs per side of the brain. The primary end point was a change in the

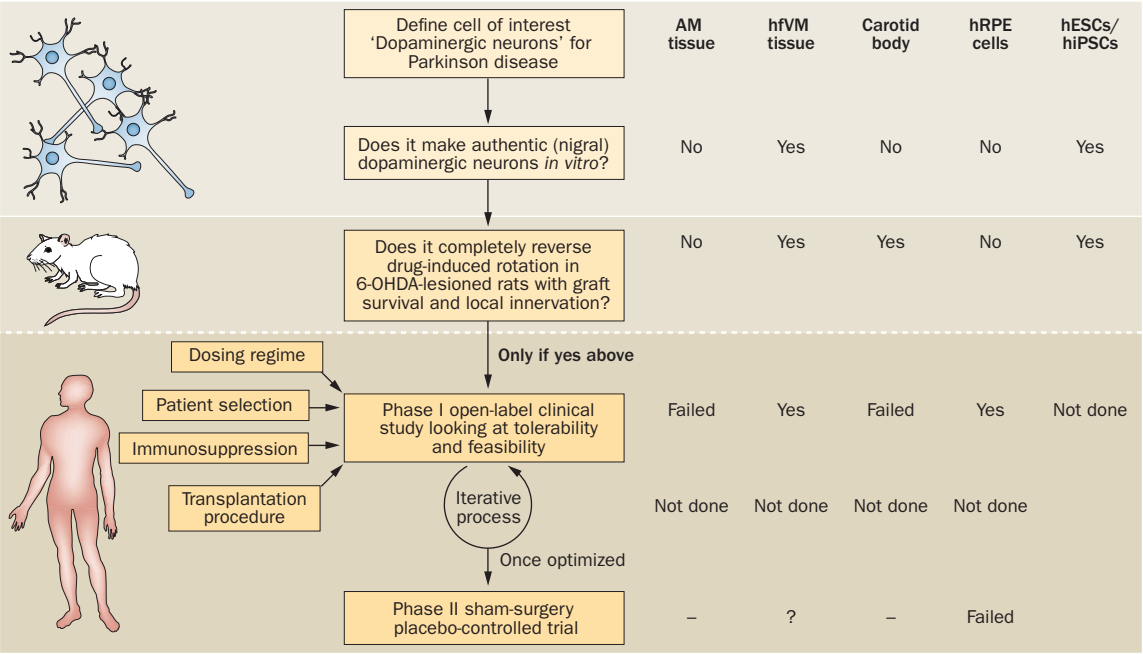

Figure 3 | Processes followed and outcomes recorded when taking different 'dopaminergic' cell sources from the laboratory to clinical trials. Five sources have been studied, but only two—fVM tissue and hESCs/iPSCs—have proven to provide authentic midbrain dopaminergic neurons. Although three sources have produced reversal of behavioural deficits in the 6-OHDA rat model, only fVM tissue and RPE cells showed clinical benefits in phase I open-label trials. Optimal procedures have not been identified for any of these sources of cells, but such an optimization process is ongoing for fVM tissue in the TRANSEURO trial. Abbreviations: 6-OHDA, 6-hydroxydopamine; AM, adrenal medullary; hESCs, human embryonic stem cells; hfVM, human fetal ventral mesencephalic; hiPSCs, human induced pluripotent stem cells; hRPE, human retinal pigmentary epithelial.

defined Unified Parkinson's Disease Rating Scale (UPDRS) score in the 'off' state 2 years post-grafting. Cyclosporin A was given, but only for 6 months post-transplantation. Again, no significant benefit was observed when all three groups were compared, although there was a trend towards improvement in patients who received transplants derived from four fVMs, and ^{18}F-dopa PET revealed a significant effect on dopamine levels in both transplantation groups. In addition, 56.5% of the transplanted patients developed GIDs, which in some cases were sufficiently severe to necessitate further neurosurgery. Why so many patients developed such a complication remained unclear, but one explanation—as an alternative to the 'hot spot' theory outlined above—may relate to co-grafted serotonergic neurons releasing dopamine in an unregulated fashion.[67–69] This latter theory has gained favour through preclinical work in animal models of GIDs,[70,71] by looking at postmortem transplants,[72] and from an acute interventional study in which sarozitan was used to inhibit serotonergic neurons.[68]

Interestingly in this second trial, although the primary end point failed to reach significance, patients with less-advanced disease did benefit significantly from transplantation. Furthermore the slope of the graft effects was suggested to have changed when the immunosuppression was discontinued after 6 months, leading some to conclude that a partial rejection response occurred at this time point, thereby compromising the long-term efficacy of the graft.

These two trials[60,61] reached the same conclusion, namely, that human fVM transplants did not provide significant improvements in patients with PD, especially when compared with newer therapies for PD such as DBS, and produced unacceptable adverse effects including GIDs.[73] However, it was also clear from these trials that some patients did do well, as had been seen in the open-label studies, and that longer-term follow-up of some of the patients gave a more encouraging signal of efficacy.[74] Nevertheless, the consensus at the time of publication of the second trial was that this approach should not be pursued.

In Europe, this conclusion was felt to be somewhat premature given some of the shortcomings in the trials, all of which reflected the fact that the reparative approach had not yet been optimized. As a result, a working group was set up to re-analyse the available clinical data on human fVM transplantation to see what conclusions could be drawn about this whole approach, and in particular to attempt to identify why some patients had significantly benefited from this therapy. Although the analysis was limited by restricted data availability,[75] several factors emerged that were associated with positive outcomes. These factors included younger age with less-advanced disease clinically; preserved ventral striatal dopaminergic innervation on ^{18}F-dopa PET;[76] no significant disabling levodopa-induced dyskinesias pre-grafting; receipt of fVM tissue from three to four fetuses per side, yielding grafts with 100,000 or more dopaminergic nigral

Box 1 | Stem cell sources being considered for cell grafting in PD

Embryonic stem cells
Pluripotent stem cells derived from the inner cell mass of early-stage preimplantation embryos that provide an unlimited supply of cells. These cells have been shown to differentiate into midbrain dopaminergic neurons[121,122] and to provide similar efficacy to fVM transplants in preclinical studies.[123]

iPSCs
Pluripotent stem cells reprogrammed from adult somatic cells, such as skin fibroblasts, by defined factors. This source of cell allows autologous grafting and provides an unlimited supply of cells. Long-term survival and function of autologous iPSC-derived midbrain-like dopaminergic neurons has recently been reported in nonhuman primates.[140]

Mesenchymal stem cells
Multipotent cells derived from the bone marrow that can differentiate into various cells of the mesodermal lineage, but also have the capacity to differentiate into epithelial, endothelial and neuronal cells. In preclinical studies, they have been shown to differentiate into tyrosine hydroxylase-expressing cells,[142] but their capacity to make true midbrain dopaminergic neurons is unproven. Thus, although benefits have been reported in animal models of PD,[141,142] the quality of the response is insufficient to allow these cells to go to proper clinical trials.

Expanded neural precursor cells
Precursor cells from the fVM expanded in culture that can generate midbrain dopaminergic neurons and provide behavioural recovery on grafting in animal models of PD.[143] To date, they have been found to have limited proliferative potential, so the midbrain dopaminergic neuronal yield from such a source is insufficient to allow clinical trials to be considered.

Induced neurons
Neurons obtained by direct reprogramming of somatic cells by defined factors. Dopaminergic neurons have been reprogrammed from fibroblasts,[144,145] but reprogramming of true midbrain dopaminergic neurons has yet to be achieved. This source would allow autologous grafting, as well as greatly reducing graft overgrowth and/or tumour formation risks associated with grafts from stem cell sources.

Abbreviations: fVM, fetal ventral mesencephalic; iPSCs, induced pluripotent stem cells; PD, Parkinson disease.

neurons;[77–79] receipt of adequate immunotherapy; and a grafting technique that allowed the transplanted dopaminergic cells to homogenously innervate the striatum into which they were placed. On the basis of these findings, a new human fVM trial in PD, known as TRANSEURO,[80] was planned (see below).

1990s onwards—other cell sources

From the very beginning, while the clinical work with human fVM tissue was being undertaken, other cell sources were being investigated and taken to clinical trials (Figure 2 and Table 1) owing to issues of tissue availability and the ethical problems inherent in using fetal tissue. These alternative approaches included xenografts of porcine ventral mesencephalic tissue,[81–83] autografted carotid body cells,[84,85] and retinal pigmentary epithelial (RPE) cells linked to specific microcarriers (Spheramine).[86–89] In all cases, the preclinical data did not demonstrate that the transplanted cells had a reproducible, significant effect that was at least comparable to the effects previously reported with allografted fVM tissue (Figure 3). Thus, it is not surprising that when the clinical trials were undertaken with these cells, they all produced negative results.[90] Therefore, like the results with adrenal medullary grafts, these trials should not be equated and amalgamated with those conducted with human fVM tissue.

A number of other approaches have been pursued to find a better source of cells for grafting that in some ways recapitulates the advantages of using of fVM tissue. One promising strategy involves the short-term expansion of fVM tissue and the dopaminergic neuroblasts within it,[91] although this approach is still hampered by the problems of using human fetuses, as well as issues of good manufacturing practice (GMP). An alternative source that has gained prominence over the years is stem cells (Box 1), given that their use would avoid issues of tissue availability and, depending on the source of the cells, be less ethically contentious.[92] Learning from the clinical trials using different types of dopamine-producing cells, one would predict that to achieve a successful clinical outcome, these cells must have the potential to be made into authentic mesencephalic dopaminergic neurons of the A9 phenotype, which can be found in the pars compacta of the substantia nigra. A number of different stem cell sources have been pursued (Figure 2), but human embryonic stem cells (hESCs) have shown the most promise to date.[93]

With the establishment of hESCs in 1998,[94] new possibilities emerged for obtaining an unlimited source of any cell type in the body (Figure 4). Soon after the first hESC lines were generated,[94,95] protocols for generating neurons via spontaneous differentiation and embryoid body formation were rapidly established.[96–98] Neurons that synthesized glutamate or γ-aminobutyric acid were present in these cultures, but few if any neurons expressing tyrosine hydroxylase (TH)—the rate limiting enzyme for dopamine synthesis—could be detected.[97,98] What became evident from these early studies was that *in vitro* differentiation of neural progenitors from hESCs seemed to recapitulate spatial and temporal aspects of early brain development, and that analogies to mouse ESCs could be drawn.

The initial strategies for the generation of dopaminergic neurons from hESCs were based on developmental principles and experience with mouse ESCs.[99,100] A number of protocols were developed in which hESC-derived neural progenitor cells were patterned in co-culture with murine stromal cell lines such as PA6 and MS5,[101–105] co-cultured with astrocytes,[106] or cultured with fibroblast growth factor 8 and sonic hedgehog.[107–109] These protocols all gave rise to TH-expressing dopaminergic neurons, albeit in varying numbers, thereby providing important evidence that hESCs could be patterned into dopaminergic neurons using developmental cues and/or feeder cells. Some of these early hESC differentiation protocols produced relatively high numbers of TH-positive neurons that were capable of releasing dopamine, but none of them generated cells co-expressing two transcription factors required for proper midbrain dopaminergic neuron specification, namely, FOXA2 and LMX1A. This finding could help to explain why these grafts showed only modest, if any, effects in transplantation models. Furthermore, the incomplete and non-synchronized differentiation of the cells led to tumour formation *in vivo* in some cases.[102,104,106] Nevertheless, these early studies provided important

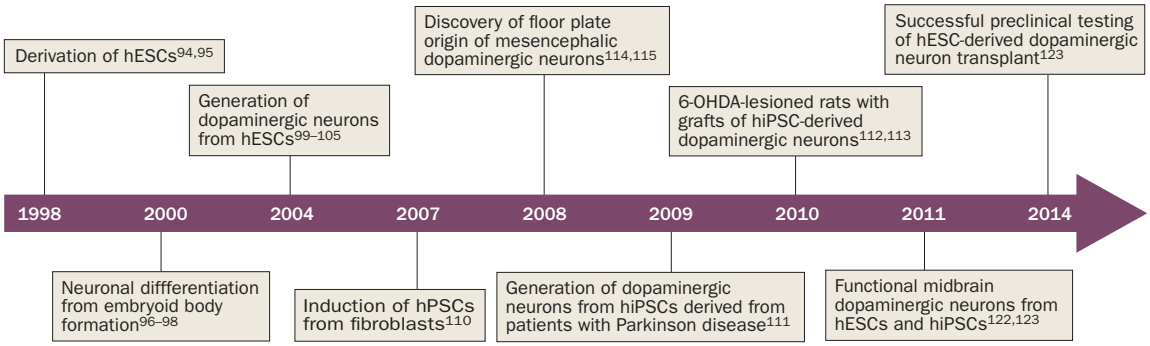

Figure 4 | Timeline of stem cell discoveries and their application to Parkinson disease. Abbreviations: 6-OHDA, 6-hydroxydopamine; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; hPSCs, human pluripotent stem cells.

proof-of-principle data that hESCs can be patterned using region-specific developmental cues, leading to the production of dopamine-producing neurons that can survive transplantation into the adult rodent brain.

In 2006, the demonstration that pluripotency can be induced in human fibroblasts sparked a major revolution in the field.[110] Much hope was placed on these induced pluripotent stem cells (iPSCs) as a source of patient-specific and disease-specific neurons, especially as, in theory, this approach would avoid many of the ethical issues associated with making hESC lines.[92] Indeed, it soon became clear that iPSCs responded very similarly to hESCs in terms of developmental patterning cues, and that they could, therefore, be differentiated into dopaminergic neurons using similar protocols.[111] However, like the hESC-derived dopaminergic neurons, the midbrain properties of the cells were unclear, and their *in vivo* performance in standard animal models of PD was modest.[112,113]

Around the same time, an unexpected discovery was made, that dopaminergic neurons have a cellular origin that differs from all other neurons in the brain. Two reports clearly demonstrated that the mesencephalic dopaminergic neurons are derived from floor plate cells and not from neuroepithelial progenitors.[114,115] The floor plate consists of a group of cells located in the ventral midline of the neural tube, and was traditionally considered to be non-neurogenic.[116] This new insight into the unique cellular origin of mesencephalic dopaminergic neurons, combined with more-precise molecular insight into their differentiation[117] and better neuralization strategies,[118] led to the development of a new generation of differentiation protocols.[119]

In light of this revised understanding of the developmental origin of mesencephalic dopaminergic neurons,[114,115] new protocols were developed that were based on a floor plate intermediate,[120] combined with patterning approaches that employed extrinsic developmental cues in a dose-dependent manner similar to that which had been used in earlier protocols.[106,107] This refined approach for differentiation and patterning enabled authentic and functional midbrain dopaminergic neurons to be obtained from both hESCs and human iPSCs.[121,122] The initial report, using a protocol based on a FOXA2-expressing floor plate intermediate, showed highly efficient induction of dopaminergic neurons with a midbrain molecular profile.[122] When grafted into rodent models of PD, these neurons showed more robust survival and function compared with dopaminergic neurons generated via a PAX6-expressing neuroepithelial intermediate.[121,122]

Since the initial publication, rigorous preclinical testing in animal models of PD has shown that these floor plate-derived dopaminergic neurons can function with equal potency and efficacy to fetal dopaminergic neurons.[123] Furthermore, they have a remarkable capacity for long-distance, target-specific fibre outgrowth.[123,124] In addition, these fast, efficient and synchronized differentiation protocols seem to have circumvented the problems of tumour formation and neural overgrowth seen with the older protocols.[121–123,125] As such, these protocols may obviate the need for a positive or negative cell selection step in the cell production process for any clinical trial.

A growing number of studies show that the refined differentiation strategies result in hESC-derived dopaminergic neurons that survive, innervate, integrate and provide functional recovery[121–125] with a temporal course of effects comparable to that seen with fetal dopaminergic neurons.[126] Moreover, this all occurs in the absence of tumours or neural overgrowth. Consequently, this field has now reached a point where clinical translation seems feasible. To take hESC-derived dopaminergic neurons to patients, however, an essential requirement is a scalable cell production process that adheres to GMP, with robust procedures for banking and distribution of the cell product that allow it to be cryopreserved, shipped and thawed[93] without altering the properties of the cells in any way. This resulting product must show survival, sufficient innervation and efficacy in preclinical models, and must also have a documented safety profile that adheres to regulatory guidelines. Though a daunting task, these criteria have already been fulfilled by several pluripotent cell-based products,[127,128] which are likely to be followed by many more in years to come.

2010s—fVM grafts and new initiatives

Re-evaluation of the results of the human fVM studies, along with the development of new protocols that

generate seemingly authentic nigral A9 dopaminergic cells from stem cell sources, has led to a new optimism regarding the cell replacement approach. In 2008, however, further questions were raised following the first reports that patients who had received human fVM grafts had postmortem evidence of Lewy body pathology in the transplant.[129,130] This finding has subsequently been confirmed in a number of follow-up studies,[131,132] with evidence that the burden of pathology increases with time after implantation. These unexpected observations have led to new theories surrounding the pathogenesis and spread of pathology in PD, with the emerging hypothesis that α-synuclein can act in a prion-like fashion.[133] However, for reasons that are not yet clear, this phenomenon only ever affects a small percentage of the transplanted dopaminergic cells, with no more than 10–15% of cells exhibiting Lewy body pathology 15–25 years after grafting.[134] Thus, although the graft might ultimately be compromised many decades after implantation, the available clinical and postmortem data indicate that the integrity of the graft is maintained for at least 20 years.[51] This discovery of α-synuclein pathology in the transplanted dopaminergic cells was unexpected, but it does not negate the value of this approach to treat PD.

In 2009, the European Union funded TRANSEURO,[80] a new trial using human fVM tissue in patients with PD. This trial has adopted a systematic and rigorous approach, with a series of well-defined criteria for patient selection, tissue dissection, preparation, grafting and immunosuppression, and trial design (Figure 5). This study will involve 20 patients, who will receive neural transplants derived from three or more human fVM grafts per side. The tissue will be prepared to GMP standards, and the primary end point at 3 years will be the UPDRS motor score in a defined 'off' state. The trial design also includes a large number of other secondary clinical and imaging outcome measures. The first graft was performed in May 2015.

As this trial is being undertaken, a new parallel effort—the so-called GForce-PD initiative—has commenced that brings together all the leading groups working on stem cell-derived dopaminergic cells for use in PD.[135,136] This initiative has been established to enable the free exchange of information and expertise between the relevant teams in Europe, North America and Japan, with the aim of developing a more coordinated approach to take this therapy to first-in-human studies. GForce-PD is seen as a template for the future development and employment of any new invasive experimental therapy, in that it seeks to bring together all the relevant parties to synergize their efforts, to rigorously assess the safety and efficacy of stem cell-derived dopaminergic neurons in preclinical models, and to avoid undertaking premature trials ahead of the scientific data—a problem that has plagued this area of restorative neurotherapeutics.

Conclusions

The recognition that patients with PD can be treated effectively with dopaminergic drugs for many years highlights the fact that targeting of this part of the pathology can make a huge difference to patients from a clinical perspective. However, these drugs ultimately fail owing to continued nigral dopaminergic cell loss, the non-physiological mode of delivery of the drugs used to replace the dopamine, and the increasing prominence of extranigral pathology as the disease progresses. Dopaminergic cell grafts cannot resolve the latter problem, but they can assist in the other two respects, and human fVM allografts have been shown to be effective for decades in some patients. This source of cells has produced inconsistent results, however, due to the unique way in which the tissue is prepared for

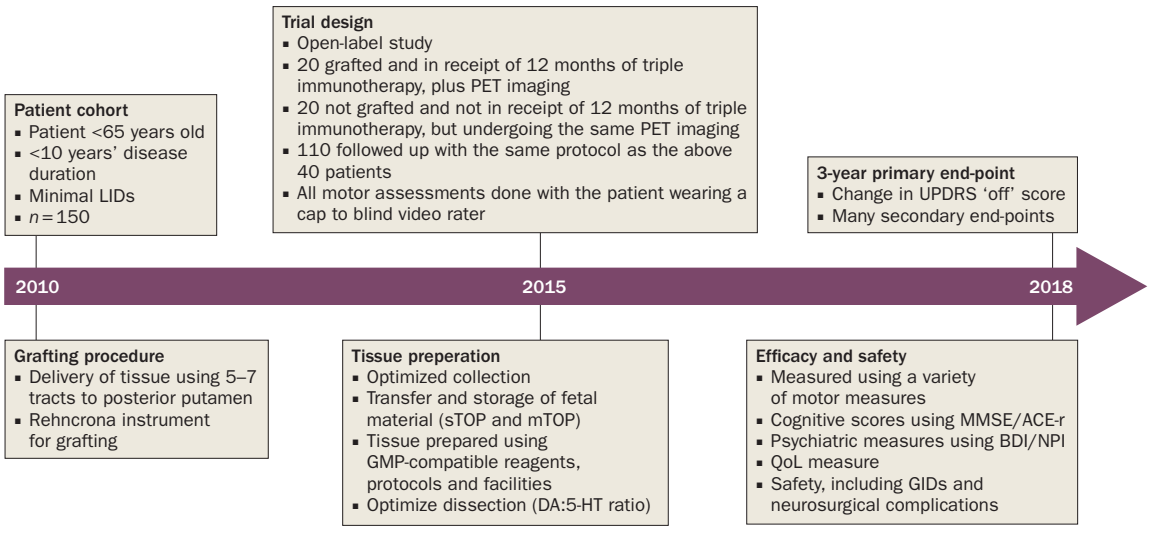

Figure 5 | The TRANSEURO hfVM tissue trial for the treatment of Parkinson disease. Abbreviations: 5-HT, serotonergic; ACE-r, Addenbrooke's Cognitive Examination—revised; BDI, Beck Depression Inventory; DA, dopaminergic; GIDs, graft-induced dyskinesias; hfVM, human fetal ventral mesencephalic; LIDs, levodopa-induced dyskinesias; MMSE, Mini-Mental State Examination; mTOP, medical termination of pregnancy; NPI, Neuropsychiatric Inventory; QoL, quality of life; UPDRS, Unified Parkinson's Disease Rating Scale; sTOP, surgical termination of pregnancy.

every patient, coupled to issues of trial design, patient selection, graft implantation and immunotherapy. Furthermore, all cell-based therapies for PD—and, thus, the conclusions drawn from them—have tended to be grouped together, creating the impression that they all work in the same way, with the same degree of preclinical data supporting their adoption in clinical trials. In many cases, however, the cell being trialled has limited preclinical data to support its use. Consequently, confusion prevails regarding what has actually been shown with this therapeutic approach, and what the results mean as we enter a new era of stem cell-based dopaminergic cells for use in patients with PD.

In this Review, we have summarized the history of neural grafting in PD, the mistakes that have been made *en route*, and the lessons that have been learned, so that we can plan for the next generation of cell-based therapies for PD with greater confidence and understanding. In so doing, we can better interpret any data and avoid rushing to premature conclusions and inaccurate statements on the true efficacy and potential of this type of therapeutic approach, and thereby avoid proceeding to clinical trials ahead of the preclinical data. Finally, it is important to remember that these therapies will, ultimately, have to compete with newer but better-established therapies for PD, such as DBS and Duodopa® (AbbVie, North Chicago, IL, USA). Therefore, the size and durability of any therapeutic effect of the cell transplants will need to be weighed against the cost of this therapy.

1. Spillantini, M. G. *et al.* α-Synuclein in Lewy bodies. *Nature* **388**, 839–840 (1997).
2. Damier, P., Hirsch, E. C., Agid, Y. & Graybiel, A. M. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain* **122**, 1437–1448 (1999).
3. Braak, H. *et al.* Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* **24**, 197–211 (2003).
4. Jenner, P. Dopamine agonists, receptor selectivity and dyskinesia induction in Parkinson's disease. *Curr. Opin. Neurol.* **16** (Suppl. 1), S3–S7 (2003).
5. Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H. & Brotchie, J. M. The pharmacology of L-DOPA-induced dyskinesia in Parkinson's disease. *Pharmacol. Rev.* **65**, 171–222 (2013).
6. Thompson, W. G. Successful brain grafting. *N. Y. Med. J.* **51**, 701–702 (1890).
7. Olson, L. & Seiger, A. Brain tissue transplanted to the anterior chamber of the eye: 2. Fluorescence histochemistry of immature catecholamine- and 5-hydroxytryptamine neurons innervating the rat vas deferens. *Cell Tissue Res.* **158**, 141–150 (1975).
8. Olson, L. & Seiger, A. Development and growth of immature monoamine neurons in rat and man *in situ* and following intraocular transplantation in the rat. *Brain Res.* **62**, 353–360 (1973).
9. Olson, L. & Seiger, A. Brain tissue transplanted to the anterior chamber of the eye. 1. Fluorescence histochemistry of immature catecholamine and 5-hydroxytryptamine neurons reinnervating the rat iris. *Z. Zellforsch. Mikrosk. Anat.* **135**, 175–194 (1972).
10. Barker, R. & Dunnett, S. The biology and behaviour of intracerebral adrenal transplants in animals and man. *Rev. Neurosci.* **4**, 113–146 (1993).
11. Ungerstedt, U., Ljungberg, T. & Steg, G. Behavioral, physiological, and neurochemical changes after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. *Adv. Neurol.* **5**, 421–426 (1974).
12. Ungerstedt, U. & Arbuthnott, G. W. Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res.* **24**, 485–493 (1970).
13. Ungerstedt, U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmacol.* **5**, 107–110 (1968).
14. Hudson, J. L. *et al.* Correlation of apomorphine- and amphetamine-induced turning with nigrostriatal dopamine content in unilateral 6-hydroxydopamine lesioned rats. *Brain Res.* **626**, 167–174 (1993).
15. Freed, W. J. *et al.* Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. *Nature* **292**, 351–352 (1981).
16. Perlow, M. J. *et al.* Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* **204**, 643–647 (1979).
17. Hoffer, B., Freed, W., Olson, L. & Wyatt, R. J. Transplantation of dopamine-containing tissues to the central nervous system. *Clin. Neurosurg.* **31**, 404–416 (1983).
18. Freed, W. J. *et al.* Restoration of dopaminergic function by grafting of fetal rat substantia nigra to the caudate nucleus: long-term behavioral, biochemical, and histochemical studies. *Ann. Neurol.* **8**, 510–519 (1980).
19. Björklund, A., Stenevi, U., Dunnett, S. B. & Iversen, S. D. Functional reactivation of the deafferented neostriatum by nigral transplants. *Nature* **289**, 497–499 (1981).
20. Björklund, A., Dunnett, S. B., Stenevi, U., Lewis, M. E. & Iversen, S. D. Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimotor testing. *Brain Res.* **199**, 307–333 (1980).
21. Björklund, A. & Stenevi, U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res.* **177**, 555–560 (1979).
22. Björklund, A., Stenevi, U., Schmidt, R. H., Dunnett, S. B. & Gage, F. H. Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cell suspensions implanted in different brain sites. *Acta Physiol. Scand. Suppl.* **522**, 9–18 (1983).
23. Brundin, P., Barker, R. A. & Parmar, M. Neural grafting in Parkinson's disease: problems and possibilities. *Prog. Brain Res.* **184**, 265–294 (2010).
24. Barker, R. A. What have open label studies of cell based therapies for Parkinson's disease told us, if anything? *Basal Ganglia* **4**, 85–87 (2014).
25. Backlund, E. O. *et al.* Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. *J. Neurosurg.* **62**, 169–173 (1985).
26. Freed, W. J., Poltorak, M. & Becker, J. B. Intracerebral adrenal medulla grafts: a review. *Exp. Neurol.* **110**, 139–166 (1990).
27. Madrazo, I. *et al.* Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N. Engl. J. Med.* **316**, 831–834 (1987).
28. Moore, R. Y. Parkinson's disease—a new therapy? *N. Engl. J. Med.* **316**, 872–873 (1987).
29. Allen, G. S., Burns, R. S., Tulipan, N. B. & Parker, R. A. Adrenal medullary transplantation to the caudate nucleus in Parkinson's disease. Initial clinical results in 18 patients. *Arch. Neurol.* **46**, 487–491 (1989).
30. Drucker-Colin, R. *et al.* Adrenal medullary tissue transplants in the caudate nucleus of Parkinson's patients. *Prog. Brain Res.* **78**, 567–574 (1988).
31. Goetz, C. G. *et al.* Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. *N. Engl. J. Med.* **320**, 337–341 (1989).
32. Goetz, C. G. *et al.* Adrenal medullary transplant to the striatum of patients with advanced Parkinson's disease: 1-year motor and psychomotor data. *Neurology* **40**, 273–276 (1990).
33. Jankovic, J. *et al.* Clinical, biochemical, and neuropathologic findings following transplantation of adrenal medulla to the caudate nucleus for treatment of Parkinson's disease. *Neurology* **39**, 1227–1234 (1989).
34. Jiao, S. S. *et al.* Study of adrenal medullary tissue transplantation to striatum in parkinsonism. *Prog. Brain Res.* **78**, 575–580 (1988).
35. Jiao, S. S. *et al.* Adrenal medullary autografts in patients with Parkinson's disease. *N. Engl. J. Med.* **321**, 324–327 (1989).
36. Kelly, P. J. *et al.* Adrenal medullary autograft transplantation into the striatum of patients with Parkinson's disease. *Mayo Clin. Proc.* **64**, 282–290 (1989).
37. Lindvall, O. *et al.* Transplantation in Parkinson's disease: two cases of adrenal medullary grafts to the putamen. *Ann. Neurol.* **22**, 457–468 (1987).
38. Ostrosky-Solis, F. *et al.* Neuropsychological effects of brain autograft of adrenal medullary tissue for the treatment of Parkinson's disease. *Neurology* **38**, 1442–1450 (1988).
39. Goetz, C. G. *et al.* United Parkinson Foundation Neurotransplantation Registry on adrenal medullary transplants: presurgical, and 1- and 2-year follow-up. *Neurology* **41**, 1719–1722 (1991).
40. Hurtig, H., Joyce, J., Sladek, J. R. J. & Trojanowski, J. Q. Postmortem analysis of adrenal-medulla-to-caudate autograft in a patient with Parkinson's disease. *Ann. Neurol.* **25**, 607–614 (1989).

41. Kompoliti, K., Chu, Y., Shannon, K. M. & Kordower, J. H. Neuropathological study 16 years after autologous adrenal medullary transplantation in a Parkinson's disease patient. *Mov. Disord.* **22**, 1630–1633 (2007).
42. Kordower, J. H., Cochran, E., Penn, R. D. & Goetz, C. G. Putative chromaffin cell survival and enhanced host-derived TH-fiber innervation following a functional adrenal medulla autograft for Parkinson's disease. *Ann. Neurol.* **29**, 405–412 (1991).
43. Waters, C., Itabashi, H. H., Apuzzo, M. L. & Weiner, L. P. Adrenal to caudate transplantation —postmortem study. *Mov. Disord.* **5**, 248–250 (1990).
44. Lindvall, O. *et al.* Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch. Neurol.* **46**, 615–631 (1989).
45. Lindvall, O. *et al.* Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* **247**, 574–577 (1990).
46. Brundin, P. *et al.* Bilateral caudate and putamen grafts of embryonic mesencephalic tissue treated with lazaroids in Parkinson's disease. *Brain* **123**, 1380–1390 (2000).
47. Lindvall, O. *et al.* Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Ann. Neurol.* **35**, 172–180 (1994).
48. Wenning, G. K. *et al.* Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann. Neurol.* **42**, 95–107 (1997).
49. Piccini, P. *et al.* Dopamine release from nigral transplants visualized *in vivo* in a Parkinson's patient. *Nat. Neurosci.* **2**, 1137–1140 (1999).
50. Piccini, P. *et al.* Delayed recovery of movement-related cortical function in Parkinson's disease after striatal dopaminergic grafts. *Ann. Neurol.* **48**, 689–695 (2000).
51. Kefalopoulou, Z. *et al.* Long-term clinical outcome of fetal cell transplantation for Parkinson disease: two case reports. *JAMA Neurol.* **71**, 83–87 (2014).
52. Freed, C. R. *et al.* Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N. Engl. J. Med.* **327**, 1549–1555 (1992).
53. Freeman, T. B. *et al.* Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. *Ann. Neurol.* **38**, 379–388 (1995).
54. Redmond, D. E. *et al.* Cellular replacement of dopamine deficit in Parkinson's disease using human fetal mesencephalic tissue: preliminary results in four patients. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **71**, 325–359 (1993).
55. Widner, H. *et al.* Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N. Engl. J. Med.* **327**, 1556–1563 (1992).
56. Mendez, I. *et al.* Enhancement of survival of stored dopaminergic cells and promotion of graft survival by exposure of human fetal nigral tissue to glial cell line-derived neurotrophic factor in patients with Parkinson's disease. Report of two cases and technical considerations. *J. Neurosurg.* **92**, 863–869 (2000).
57. Mendez, I. *et al.* Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study. Report of three cases. *J. Neurosurg.* **96**, 589–596 (2002).
58. Widner, H. NIH neural transplantation funding. *Science* **263**, 737 (1994).
59. Kumar, R. *et al.* Double-blind evaluation of subthalamic nucleus deep brain stimulation in advanced Parkinson's disease. *Neurology* **51**, 850–855 (1998).
60. Freed, C. R. *et al.* Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **344**, 710–719 (2001).
61. Olanow, C. W. *et al.* A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann. Neurol.* **54**, 403–414 (2003).
62. Cho, C., Alterman, R., Miravite, J., Shils, J. & Tagliati, M. Subthalamic DBS for the treatment of "runaway" dyskinesias after embryonic or fetal tissue transplant. *Mov. Disord.* **20**, 1237 (2005).
63. Graff-Radford, J. *et al.* Deep brain stimulation of the internal segment of the globus pallidus in delayed runaway dyskinesia. *Arch. Neurol.* **63**, 1181–1184 (2006).
64. Herzog, J. *et al.* Deep brain stimulation in Parkinson's disease following fetal nigral transplantation. *Mov. Disord.* **23**, 1293–1296 (2008).
65. Hagell, P. *et al.* Dyskinesias following neural transplantation in Parkinson's disease. *Nat. Neurosci.* **5**, 627–628 (2002).
66. Ma, Y. *et al.* Dyskinesia after fetal cell transplantation for parkinsonism: a PET study. *Ann. Neurol.* **52**, 628–634 (2002).
67. Barker, R. A. & Kuan, W. L. Graft-induced dyskinesias in Parkinson's disease: what is it all about? *Cell Stem Cell* **7**, 148–149 (2010).
68. Politis, M. *et al.* Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. *Sci. Transl. Med.* **2**, 38ra46 (2010).
69. Politis, M. *et al.* Graft-induced dyskinesias in Parkinson's disease: high striatal serotonin/dopamine transporter ratio. *Mov. Disord.* **26**, 1997–2003 (2011).
70. Lane, E. L., Winkler, C., Brundin, P. & Cenci, M. A. The impact of graft size on the development of dyskinesia following intrastriatal grafting of embryonic dopamine neurons in the rat. *Neurobiol. Dis.* **22**, 334–345 (2006).
71. Winkler, C., Georgievska, B., Carlsson, T., Lacar, B. & Kirik, D. Continuous exposure to glial cell line-derived neurotrophic factor to mature dopaminergic transplants impairs the graft's ability to improve spontaneous motor behavior in parkinsonian rats. *Neuroscience* **141**, 521–531 (2006).
72. Mendez, I. *et al.* 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 (2005).
73. Krack, P., Poepping, M., Weinert, D., Schrader, B. & Deuschl, G. Thalamic, pallidal, or subthalamic surgery for Parkinson's disease? *J. Neurol.* **247** (Suppl. 2), II122–II134 (2000).
74. Ma, Y. *et al.* Dopamine cell implantation in Parkinson's disease: long-term clinical and ^{18}F-FDOPA PET outcomes. *J. Nucl. Med.* **51**, 7–15 (2010).
75. Barker, R. A., Barrett, J., Mason, S. L. & Björklund, A. Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. *Lancet Neurol.* **12**, 84–91 (2013).
76. Piccini, P. *et al.* Factors affecting the clinical outcome after neural transplantation in Parkinson's disease. *Brain* **128**, 2977–2986 (2005).
77. Kordower, J. H. *et al.* Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N. Engl. J. Med.* **332**, 1118–1124 (1995).
78. Kordower, J. H. *et al.* Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural, and metabolic studies. *J. Comp. Neurol.* **370**, 203–230 (1996).
79. Kordower, J. H. *et al.* Fetal nigral grafts survive and mediate clinical benefit in a patient with Parkinson's disease. *Mov. Disord.* **13**, 383–393 (1998).
80. TRANSEURO [online], http://www.transeuro.org.uk/ (2014).
81. Barker, R. A., Kendall, A. L. & Widner, H. Neural tissue xenotransplantation: what is needed prior to clinical trials in Parkinson's disease? Neural Tissue Xenografting Project. *Cell Transplant.* **9**, 235–246 (2000).
82. Galpern, W. R., Burns, L. H., Deacon, T. W., Dinsmore, J. & Isacson, O. Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology. *Exp. Neurol.* **140**, 1–13 (1996).
83. Schumacher, J. M. *et al.* Transplantation of embryonic porcine mesencephalic tissue in patients with PD. *Neurology* **54**, 1042–1050 (2000).
84. Arjona, V. *et al.* Autotransplantation of human carotid body cell aggregates for treatment of Parkinson's disease. *Neurosurgery* **53**, 321–328 (2003).
85. Minguez-Castellanos, A. *et al.* Carotid body autotransplantation in Parkinson disease: a clinical and positron emission tomography study. *J. Neurol. Neurosurg. Psychiatry* **78**, 825–831 (2007).
86. Bakay, R. A. *et al.* Implantation of Spheramine in advanced Parkinson's disease (PD). *Front. Biosci.* **9**, 592–602 (2004).
87. Stover, N. P. *et al.* Intrastriatal implantation of human retinal pigment epithelial cells attached to microcarriers in advanced Parkinson disease. *Arch. Neurol.* **62**, 1833–1837 (2005).
88. Stover, N. P. & Watts, R. L. Spheramine for treatment of Parkinson's disease. *Neurotherapeutics* **5**, 252–259 (2008).
89. Watts, R. L. *et al.* Stereotaxic intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson's disease. *J. Neural Transm. Suppl.* **65**, 215–227 (2003).
90. Gross, R. E. *et al.* Intrastriatal transplantation of microcarrier-bound human retinal pigment epithelial cells versus sham surgery in patients with advanced Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol.* **10**, 509–519 (2011).
91. Ribeiro, D. *et al.* Efficient expansion and dopaminergic differentiation of human fetal ventral midbrain neural stem cells by midbrain morphogens. *Neurobiol. Dis.* **49**, 118–127 (2013).
92. Barker, R. A. & de Beaufort, I. Scientific and ethical issues related to stem cell research and interventions in neurodegenerative disorders of the brain. *Prog. Neurobiol.* **110**, 63–73 (2013).
93. Barker, R. A. Developing stem cell therapies for Parkinson's disease: waiting until the time is right. *Cell Stem Cell* **15**, 539–542 (2014).
94. Thomson, J. A. *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147 (1998).
95. Reubinoff, B. E., Pera, M. F., Fong, C. Y., Trounson, A. & Bongso, A. Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat. Biotechnol.* **18**, 399–404 (2000).

96. Itskovitz-Eldor, J. *et al.* Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol. Med.* **6**, 88–95 (2000).
97. Reubinoff, B. E. *et al.* Neural progenitors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1134–1140 (2001).
98. Zhang, S. C., Wernig, M., Duncan, I. D., Brustle, O. & Thomson, J. A. *In vitro* differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1129–1133 (2001).
99. Kawasaki, H. *et al.* Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron* **28**, 31–40 (2000).
100. Kim, J. H. *et al.* Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* **418**, 50–56 (2002).
101. Brederlau, A. *et al.* Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of *in vitro* differentiation on graft survival and teratoma formation. *Stem Cells* **24**, 1433–1440 (2006).
102. Park, C. H. *et al. In vitro* and *in vivo* analyses of human embryonic stem cell-derived dopamine neurons. *J. Neurochem.* **92**, 1265–1276 (2005).
103. Perrier, A. L. *et al.* Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **101**, 12543–12548 (2004).
104. Sonntag, K. C. *et al.* Enhanced yield of neuroepithelial precursors and midbrain-like dopaminergic neurons from human embryonic stem cells using the bone morphogenic protein antagonist noggin. *Stem Cells* **25**, 411–418 (2007).
105. Zeng, X. *et al.* Dopaminergic differentiation of human embryonic stem cells. *Stem Cells* **22**, 925–940 (2004).
106. Roy, N. S. *et al.* Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat. Med.* **12**, 1259–1268 (2006).
107. Cooper, O. *et al.* 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 (2010).
108. Yan, Y. *et al.* Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells* **23**, 781–790 (2005).
109. Yang, D., Zhang, Z. J., Oldenburg, M., Ayala, M. & Zhang, S. C. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in parkinsonian rats. *Stem Cells* **26**, 55–63 (2008).
110. Takahashi, K., Okita, K., Nakagawa, M. & Yamanaka, S. Induction of pluripotent stem cells from fibroblast cultures. *Nat. Protoc.* **2**, 3081–3089 (2007).
111. Soldner, F. *et al.* Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* **136**, 964–977 (2009).
112. Hargus, G. *et al.* 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 (2010).
113. Kikuchi, T. *et al.* 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 (2011).
114. Bonilla, S. *et al.* Identification of midbrain floor plate radial glia-like cells as dopaminergic progenitors. *Glia* **56**, 809–820 (2008).
115. Ono, Y. *et al.* Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. *Development* **134**, 3213–3225 (2007).
116. Placzek, M. & Briscoe, J. The floor plate: multiple cells, multiple signals. *Nat. Rev. Neurosci.* **6**, 230–240 (2005).
117. Arenas, E., Denham, M. & Villaescusa, J. C. How to make a midbrain dopaminergic neuron. *Development* **142**, 1918–1936 (2015).
118. Chambers, S. M. *et al.* Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* **27**, 275–280 (2009).
119. Tabar, V. & Studer, L. Pluripotent stem cells in regenerative medicine: challenges and recent progress. *Nat. Rev. Genet.* **15**, 82–92 (2014).
120. Fasano, C. A., Chambers, S. M., Lee, G., Tomishima, M. J. & Studer, L. Efficient derivation of functional floor plate tissue from human embryonic stem cells. *Cell Stem Cell* **6**, 336–347 (2010).
121. Kirkeby, A. *et al.* Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. *Cell Rep.* **1**, 703–714 (2012).
122. Kriks, S. *et al.* Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* **480**, 547–551 (2011).
123. Grealish, S. *et al.* 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 (2014).
124. Grealish, S. *et al.* Monosynaptic tracing using modified rabies virus reveals early and extensive circuit integration of human embryonic stem cell-derived neurons. *Stem Cell Rep.* http://dx.doi.org/10.1016/j.stemcr.2015.04.011.
125. Steinbeck, J. A. *et al.* Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. *Nat. Biotechnol.* **33**, 204–209 (2015).
126. Rath, A. *et al.* Survival and functional restoration of human fetal ventral mesencephalon following transplantation in a rat model of Parkinson's disease. *Cell Transplant.* **22**, 1281–1293 (2013).
127. Alper, J. Geron gets green light for human trial of ES cell-derived product. *Nat. Biotechnol.* **27**, 213–214 (2009).
128. Kanemura, H. *et al.* Tumorigenicity studies of induced pluripotent stem cell (iPSC)-Derived retinal pigment epithelium (RPE) for the treatment of age-related macular degeneration. *PLoS ONE* **9**, e85336 (2014).
129. Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B. & Olanow, C. W. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat. Med.* **14**, 504–506 (2008).
130. Li, J. Y. *et al.* Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat. Med.* **14**, 501–503 (2008).
131. Chu, Y. & Kordower, J. H. Lewy body pathology in fetal grafts. *Ann. N. Y. Acad. Sci.* **1184**, 55–67 (2010).
132. Li, J. Y. *et al.* Characterization of Lewy body pathology in 12- and 16-year-old intrastriatal mesencephalic grafts surviving in a patient with Parkinson's disease. *Mov. Disord.* **25**, 1091–1096 (2010).
133. Guo, J. L. & Lee, V. M. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nat. Med.* **20**, 130–138 (2014).
134. Hallett, P. J. *et al.* Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. *Cell Rep.* **7**, 1755–1761 (2014).
135. Abbott, A. Fetal-cell revival for Parkinson's. *Nature* **510**, 195–196 (2014).
136. GForce-PD [online], http://www.gforce-pd.com/ (2015).
137. Hirsch, E. C., Duyckaerts, C., Javoy-Agid, F., Hauw, J. J. & Agid, Y. Does adrenal graft enhance recovery of dopaminergic neurons in Parkinson's disease? *Ann. Neurol.* **27**, 676–682 (1990).
138. Peterson, D. I., Price, M. L. & Small, C. S. Autopsy findings in a patient who had an adrenal-to-brain transplant for Parkinson's disease. *Neurology* **39**, 235–238 (1989).
139. Olanow, C. W. *et al.* Autologous transplantation of adrenal medulla in Parkinson's disease. 18-month results. *Arch. Neurol.* **47**, 1286–1289 (1990).
140. Hallett, P. J. *et al.* Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. *Cell Stem Cell* **16**, 269–274 (2015).
141. Delcroix, G. J. *et al.* The therapeutic potential of human multipotent mesenchymal stromal cells combined with pharmacologically active microcarriers transplanted in hemi-parkinsonian rats. *Biomaterials* **32**, 1560–1573 (2011).
142. Offen, D. *et al.* Intrastriatal transplantation of mouse bone marrow-derived stem cells improves motor behavior in a mouse model of Parkinson's disease. *J. Neural Transm. Suppl.* 133–143 (2007).
143. Sanchez-Pernaute, R., Studer, L., Bankiewicz, K. S., Major, E. O. & McKay, R. D. *In vitro* generation and transplantation of precursor-derived human dopamine neurons. *J. Neurosci. Res.* **65**, 284–288 (2001).
144. Caiazzo, M. *et al.* Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **476**, 224–227 (2011).
145. Pfisterer, U. *et al.* Direct conversion of human fibroblasts to dopaminergic neurons. *Proc. Natl Acad. Sci. USA* **108**, 10343–10348 (2011).

Acknowledgements

The authors' own work is supported by grants from Neurostemcellrepair (grant no. 602278) and the Swedish Research Council (grants K2012-99X-22324-01-5 and K2014-61X-20391-08-4) and TRANSEURO, and by the National institute for Health Research (NIHR)-funded Biomedical Research Centre in Cambridge, UK. M.P. is funded from the European Research Council ERC Grant Agreement no. 309712. We would also like to thank Hakan Widner, Olle Lindvall and Anders Björklund for their advice and input, especially relating to information on the patients grafted in Lund.

Author contributions

All authors researched data for the article and reviewed and/or edited the manuscript before submission. R.A.B. and M.P. discussed the content and wrote the article.