nature medicine

PERSPECTIVE

https://doi.org/10.1038/s41591-019-0507-2

Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease

Roger A. Barker[1,2] and TRANSEURO consortium[3]

Clinical studies of Parkinson's disease (PD) using a dopamine cell replacment strategy have been tried for more than 30 years. The outcomes following transplantation of human fetal ventral mesencephalic tissue (hfVM) have been variable, with some patients coming off their anti-PD treatment for many years and others not responding and/or developing significant side effects, including graft-induced dyskinesia. This led to a re-appraisal of the best way to do such trials, which resulted in a new European-Union-funded allograft trial with fetal dopamine cells across several centers in Europe. This new trial, TRANSEURO (NCT01898390), is an open-label study in which some individuals in a large observational cohort of patients with mild PD who were undergoing identical assessments were randomly selected to receive transplants of hfVM. The TRANSEURO trial is currently ongoing as researchers have completed both recruitment into a large multicenter observational study of younger onset early-stage PD and transplantation of hfVM in 11 patients. While completion of TRANSEURO is not expected until 2021, we feel that sharing the rationale for the design of TRANSEURO, along with the lessons we have learned along the way, can help inform researchers and facilitate planning of transplants of dopamine-producing cells derived from human pluripotent stem cells for future clinical trials.

PD is a chronic neurodegenerative disorder that is characterized by the loss of nigrostriatal dopaminergic neurons and the development of a movement disorder typically in the seventh to eighth decade of life. Pathologically, the disease is defined by the accumulation of alpha synuclein in Lewy bodies and Lewy neurites, which extends across many areas of the central nervous system (CNS) and involves the enteric and autonomic nervous systems[1]. This widespread pathology explains many of the non-motor abnormalities that patients with PD experience, only some of which are responsive to dopaminergic medications[2]. Nevertheless, the core motor deficits of bradykinesia and rigidity are responsive to dopaminergic-replacement therapies, and patients typically do very well when treated with such drugs in the early stages of disease. However, oral dopaminergic drugs cause both short- and long-term problems. These include off-target effects that lead to neuropsychiatric and autonomic problems as well as dyskinesia through the non-physiological stimulation of dopaminergic receptors in the striatum. As a result, there has long been an interest in using different approaches, including gene- and cell-based therapies, to selectively target the loss of dopamine at the site of greatest depletion, namely the putamen[3,4]. These therapies have now both been trialed in patients, resulting in mixed benefits.

In 2006, a new international initiative was conceived and was followed by a series of meetings to re-evaluate the merit of therapies for PD based on dopamine-producing cells. This was deemed necessary given the contrasting outcomes of the two National Institutes of Health (NIH)-funded hfVM allograft trials in patients with PD that were published in 2001 and 2003 (refs. [5,6]) compared with results from earlier open-label studies using similar tissue (reviewed in ref. [7]). These NIH-funded double-blind placebo-controlled studies reported no benefits in patients who received grafts versus those who underwent sham operations, and in addition, significant numbers of patients developed side effects in the form of graft-induced dyskinesia (GID). These results were at odds with earlier open-label studies that reported long-term benefits, which led to the need to explore how such disparate results could be reconciled.

The meetings invited all the main investigators involved in these trials and sought to critically appraise the previous work, with the aim of deciding whether this therapeutic approach had a future and, if so, how best to move it forward for treatment of PD. On the basis of these discussions and an analysis of the raw data collated from all major hfVM trials in PD, this group identified factors that were thought to explain some of the differences in patients who had a positive outcome following this intervention. In particular, it appeared that disease stage at the time of grafting was critical. This in turn led to the hypothesis that patients at an earlier disease stage with no significant ventral striatal dopaminergic denervation and negligible dyskinesia may benefit the most from hfVM grafts and they may be less likely to develop GID.

On the basis of this reasoning, TRANSEURO, a new European Union-funded trial in Europe using hfVM tissue for transplant, was initiated (NCT01898390). This tissue was chosen for implantation in 2010 (when the trial began) as there were no published protocols for making authentic and functional midbrain dopaminergic neurons derived from human stem cells. The TRANSEURO multicenter study had two major arms: (1) an observational study charting the natural history of younger onset, early-stage PD ($n > 100$ patients; the group of people thought to be optimal for dopamine cell therapies); and (2) a transplant arm that included patients randomly selected from the observational cohort (provided they had continued adherence to eligibility criteria).

The observational study is still ongoing, and a significant proportion of the cohort has been followed up to the present day. The transplant arm has now been completed, and 11 patients received a graft over a 3-year period (2015–2018). The outcome of these hfVM transplants will be evaluated in 2021 using the predefined primary

[1]John van Geest Centre for Brain Repair, Department of Clinical Neurosciences and WT-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK. [2]John van Geest Centre for Brain Repair, Cambridge University, Cambridge, UK. [3]A full list of members appears at the end of the paper. e-mail: rab46@cam.ac.uk

endpoint of the trial, which is change in the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III scores for the patients in the defined 'OFF' medication state at 36 months following the second transplant compared to their baseline pretransplant scores. We will also compare the trajectory of the transplant group against that of patients in the well-matched, contemporaneously studied control group of the TRANSEURO study.

In this Perspective, we describe both the observational and the transplant arms of the TRANSEURO trial in terms of how they were designed, as well as some of the data from the observational cohort justifying our approach. We also discuss some of the major issues that arose during this work, which are likely to be important and relevant to the new stem-cell-based therapies that are soon to enter clinical trials for PD. We think that there is a need to present such information at this stage since the primary endpoint in the TRANSEURO transplant trial will be reported at a time when several clinical trials of transplants using stem-cell-derived dopamine-producing cells for PD will have already started[8].

The TRANSEURO study

Observational study. The recruitment of patients with idiopathic PD started in December 2010 and continued until the end of 2013 at several sites: Addenbrooke's Hospital, Cambridge, UK; Imperial College, London, UK; the National Hospital for Neurology and Neurosurgery, London, UK; the University of Cardiff, Cardiff, UK; Skåne University Hospital, Lund, Sweden; Freiburg University, Freiburg, Germany; and the Assistance Publique-Hôpitaux de Paris, Paris, France. Inclusion and exclusion criteria are detailed in Box 1.

After recruitment, patients were seen every 6 months in the OFF medication state, and a detailed number of assessments were undertaken (see Fig. 1 for details) that were selected to objectively capture motor, cognitive, psychiatric and other non-motor symptoms as well as quality-of-life measures. These were chosen to ensure that: (1) the most widely recognized clinical assessments were included for ease of cross-study comparisons; (2) all included measures were validated and (3) all measures could be completed in a timely manner and were acceptable to the participants.

Given the practical difficulties associated with observer bias, all motor assessments were videotaped while participants wore caps to hide any clues regarding surgery. This allows for blinding of independent raters, who will score the videos at a later date, to treatment allocation of the participants.

Finally, despite randomization, surgical trials involving small numbers of patients can be vulnerable to outlying data from individuals with conspicuously fast or slow rates of disease progression and/or atypical responses to interventions, some of which have a genetic basis. To try and partially mitigate this, all participants are currently being genotyped for the common, known PD-associated genes, some of which have been documented to play a role in the rate of disease progression[9].

Throughout the study, medication for the patients was managed according to best medical practice, and no changes or alterations were made explicitly for the execution of this trial, but only as clinically indicated by the treating physician. Patients enrolled in the observational study were informed that they may be selected for the transplant study, but this was not guaranteed. They were also informed that if they were not included in the transplant part of this study that they may still be suitable in the future for other experimental cell- and gene-based dopamine therapies for their PD as well as for deep brain stimulation.

Transplant study. The inclusion and exclusion criteria for transitioning into the transplant arm of TRANSEURO were reapplied to a subset of patients who were selected at random to either (1) form the transplant arm of the trial or (2) act as a matched control population in terms of clinical assessments and positron-emission tomography (PET) imaging. Some additional measures were added (see Box 1 and Fig. 1). These criteria were carefully chosen to ensure that patients participating in the trial were likely to be at the lowest risk from the transplantation procedure and yet have the highest chance of receiving clinical benefit, while being representative of the main population of patients with PD in the early stages of the disease. Given the longitudinal nature of the project and concerns regarding issues such as GID, it was essential during the process of patient selection for transplantation that the criteria at baseline were reapplied at the time of grafting to avoid recruiting patients who had developed significant levodopa (L-dopa)-induced dyskinesias (LID) during the observational follow-up period. This period of observational follow-up was extremely valuable in that it allowed for the assessment of the rate of progression of motor severity of early PD using the MDS-UPDRS Part III in both the OFF and ON medication conditions. The MDS-UPDRS Part III is a well-validated standard clinical assessment tool used to measure motor function in PD, which is why we adopted it in TRANSEURO. Moreover, the re-application of criteria ahead of transplantation allowed for an increasing confidence of the accuracy of the diagnosis of PD by introducing a threshold of a documented 33% response to L-dopa between ON versus OFF assessments.

Finally, the assessment of patients in the transplant arm of TRANSEURO was identical to that undertaken in the observational arm. The chosen primary outcome is the change in MDS-UPDRS Part III motor score after a defined period of medication withdrawal (practically defined as OFF medication) at 36 months

Box 1 | Inclusion and exclusion criteria for patients in the observational TRANSEURO study

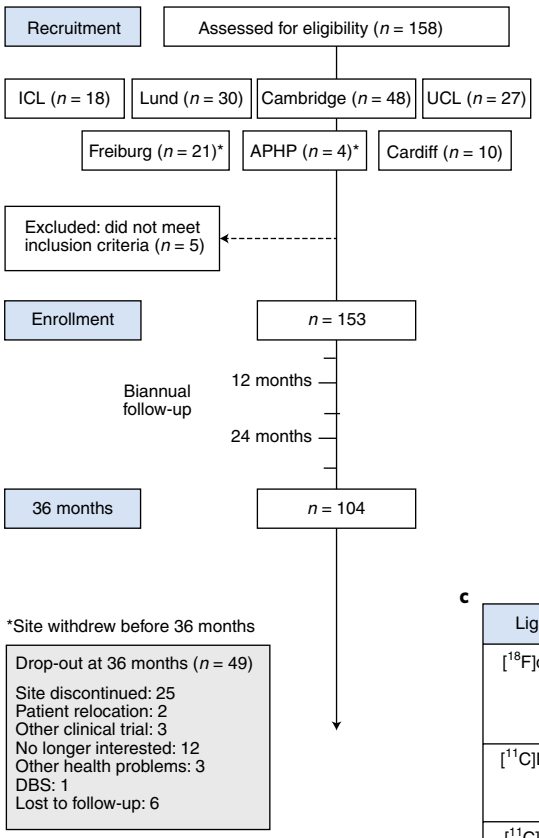
Additional criteria for the transplant cohort are bolded.
Inclusion criteria.

- PD as defined by the Queen Square Brain Bank criteria
- Disease duration **≥2 yr and ≤13 yr**
- Aged ≥30 yr and **≤68 yr** at the time of grafting
- Hoehn & Yahr stage 2 or better when in ON medication state
- On no therapy or only receiving standard anti-PD treatment
- No significant L-dopa-induced dyskinesia
- **Significant ≥33% improvement in their UPDRS part III motor score in response to an acute dose of L-dopa as they move from OFF to ON**
- **Preserved [^{18}F]dopa signal in ventral striatum**

Exclusion criteria.

- Atypical parkinsonism, including F-dopa PET patterns consistent with this
- Mini mental state examination (MMSE) score of <24 **(<26)** or evidence for dementia using DSM-IV criteria
- Unable to copy normally and accurately two interlocking pentagons and a semantic fluency score of <20 over 90 s
- Ongoing major medical or psychiatric disorder, including depression and psychosis
- Other concomitant treatment with neuroleptics
- Significant drug-induced dyskinesia (>2 for any body part on the AIMS scale)
- Previous neurosurgery
- Unable to be imaged using MRI
- **Clinically insignificant response to L-dopa**
- **Any contraindication to immunosuppression therapy**
- **Patients on anticoagulants**
- **Patients who are left-handed**

a



b

Motor	• MDS-UPDRS Parts I–IV † • Hauser diary card † • Nine-hole pegboard † • Timed sit–stand–walk task †	• Thirty second tap test † • Abnormal Involuntary Movement Scale † • Rush Dyskinesia Scale †
Cognitive	• ACE-R (or MOCA) † • CANTAB OTS • CANTAB RTI • CANTAB PRM • CANTAB PAL • Hopkins Verbal Learning Task	• Verbal fluency • Stroop • Digit span • Warrington Graded Naming Task • WAIS-R similarities
Psychiatric	• QUIP † • Beck Depression Inventory †	• Apathy Evaluation Scale
Other non-motor & QOL	• PDQ-39 • EQ-5D • Life Satisfaction Scale	• Clinical Global Impression Scale • PD Non-Motor Scale • PD Sleep Scale
Other information	• Physical examination • Concomitant mediations †	• Adverse events † • Hospitalizations †

† Assessed at 6-month interval

c

Ligand	Target	Marker	Corresponding clinical results
[^{18}F]dopa	AADC	Indirect measure of DA synthesis capacity and storage	DA-rich graft survival and growth
[^{11}C]DASB	5-HT transporter	Pre-synaptic 5-HT terminal integrity and SERT availability	Graft derived 5-HT innervation and growth GID
[^{11}C]PE2I	DAT	Pre-synaptic DA terminal integrity and DAT availability	DA-rich graft survival and growth, expression of DAT by grafted cells GID

Fig. 1 | Overview of the TRANSEURO protocol. a, The number of patients recruited by each center, the number enrolled and the number who were followed up in the observational arm. Reasons for drop-out at 36 months are shown. **b**, Complete list of assessments undertaken by all patients every 12 months. At 6 months, a more limited set of assessments were undertaken. ACE-R/MOCA, Addenbrooke's cognitive examination revised (2005)/Montreal cognitive assessment. CANTAB, Cambridge neuropsychological test automated battery. OTS, one touch Stocking. RTI, reaction time. PRM, pattern recognition memory. PAL, paired associates learning. WAIS-R, Wechsler adult intelligence scale revised. QUIP, questionnaire for impulsive-compulsive disorders in Parkinson's disease. PDQ-39: 39-item Parkinson's disease questionnaire. EQ-5D, EuroQoL 5-dimension, 5-level questionnaire. QOL, quality of life. **c**, PET imaging schedule adopted and the reasons underlying the schedule. DA, dopamine.

after their final transplant compared to their baseline score as well as those patients who were not grafted (Box 2). The longitudinal 'observational' data obtained prior to transplant has allowed this measure to be selected as the primary outcome with confidence, with the additional advantage that any change in MDS-UPDRS Part III trajectory could be assessed according to treatment allocation.

Imaging studies done as part of this study. Anatomical imaging using magnetic resonance imaging (MRI) was undertaken in the selected transplant cohort ahead of grafting to plan for implantation surgery. MRI scanning was also routinely done in the immediate post-operative period to assess for (1) graft placement and (2) any complications, such as hemorrhages or other abnormalities.

In addition, MRI and PET imaging studies were undertaken to examine functional aspects of the transplant, in particular its ability to normalize network activity and dopaminergic content, as assessed with [^{18}F]dopa, and its dopamine transporter (DAT) expression, as assessed with [^{11}C]PE2I, as well as its contamination with 5-HT neurons (using [^{11}C]DASB PET imaging) as there is a possible role for serotonin in the genesis of GID[10] (Fig. 1). In addition, we used [^{18}F]dopa PET imaging as a criterion for transplant arm inclusion or exclusion (Box 1) as it has previously been reported that significant preoperative ventral striatal dopamine loss is associated with less successful outcomes in patients who have received hfVM transplants[11].

The functional and structural imaging studies using MRI were done to determine whether a transplant could restore cortical networks back to a more normal state[12]. This assessment included resting state network activity (measured with resting state functional MRI (fMRI)), motor task activation (measured with multi-echo fMRI) and diffusion tensor imaging. This PET and MRI imaging battery was repeated every 18 months in the patients with the grafts.

One additional criterion for transplantation was introduced for pragmatic reasons to accommodate the imaging protocols: dominant handedness. Given the impact that dominant handedness has on functional imaging using fMRI, the decision was made to only perform transplantation on individuals with a dominant right hand. While this restriction was not desirable from the clinical perspective, the compromise was made in light of the importance of the imaging data.

hfVM tissue preparation for transplantation. The preparation of the hfVM tissue for grafting into patients in the TRANSEURO trial was modified such that a set of standard operating procedures (SOPs) that were more defined than those of previous trials were followed. Tissue dissection and preparation were completed in

Box 2 | The primary and secondary end points of the TRANSEURO transplant trial

Primary endpoint.

- The change in motor MDS-UPDRS in a defined OFF medication state at 36 months post transplantation; the OFF state being defined as receiving no dopamine therapy for 12 h before assessment or 36 h in the case of long-acting dopamine agonists (for example, ropinirole slow-release)

Secondary endpoints.

- Change in timed motor tasks at 36 months post transplantation
- The number of patients with dyskinesia (including LID and GID) at 36 months post transplantation
- L-dopa equivalence medication doses at 36 months post transplantation
- Number of patients on L-dopa therapy at 36 months post transplantation
- The amount of OFF time 36 months post transplantation
- Quality of life as assessed by PDQ-39 and calculated 'overall outcome changes' 36 months post transplantation
- Changes in F-DOPA PET in transplanted patients 36 months post transplantation

Safety Endpoints.

- The number of adverse events and serious adverse events associated with the neural transplant
- Laboratory parameters — any reported changes in hematology, biochemistry or urinalysis measures outside the normal range
- Other Safety parameters — vital signs, physical exam (new abnormalities are recorded as an adverse event)

Table 1 | The timetable of transplants and the reasons why planned surgeries were cancelled

	2015	2016	2017	2018*	Total
Theater slots	30	62	31	5	128
Completed procedures	7	9	4	1	21
Cancelled (due to)	23	53	27	4	107
Tissue supply	15	44	24	4	87
Tissue viability	1				1
Scheduling issues	2	6	3		11
Instruments	3				3
GMP airflow	2				2
Localization queries		2			2
Oncology case		1			1

Twenty-one transplant surgeries were completed across the two sites. This included ten bilateral grafts that were done sequentially (that is, at two different surgical operations), and one patient elected not to have a second transplant after their unilateral surgery. *Final procedure March 2018

either a good manufacturing practice (GMP) facility (in the United Kingdom) or a clean room (in Sweden). The SOPs were validated extensively in a series of preclinical in vitro and in vivo studies, including animal models of PD[13,14], to ensure that the tissue could consistently and reproducibly be dissected to yield the number of dopamine cells thought to be needed to repair the putamen in the PD brain (>100,000 cells per side grafted). Also, for the first time to our knowledge, we used hfVM tissue in a clinical trial that had been collected from medical and surgical terminations of pregnancy on the basis of pre-clinical validation showing that tissue collected via either route was equivalent in terms of dopaminergic cell yields following transplantation[15] (unpublished data).

Given the source of the cells in the TRANSEURO trial, all donors and recipients had HIV, hepatitis B, hepatitis C, human T-lymphotropic virus-1, toxoplasma and syphilis testing to ensure that no viral or spirochete transmission occurred and that there were no additional risks for disease reactivation in the host through immunosuppression in the post-operative period. Furthermore, donor tissue and recipients had to receive cytomegalovirus serology tests to ensure optimal donor matching to minimize any possible infective risk.

An absolute requirement for transplantation was that at least three hfVM samples from fetuses aged between 6 and 8 weeks post conception were available per side of the brain that underwent grafting surgery. This was based on previous data from open-label trials indicating that around 100,000 surviving dopaminergic neurons in the grafted putamen were needed for major clinical improvement and that a minimum of three hfVMs are needed to reach this number[16,17]. The tissue was also stored for no more than 4 days after dissection in Hibernate E and lazaroids[18], and the viability of the cell preparation on the day of surgery had to be >80%. These requirements meant that over the 3-year course of the transplant trial, many scheduled surgeries were cancelled because of insufficient amounts of tissue and on one occasion because of poor tissue viability (Table 1).This emphasizes the need to have a more readily available source of cells for grafting that does not rely on the unpredictable harvesting of fresh human fetal tissue. Indeed, we considered abandoning the transplant trial given the major logistical problems we encountered. While this was not done, we decided to continue to perform grafts in patients until either all 20 patients originally selected had received bilateral transplantation of hfVM or a 3-year period from the time of the first transplant had elapsed. The latter was reached first, and thus only 11 patients in the transplant arm of the TRANSEURO study received a graft.

The transplantation procedure. The transplantation approach targeting the putamen was devised on the basis of data from previous trials and the level of innervation seen around individual deposits of hfVM[17]. Thus, we adopted an established surgical implantation that had shown good post mortem evidence of coverage of the putamen with the grafted dopamine cells in terms of innervation.

Two unilateral stereotactic procedures per patient were performed while the patient was under general anesthesia, with five tracts per hemisphere: two were grafted into the pre-commissural putamen and three into the post-commissural putamen. Eight deposits per tract were made with 2.5 µl of cell suspension placed at each deposit along the needle tract, beginning at the bottom of each tract. A total volume of 100 µl of cell suspension was grafted per procedure. The tissue was delivered using a modified version of the Rehncrona instrument that was developed for the original Lund transplant studies of the 1980s and 1990s. The interval between the two surgeries varied from 4 to 35 weeks.

Broad-spectrum antibiotics were administered at the time of surgery to prevent introduction of infection.

The immunosuppressive regimen adopted. Immunosuppressive therapy was administered for 12 months post transplantation and comprised a standard triple therapy (see next paragraph). This was in line with what had been used in previous allograft trials of hfVM tissue in PD, in particular the Lund program.

Immunosuppression began the day before the first graft and consisted of: ciclosporin, 2 mg/kg twice a day (resulting in serum levels between 100–200 ng/ml); azathioprine, 2 mg/kg per day; and prednisolone, 40 mg per day, reduced to 5 mg per day by 12 weeks post

grafting. To mitigate possible side effects, the following agents were also administered daily: omeprazole and calcichew; co-trimoxazole three times a week; and alendronic acid once a week.

The follow-up, timing of primary endpoints and choice of comparator in the transplant trial. Transplantation of immature dopaminergic neuroblasts brings with it a need for long-term follow-up to monitor efficacy and possible delayed side effects. In the case of hfVM grafts, maximum benefit is probably not seen for several years — possibly as many as 3–5 years[19]. A primary endpoint that is sufficiently far from the time of grafting is therefore needed. We decided on a 3-year primary endpoint in TRANSEURO, which also reduces the likelihood of any placebo effects related to the surgery.

The optimal primary endpoint is debatable, but given that we are trialing a dopamine cell therapy, it makes sense to use an endpoint that is known to be very sensitive to this aspect of the pathology in PD[20]. As such, we elected to use the MDS-UPDRS Part III motor score of patients in the defined OFF period — while also collecting a large number of secondary endpoints (Box 2).

In addition to the trial steering committee, we set up an independent data safety monitoring committee that was involved in the transplant study and was asked to comment on adverse events and whether the trial should be stopped or suspended on the basis of such events or if it could continue.

Analysis of the observational data. The clinical outcomes were analyzed for trends over time using multilevel models, allowing each patient to have their own rate of decline (varying intercept and varying slope model). The main parameter of interest is the average rate of change across all patients, and only a linear effect of time was included. The models were fitted with Bayesian statistical software[21] and the brms R package[22]. The output of these models is the estimated annual rate of change and a 95% confidence interval (CI). *P* values (Fig. 2) indicate the probability that the effect is in the opposite direction (that is, patients improve over time). For each outcome, the time at which the 95% CI excludes the baseline value is calculated and represents when a change from baseline can be detected, providing an estimate of an outcome's sensitivity to detect changes. We also assessed whether age and disease duration (at baseline) could predict the rate of decline of MDS-UPDRS Part III, using a simple linear regression, but incorporating the uncertainty in the estimated rate of change values (Fig. 2).

Lessons learned from TRANSEURO and its implications

Observational study. The number of patients originally recruited to each site is listed in Fig. 1, and the numbers at each follow-up visit and the reasons for drop-out are given. These data reveal a number of issues that will inform studies moving forward. First, recruiting patients at this stage and age with PD is not problematic, but retaining them in studies is harder when the assessment protocols are long. This is compounded by the need for them to be assessed in the OFF medication state and the fact that there is no guarantee that they will be randomized into the treatment arm of the study. Furthermore, recruiting patients across multiple international sites generated its own problems in terms of: (1) differences in national regulation and status of observational studies; (2) oversight of assessments and staff; and (3) stability of that center as a research site. All of these are major issues when setting up long-term studies of this type that take many years to complete. In TRANSEURO, at one of the centers, the principal investigator resigned and his team dissipated, and thus we lost the ability to follow-up the entire patient cohort from this site.

We did find that the cohort we chose to study were well suited for trials of this type, as we had predicted, and there were no major problems recruiting such patients. In particular, we found (Fig. 2):

- Patients progressed in a linear fashion over a 3-year period with respect to their scores on the MDS-UPDRS Part III in the defined OFF state. This should therefore allow for disease modifications and/or deviations to be easily seen with any intervention over this time frame, including dopamine cell transplants. In particular, we found the total MDS-UPDRS motor score significantly increased by 3.9 points a year (95% CI = 3.0–4.8) with a change from baseline being detected at 7 months, demonstrating that the measure is sensitive to temporal disease progression.
- Figure 2 shows the estimated monthly rate of change in the MDS-UPDRS Part III motor score by age; error bars are standard errors, and the uncertainty in the rate of change is propagated into this analysis. Older patients deteriorated at a faster rate than younger patients, with the average 65-year-old deteriorating at nearly twice the rate as the average 40-year-old ($P = 0.023$). However, the considerable patient-to-patient variability means that the chance of a randomly selected 65-year-old deteriorating at a faster rate than a randomly selected 40-year-old is only 70%. There was little evidence of an association between disease duration and clinical outcomes.
- Patients did not develop any major cognitive problems over this time, which is what we anticipated given the inclusion and exclusion criteria that we adopted for patient recruitment. In particular, the total score for the Revised Addenbrooke's Cognitive Examination remained stable over the entire 36-month period and was well outside the cognitively impaired range.
- Patients did not develop significant dyskinesia, as assessed by the Abnormal Involuntary Movement Scale (AIMS). In fact, LID was seen in very few patients and then with minimal impact and only at the end of the 3-year observational period.

Implications. We would recommend using this nested trial approach for first-in-human studies of dopaminergic cells derived from stem cells, as it has many merits, such as inclusion of controls with natural history that is well-matched to patients for comparison against intervention and allowing for patients to act as their own controls. However, the extent of testing should be carefully considered with respect to what key information is actually needed in such a trial and how much information needs to be collected in the OFF medication state. This was especially problematic for the participants in our study and was the reason that several of them dropped out of the trial (Fig. 1). The reason for examining patients in the OFF medication state relates to the major fluctuation in motor features that occurs in some individuals even in early disease as a result of L-dopa replacement. One solution to this would have been to restrict recruitment to patients free from dopaminergic replacement. However, this would have placed a major restriction on the longitudinal aspects of the trial as well as created ethical concerns when it came to the transplant part of the study.

In retrospect, restricting the recruitment and follow-up of patients to one or two sites makes logistical and regulatory sense given some of the issues that we found in the setting up and execution of our observational study, which included issues with sponsorship and trial classification (Clinical Trial of an Investigational Medicinal Product (CTIMP) versus non-CTIMP).

Finally, the criteria for patient selection and assessment tools we have developed in TRANSEURO would seem to be a useful platform to build upon when moving forward. Whether there are additional measures that could be used should be further explored — especially around wearable devices, which currently hold great promise for monitoring disease progression. However, there are no major limitations with the assessments we have used to date in that they have enabled us to select what we think may be the optimal group for this type of intervention.

Transplant study. Eleven patients were randomly selected to undergo transplantation surgery out of 150 patients originally

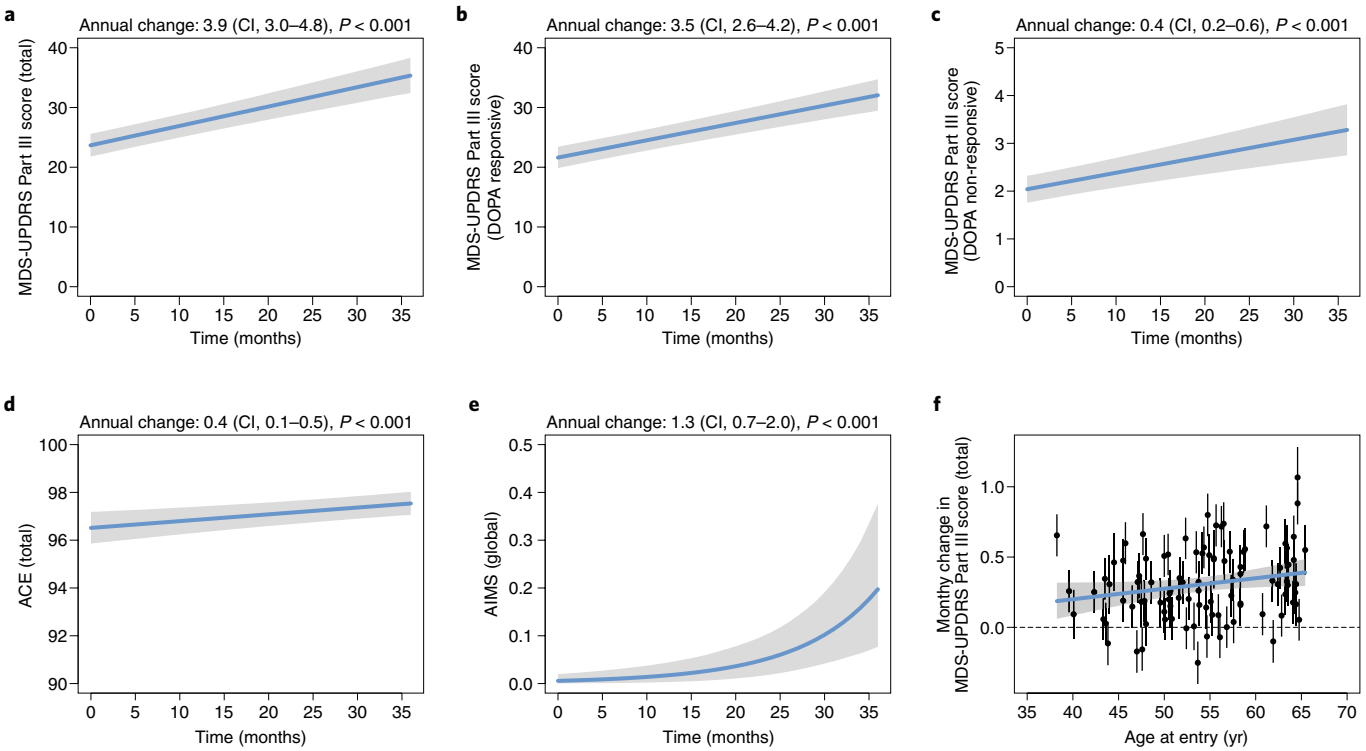


Fig. 2 | Data from the observational cohort at 36 months. a–e, The average change over time across all patients and 95% CIs (shaded regions) for key clinical outcomes. The annual rate of change (±95% CI) is reported above each graph. **f**, Age at entry and rate of decline; older patients deteriorate faster ($P = 0.023$). ACE, Addenbrooke's cognitive examination revised version.

recruited to the observational study. Originally, 20 patients were to receive a graft, but because of major issues with tissue supply (see Table 1), the number was reduced. This issue of tissue supply was the single biggest problem we encountered in this trial.

The principle of selecting patients from a cohort that has already been recruited and is being followed up over the long term and then applying the same assessment protocol to the patients who received grafts seems logical in early stage trials as it: (1) helps to ensure there are good pre-intervention clinical data defining the disease trajectory for individual patients (that is, they act as their own controls); (2) increases the likelihood that the diagnosis of PD is correct, given that misdiagnosis becomes evident as patients evolve and their response to dopaminergic medication becomes clearer; (3) guarantees a good contemporaneous comparator cohort that can be used to assess the intervention without the need for sham surgery early on in trials, which will be an issue in the first-in-human stem cell clinical trials for PD; and (4) ensures that any practice effects with tests have plateaued by the time of intervention.

Implications. It is clear that trials with hfVM tissue are not viable going forward given the problems of tissue supply (see Table 1). Thus, stem-cell-derived cells are essential if this field is to progress. We also believe that recruiting suitable individuals from an observational cohort of patients who have been followed-up for at least 12–24 months before any planned intervention would be ideal. Finally, patients should be tested on an assessment protocol that can not only be used to follow disease course, but also serve to detect a signal of graft efficacy, and it should ideally involve scoring from video recordings of patients wearing caps to minimize investigator bias in these assessments.

Imaging in TRANSEURO. We have confirmed that the two imaging markers assessing the dopaminergic system correlated well with clinical measures, such as the MDS-UPDRS motor scores, and both tracked disease progression in the observational cohort. In particular, the DAT ligand [^{11}C]PE2I seemed to have a greater predictive value and sensitivity for detecting differences in motor impairment than aromatic amino acid decarboxylase (AADC) imaging using [^{18}F]DOPA. Furthermore, DAT decline seemed to be closely associated with the decline in motor progression over time, whereas no such relationship was found with AADC, suggesting that [^{11}C]PE2I is a more objective biomarker than [^{18}F]dopa for investigating the effects of novel interventions[23].

Implications. Ideally, one would want to be able to image everyone in the observational cohort as well as after grafting for all individuals who underwent that procedure, but this is not financially viable using PET imaging because of the costs of the scans (typically >£5,000 per scan in the United Kingdom). Thus, we elected to scan only patients chosen to receive a graft along with a matched-control patient. We found that several measures of dopaminergic function were well suited for assessing dopaminergic cell transplants, including [^{11}C]PE2I and [^{18}F]dopa. For assessing stem-cell-derived transplants, [^{11}C]DASB scanning (detecting serotoninergic neurons) will most likely not be needed given that such stem cell products can be generated at a purity that they should not contain significant numbers of 5-HT neurons. The need for fMRI studies is debatable for first-in-human studies, but structural MRI scans will be needed to monitor graft placement and growth as well as to facilitate the processing and analysis of PET data post transplantation.

The transplantation procedure. The surgical approach for transplantation to the putamen was not seen as a critical issue given that we had post mortem evidence to show that the method we were adopting resulted in good dopamine cell survival and innervation across the striatum with the graft (for example, ref. [17]). However, what did emerge as a major consideration was the instrument to be used for tissue delivery since the original studies using cell-suspen-

sion approaches employed a non-certification (CE)-marked device developed in-house in Lund by S. Rehncrona and J. Legradi[24].

This instrument had been shown to deliver cells in the volume and with the accuracy needed for work of this type, but the absence of a CE marking meant that it could not be used at other hospitals. Thus, at the surgical center in the United Kingdom, a new device had to be made in-house that was based on this original instrument, which was not without problems, especially with respect to the ease with which the delivery device could be used within the outer guide sheath. This will be a major issue going forward, as the routine clinical use of any stem-cell-based dopamine product for PD will require an instrument that is: (1) easy to use, disposable and safe; (2) available to use at any surgical centre/hospital (thus CE marked for European use and approved by the Food and Drug Administration for use in the United States) and (3) shown not to adversely affect the viability of cells delivered to the striatum. Work to build this instrument is now underway.

Implications. Studies using new stem-cell-based dopamine therapies will need to carefully consider the delivery method. This is not so much referring to target sites and volume, but rather the actual instrument used for delivery. Evidence must be provided that such an instrument is compatible not only with most neurosurgical centers' practice, but also with the survival of implanted cells and their capacity to reinnervate the denervated striatum.

The immunosuppressive regimen. The regimen for preventing rejection of the transplant is still debated, as is evident from previous hfVM trials in which treatment has varied from the use of no immunosuppression[5], monotherapy with ciclosporin A (CyA) for 6 months[6] and triple therapy with CyA, azathioprine and steroids for at least 12 months post grafting[25]. While no firm conclusions can be drawn, the duration and completeness of immunosuppression appear to be important for optimal, long-term graft survival. However, the choice of which regimen to adopt is not clear, especially given the problems in monitoring any rejection process in an intracerebrally placed graft. This, coupled to the fact that it is known that (1) human fetal tissue expresses low levels of major histocompatibility complex antigens, which are upregulated in the presence of inflammatory cytokines (as is found at the graft site)[26], (2) post-mortem studies in patients with grafts in the early post implantation period have an inflammatory infiltrate around the grafts[16] and (3) long-term graft survival can be obtained in the absence of long-term immunosuppression[17,19], led us to adopt triple therapy of the type used previously. This was given for 12 months post grafting. In addition, the side effects of such a short-term immunosuppressive regimen are relatively benign, and also, many of the long-term complications associated with these drugs (for example, increased risk of solid tumor development with CyA) are avoided. In our trial, we only had three major problems with this regimen: one patient developed an azathioprine-related colitis, which required this drug to be stopped; another patient developed a Kaposi sarcoma that resolved once the immunotherapy was discontinued; and a third showed mild signs of hepatotoxicity (increased aspartate aminotransferase values) that completely resolved once the azathioprine was discontinued and replaced by mycophenolate.

Implications. For future stem cell trials, there seems to be a need for immunosuppressive treatment to be continued for 12 months post grafting[27,28]. The optimal regimen is unclear, and it also cannot be assumed that the graft composition and the presentation of antigens are the same in fetal and stem-cell-derived grafts. Some form of combination therapy, as is used in solid organ transplant programs, would be best, although improved ways to measure intracerebral graft rejection are still urgently needed.

Follow-up, timing of primary endpoints and choice of comparator. The selection of the outcome measure must include some readout around the dopamine-responsive aspects of PD, given this is what the therapy is designed to treat. Motor measures in the OFF medication state are optimal for doing this, although changes in scores in the ON medication state and non-motor symptoms will enable assessment of any impact that the grafts have on non-dopaminergic aspects of disease progression and non-motor dopaminergic features of PD.

It is unclear what constitutes a major quantitative improvement, but we estimate that at least a 30–50% improvement post grafting relative to baseline is needed in the MDS-UPDRS Part III motor OFF score for this therapy to be viewed as competitive given its invasive nature, irrespective of looking for an effect that is greater than that which could be explained through any placebo effect[29]. While this was a reason for not using sham surgery in TRANSEURO, it was not the main one. The major reason for having no sham surgery was that this trial was not undertaken to prove that this therapy could be taken forward for clinical adoption as a standard of care; rather, it was done to try and establish the trial framework for the next generation of stem-cell-derived dopaminergic cells for PD. Namely it was undertaken as a further proof-of-principle study. In addition, there were also ethical concerns, given that patients in a non-interventional sham surgery arm would receive immunosuppressive drugs and be tied into a trial that would prevent them from having other possible experimental treatments for at least 3 years.

Implications. The first new trials with stem-cell-derived dopamine cells will need to address tolerability and feasibility, with an emphasis on safety rather than efficacy. However, we would recommend long-term follow-up for all patients recruited into cell therapy trials, ideally indefinitely, and that these patients make declarations of intent for post mortem brain donation so that the long-term benefits and histological effects of these (irreversible) interventions can be best described. We would also recommend that a variety of endpoints are chosen that primarily focus on the dopaminergic aspects of PD since this is what is being treated, but that these endpoints also include cognitive, motor and quality-of-life measures. At some point, a double-blind sham surgery trial should be considered, or at least if not sham surgery, some form of competing invasive therapeutic. Exactly when this should be done is unresolved, as the Food and Drug Administration would recommend that this be a part of the first-in-human study, while we and others would advocate that this is best done once one has worked out how to optimally deliver the right dose of cells to the right patient.

Conclusion

In this Perspective, we have summarized the rationale and structure of the clinical trial design for both the observational natural history and hfVM transplant arms of TRANSEURO and have detailed the lessons we have learned en route. We describe how all this can be used to optimize stem-cell-based dopamine replacement trials entering the clinic. Importantly, stem-cell-derived neurons have the potential to provide solutions to two of the major problems highlighted by the TRANSEURO trial that hinder the further development of hfVM transplantation toward a clinically competitive treatment for PD. First, the stem-cell-derived neurons will be available in large numbers, and each transplantation session can, therefore, be safely planned in advance, avoiding the multiple cancellations of surgeries due to lack of hfVM tissue and markedly increasing the number of patients that receive a graft. Second, compared with the variability in outcomes of the grafts comprising hfVM tissue from several donors of different ages used in the TRANSEURO trial, outcome variability will conceivably be much less following the transplantation of well-characterized stem-cell-derived cell populations. It is important to emphasize, though, that transplantation of stem-cell-

derived dopaminergic neurons to the striatum, even if it leads to improvement of motor features, will never be a cure for PD. The degeneration of other neuronal systems, as is seen in all patients with PD, will continue, and dopamine-resistant motor features and non-motor symptoms will most likely not be affected by the intrastriatal dopaminergic grafts. A stem-cell-based dopamine-replacement therapy will only be clinically competitive in the long term for patients with PD if the motor improvements outweigh the worsening of non-motor symptoms.

Received: 19 February 2019; Accepted: 3 June 2019;
Published online: 1 July 2019

References

1. Goedert, M., Spillantini, M. G., Del Tredici, K. & Braak, H. 100 years of Lewy pathology. *Nat. Rev. Neurol.* **9**, 13–24 (2013).
2. Schapira, A. H. V., Chaudhuri, K. R. & Jenner, P. Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* **18**, 509 (2017).
3. Barker, R. A., Drouin-Ouellet, J. & Parmar, M. Cell-based therapies for Parkinson disease — past insights and future potential. *Nat. Rev. Neurol.* **11**, 492–503 (2015).
4. Palfi, S. et al. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. *Lancet* **383**, 1138–1146 (2014).
5. Freed, C. R. et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **344**, 710–719 (2001).
6. 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).
7. Barker, R. A., Barrett, J., Mason, S. L. & Bjorklund, A. Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. *Lancet Neurol.* **12**, 84–91 (2013).
8. Kirkeby, A., Parmar, M. & Barker, R. A. Strategies for bringing stem cell-derived dopamine neurons to the clinic: A European approach (STEM-PD). *Prog. Brain. Res.* **230**, 165–190 (2017).
9. Liu, G. et al. Prediction of cognition in Parkinson's disease with a clinical-genetic score: a longitudinal analysis of nine cohorts. *Lancet Neurol.* **16**, 620–629 (2017).
10. Politis, M. et al. Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. *Sci. Transl. Med.* **2**, 38ra46 (2010).
11. Piccini, P. et al. Factors affecting the clinical outcome after neural transplantation in Parkinson's disease. *Brain* **128**, 2977–2986 (2005).
12. 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).
13. Piroth, T. et al. Transplantation of human fetal tissue for neurodegenerative diseases: validation of a new protocol for microbiological analysis and bacterial decontamination. *Cell Transplant.* **23**, 995–1007 (2014).
14. 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).
15. Kelly, C. M. et al. Medical terminations of pregnancy: a viable source of tissue for cell replacement therapy for neurodegenerative disorders. *Cell Transplant.* **20**, 503–513 (2011).
16. 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).
17. Li, W. et al. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. *Proc. Natl Acad. Sci. USA* **113**, 6544–6549 (2016).
18. 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).
19. Kefalopoulou, Z. et al. Long-term clinical outcome of fetal cell transplantation for Parkinson disease: two case reports. *JAMA Neurol.* **71**, 83–87 (2014).
20. Fahn, S. et al. Levodopa and the progression of Parkinson's disease. *N. Engl. J. Med.* **351**, 2498–2508 (2004).
21. Carpenter, B. et al.Stan: a probabilistic programming language. *J. Stat. Softw.* **76**, https://doi.org/10.18637/jss.v076.i01 (2017).
22. Bürkner, P. brms: An R Package for Bayesian Multilevel Models Using Stan. *J. Stat. Softw.* **80**, https://doi.org/10.18637/jss.v080.i01 (2017).
23. Li, W. et al. (11) C-PE2I and (18) F-Dopa PET for assessing progression rate in Parkinson's: A longitudinal study. *Mov. Disord.* **33**, 117–127 (2018).
24. Lindvall, O. et al. Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. *Ann. Neurol.* **31**, 155–165 (1992).
25. 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).
26. Laguna Goya, R., Busch, R., Mathur, R., Coles, A. J. & Barker, R. A. Human fetal neural precursor cells can up-regulate MHC class I and class II expression and elicit CD4 and CD8 T cell proliferation. *Neurobiol. Dis.* **41**, 407–414 (2011).
27. Liu, X., Li, W., Fu, X. & Xu, Y. The immunogenicity and immune tolerance of pluripotent stem cell derivatives. *Front. Immunol.* **8**, 645 (2017).
28. Scheiner, Z. S., Talib, S. & Feigal, E. G. The potential for immunogenicity of autologous induced pluripotent stem cell-derived therapies. *J. Biol. Chem.* **289**, 4571–4577 (2014).
29. Galpern, W. R. et al. Sham neurosurgical procedures in clinical trials for neurodegenerative diseases: scientific and ethical considerations. *Lancet Neurol.* **11**, 643–650 (2012).

Acknowledgements

The authors acknowledge D. Marsden, N. Cawthorne and T. Stone from the Cambridge University Hospital Clinical Engineering Innovation (CEI) who helped in the development of the in house delivery device in Cambridge, and K. Richardson, K. Jestice, and M. Scott from the Cambridge Cellular Therapy Laboratory, Cambridge University Hospitals NHS Foundation Trust who helped in developing the SOPs for the hfVM tissue preparations in Cambridge.

This study was supported by an EU FP7 grant (242003) as well as funding from the Cure Parkinson's Trust (RG81537), John Black Charitable Trust and Multipark. The work was also supported by NIHR funding of Biomedical Research Centres at UCL and Cambridge (146281). R.A.B. is an NIHR Senior Investigator (NF-SI-0616-10011) and a PI in the MRC/WT Stem Cell Institute (203151/Z/16/Z).

Competing interests

R.A.B. advises Living Cell Technologies, FujiFilm Cellular Dynamics Inc; BlueRock Therapeutics; Novo Nordisk; Sana Therapeutics and Cellino Biotech on their cell-based therapies for PD as well as UCB, Roche and Lundbeck on other aspects of neurodegenerative disorders of the brain. T.P. has received honorary fees from Boston Scientific for peer-to-peer workshops (related to DBS) in 2018 and 2019. T.F. advises Living Cell Technologies on their cell-based therapy, as well as Bial, Profile Pharma, Peptron and Boston Scientific on other aspects of neurodegenerative disorders of the brain. A.B. is a consultant for Novo Nordisk. M.P. is the owner of Parmar Cells AB and co-inventor on US patent applications 15/093,927 owned by Biolamina AB and EP17181588 owned by Miltenyi Biotec. Patent WO 2015/114059 A1 patents the use of BCL2 in reprogramming. S.P. advises Oxford Biomedica and Axovant

Additional information

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence should be addressed to R.A.B.

Peer review information: Joao Monteiro was the primary editor on this perspective and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

TRANSEURO consortium

Krista Farrell[2], Natalie Valle Guzman[2], Xiaoling He[2], Stanley E. Lazic[4], Sarah Moore[2], Robert Morris[5], Pamela Tyers[2], Ruwani Wijeyekoon[2], Danielle Daft[2], Sam Hewitt[2], Viswas Dayal[6,7], Thomas Foltynie[6,7], Zinovia Kefalopoulou[6,7], Philipp Mahlknecht[6,7], Nick P. Lao-Kaim[8], Paola Piccini[8],

Hjalmar Bjartmarz[9], Anders Björklund[9], Olle Lindvall[10,11], Jenny Nelander-Wahlestedt[9], Malin Parmar[9], Gesine Paul[12], Hakan Widner[12], Alistair Church[4], Stephen Dunnett[4], Kathryn Peall[4], Anne Rosser[4], Jean Marc Gurruchaga[13], Stéphane Palfi[13], Tobias Piroth[14] and Christian Winkler[14]

[4]Brain Repair Group, School of Biosciences, Cardiff University, Cardiff, UK. [5]Cambridge University Hospital NHS Foundation Trust, Addenbrookes Hospital, Hills Road, Cambridge, UK. [6]Department of Clinical and Movement Neurosciences, University College London Queen Square Institute of Neurology, London, UK. [7]The National Hospital for Neurology and Neurosurgery, London, UK. [8]Neurology Imaging Unit, Centre of Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Hammersmith Campus, Imperial College London, London, UK. [9]Wallenberg Neuroscience Center and Lund Stem Cell Center, Lund University, BMC, Lund, Sweden. [10]Laboratory of Stem Cells and Restorative Neurology, University Hospital, Lund, Sweden. [11]Lund Stem Cell Center, Lund University, BMC, Lund, Sweden. [12]Neurology Clinic, Skåne University Hospital, Lund, Sweden. [13]Department of Neurosurgery, Assistance Publique-Hôpitaux de Paris, PePsy Department, Groupe Hospitalier Henri-Mondor Albert-Chenevier, Créteil, France. [14]Laboratory of Molecular Neurosurgery, Department of Stereotactic Neurosurgery, Freiburg University, Freiburg, Germany.