DESIGNING STEM CELL-BASED DOPAMINE CELL REPLACEMENT TRIALS FOR PARKINSON'S DISEASE

Roger A Barker on behalf of the TRANSEURO consortium

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ABSTRACT

Clinical studies of dopamine cell replacement therapies for Parkinson's disease (PD) go back more than 30 years. The outcomes using transplantation of human fetal ventral mesencephalic tissue (hfVM) have been variable, with some patients coming off their anti-PD treatment for many years, while other patients have not responded and/or developed significant side effects, including graft-induced dyskinesias. This led to a re-appraisal of how trials should best be done which resulted in a new EU funded allograft trial with fetal dopamine cells across several centres in Europe. This new trial, TRANSEURO[1] (NCT01898390); is an open label study in which patients were randomly selected for transplantation out of a larger observational cohort of patients with mild PD undergoing identical assessments. The TRANSEURO trial is currently ongoing, having completed both recruitment into a large multicentre observational study of younger onset early stage PD as well as 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 lessons we have learned along the way, can help to inform researchers and facilitate planning of human pluripotent stem cell-derived dopamine cell transplants for future clinical trials.

Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder that is characterised by the loss of nigrostriatal dopaminergic neurons and the development of a movement disorder typically in the 7th to 8th 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) as well as involving the enteric and autonomic nervous systems [2]. This widespread pathology explains many of the non-motor abnormalities patients which PD experience, only some of which are responsive to dopaminergic medications [3]. 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 leading to neuropsychiatric and autonomic problems as well as dyskinesias through the non-physiological stimulation of dopaminergic receptors in the striatum. As a result, there has long been an interest in using different approaches to target selectively the loss of dopamine at the site of greatest depletion, namely the putamen, including gene and cell based therapies [4,5]. These therapies have now both been trialled in patients with mixed benefits.

In 2006, a new international initiative was conceived followed by a series of meetings to re-evaluate the merit of dopamine cell-based therapies for PD. This was deemed necessary given the contrasting outcomes of the two NIH funded human fetal ventral mesencephalic (hfVM) allograft trials in PD that were published in 2001 and 2003 [6,7]compared to earlier open-label studies using similar tissue (reviewed in [8]). These NIH funded double-blind placebo-controlled studies reported no benefits in grafted versus sham-operated patients and, in addition, significant numbers of patients developed side effects in the form of graft-induced dyskinesias (GIDs). These results were at odds with earlier open-label studies reporting 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 PD. Based on these discussions and an analysis of the raw

data collated from all major hfVM trials in PD, the 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 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 dyskinesias may benefit the most from hfVM grafts as well as being less likely to develop GIDs.

Based on this reasoning, a new EU funded transplant trial in Europe using hfVM tissue, called TRANSEURO 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 human stem cell derived midbrain dopaminergic neurons. This TRANSEURO multicentre study had two major arms; (i) an observational study charting the natural history of younger onset, early stage PD (N>100) (the group thought to be optimal for dopamine cell therapies); and (ii) a transplant arm involving patients randomly selected from this observational cohort (provided they had continued adherence to eligibility criteria)[1].

The observational study is still ongoing with a significant proportion of the cohort being followed up to the present day. The transplant arm has now been completed with 11 patients grafted over a 3 year period (2015-2018). The outcome of these hfVM transplants will be evaluated in 2021 using the predefined primary end point of the trial, which will be the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part 3 score in the defined "OFF" state. We will also compare the transplant group against the trajectory 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 in 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 end-point in the TRANSEURO transplant trial will be reported at a time when several stem cell-derived dopamine transplant clinical trials for PD will have already started[9].

The TRANSEURO study (

FIGURE 1 HERE

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; National Hospital for Neurology and Neurosurgery, London, UK; University of Cardiff, UK; Skåne University Hospital, Lund, Sweden; Freiburg University, Germany and at the Assistance Publique-Hôpitaux de Paris (APHP), France. Inclusion and Exclusion criteria are detailed in Text Box 1.

TEXT BOX 1 HERE

After recruitment, patients were seen every 6 months in the "OFF" medication state and a detailed number of assessments were undertaken (see Figure 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: (i) the most widely recognised clinical assessments were included for ease of cross study comparisons; (ii) all included measures were validated and (iii) all measures could be completed in a timely manner and were acceptable to participants.

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

Finally, despite randomisation, surgical trials involving small numbers of patients can be vulnerable to outlying data from individuals with conspicuously fast or slow rates of disease progression/ atypical responses to interventions, some of which have a genetic basis. To try and partially mitigate against this, all participants are currently being genotyped for the common known PD genes, some of which have been documented to play a role on rate of disease progression[10].

Throughout the study, the patients' medication 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 to 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 down the line for other experimental cell and gene-based dopamine therapies for their PD as well as for DBS.

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) to act as a matched control population in terms of clinical assessments and PET imaging. Some additional measures were added (see Text Box 1 and Figure 1). These criteria were carefully chosen to ensure patients participating in the trial were likely to be at the lowest risk from the transplantation procedure and yet have the highest chance of clinical benefit, while remaining representative of the main population of PD patients in the early stages of the disease. Given the longitudinal nature of the project, and concerns regarding issues such as GIDs, it was essential during the patient selection process for transplantation that the criteria at baseline were reapplied at the time of grafting to avoid recruiting patients who had developed significant L-dopa induced dyskinesias (LIDs) during the observational follow up period. This period of observational follow up

was extremely valuable in allowing for the assessment of the rate of progression of motor severity of early PD using the MDS-UPDRS part 3 in both the OFF and ON drug conditions. The MDS-UPDRS part 3 is a well validated standard clinical assessment tool used to measure motor function in PD and was the reason 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 vs OFF assessments.

Finally, the assessment of patients in the transplant arm of TRANSEURO was identical to that undertaken in the observational arm. The primary outcome chosen is the change in MDS-UPDRS part 3 motor score after a defined period of medication withdrawal (Practically defined OFF) at 36 months after their final transplant, comparing these patients with those not grafted (see Text 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 3 trajectory could be assessed according to treatment allocation.

TEXT BOX 2 HERE

Imaging studies done as part of this study

Anatomical imaging using MRI was undertaken in the selected transplant cohort ahead of grafting to plan their implantation surgery. MRI scanning was also routinely done in the immediate post-operative period to assess for (i) graft placement; (ii) any complications such as haemorrhages or other abnormalities.

In addition, MRI and PET imaging studies were undertaken to look at functional aspects of the transplant, in particular its ability to normalise network activity as well as dopaminergic content and dopamine transporter (DAT) expression and dopamine transporter (DAT) expression as assessed with ^{18}F-dopa and ^{11}C-PE2I respectively as well as contamination by 5HT neurons (using ^{11}C-DASB PET imaging) given the possible role of serotonin in the genesis of GIDs [11](see Figure 1). In addition, we used ^{18}F -dopa PET imaging as an inclusion/exclusion criterion for the transplant arm (see Text Box 1), given that it has previously been reported that significant preoperative ventral striatal dopamine loss is associated with less successful outcomes in patients receiving hfVM transplants [12].

The functional and structural imaging studies using MRI was done to ascertain whether the transplant could restore cortical networks back to a more normal state [13]. This included resting state network activity, (RSNs- fMRI), motor task activation (ME fMRI) and diffusion tensor imaging (DTI). This PET and MRI imaging battery was repeated every 18 months in the grafted patients.

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

Human fetal VM tissue (hfVM) preparation for transplantation

The preparation of the hfVM tissue for grafting in patients in the TRANSEURO trial was modified such that a more defined set of standard operating procedures (SOPs) compared to the previous trials were followed. Tissue dissection and preparation was undertaken in either a GMP facility (in the UK) 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 [14,15], 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). We also, for the first time, in a clinical trial used hfVM tissue that had been collected from medical as well as surgical terminations of pregnancy, based on pre-clinical validation showing that tissue collected via either route was equivalent in terms of dopaminergic cell yields following transplantation[16] (unpublished data).

Given the source of the cells in the TRANSEURO trial, all donors and recipients had HIV, HBV, HCV, HTLV-1, HSV, chlamydia and syphilis testing to ensure that no viral or spirochaete 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 have CMV, EBV and toxoplasma serology – to ensure optimal donor matching around possible infective risk.

An absolute requirement to be fulfilled for transplantation was that we had tissue from at least 3 hfVM specimens per side grafted, aged between 6-8 weeks post conception. This was based on previous data from open label trials indicating that around 100,000 surviving DA neurons in the grafted putamen were needed for major clinical improvement, and that a minimum of 3 hfVMs are needed to reach this number[17,18]. The tissue was also stored for no more than 4 days after dissection in Hibernate E and lazaroids[19] 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 (see Table 1).This emphasises 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, consideration was given for abandoning the transplant trial given the major logistical problems we encountered. While this was not done, a decision was made that we would continue to graft patients until either all 20 selected cases had been transplanted bilaterally or a 3 year period from the time of the first transplant had elapsed. The latter was reached first and thus only 11 patients were grafted in the transplant arm of the TRANSEURO study.

The transplantation procedure

The transplantation approach targeting the putamen was deduced using data from previous trials and the level of innervation seen around individual deposits of hfVM[18]. 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 undertaken under general anaesthesia with 5 tracts per hemisphere: 2 into the pre-commissural and 3 into the post-commissural putamen. Eight deposits per tract were made with 2.5ul of cell suspension placed at each deposit along the needle tract commencing at the bottom of the target. A total volume 100 ul of cell suspension was grafted per procedure. The tissue was delivered using a modified version of the Rehncrona instrument developed for the original Lund transplant studies of the 1980s/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 regime adopted

Immunosuppressive therapy was given for 12 months post transplantation and comprised a standard triple therapy (see below). This was in line with what had been used in previous allograft trials of hfVM tissue in PD, in particular the Lund program in the 1980s/1990s.

The immunosuppression was started the day before the 1st graft and consisted of: Ciclosporin, 2mg/kg twice a day (giving serum levels between 100-200ng/ml); Azathioprine, 2mg/kg per day; Prednisolone, 40mg per day, reducing to 5mg by 12 weeks. To cover possible side effects, the following agents were also given daily; Omeprazole and Calcichew; Co-trimoxazole 3x a week and Alendronic acid once a week.

The follow up, timing of primary end points 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 much as 3-5 years[20]. A primary end-point that is sufficiently far from the time of grafting is therefore needed. We decided to have a 3 year primary end point in TRANSEURO, which also reduces the likelihood of any placebo effects related to the surgery.

As to what is the optimal primary end point is debatable, but given that we are trialling a dopamine cell therapy-it makes sense to use an end point that is known to be very sensitive to this aspect of the pathology in PD[21]. As such, we elected to use the MDS-UPDRS part 3 motor score in the defined OFF period – while also collecting a large number of secondary end points (see Text 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 asked to comment on adverse events and whether the trial should be stopped or suspended based on such events or could continue.

Data analysis of the observational data

The clinical outcomes were analysed 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[22] and the brms R package[23] . The output of these models is the estimated annual rate of change and a 95% confidence interval (CI). P-values (see Figure 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 UPDRS Part III, using a simple linear regression, but incorporating the uncertainty in the estimated rate of change values (Figure 2).

Lessons learnt from the TRANSEURO study and its implications for future cell based trials in PD

Observational study

The number of patients originally recruited to each site is given in Figure 1, and the numbers at each follow-up visit are also recorded and the reasons for drop-out are given. These data reveal a number of issues that will inform studies moving forward. Firstly, 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 OFF treatment and the fact that there is no guarantee that they will be randomised into the treatment arm of the study. Furthermore, recruiting patients across multiple international sites generated its own problems in terms of: (a) differences in national regulation of observational studies and its application to the trial; (b) oversight of assessments and staff; (c) stability of that centre as a research site. All of these are major issues when setting up long-term studies of this type, taking many years to complete. In TRANSEURO, at one of the centres, the PI 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 (see Figure 2):

- Patients progressed in a linear fashion over a 3 year period with respect to their scores on the MDS-UPDRS part 3 in the defined OFF state. This should therefore allow for disease modifications/deviations to be easily seen with any intervention over this time frame including dopamine cell transplants. In particular, we found the total 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 UPDRS-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, as we anticipated given the inclusion/exclusion criteria we adopted for patient recruitment. In particular, the total score for the Revised Addenbrooke's Cognitive Examination (ACE-R) remained stable over the entire 36 month period and was well outside the cognitively impaired range.
- Patients did not develop significant dyskinesias as assessed by the Abnormal Involuntary Movement Scale (AIMS). In fact, LIDs were 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 stem cell derived dopamine cell studies as it has many merits in terms of a well-matched natural history control for comparison against intervention, as well as allowing for patients to act as their own controls. However, the extent of testing should be carefully considered as to what key information is actually

needed in such trials and how much of this needs to be collected in the OFF-medication state. This is especially problematic for the participants and was the cause of several of them dropping out from the trial (see Figure 1). The reason for looking at the "OFF" medication condition 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 creating ethical concerns when it came to the transplant part of the study.

In retrospect, restricting the recruitment and follow-up of patients to 1-2 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 patient selection criteria 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 for transplantation out of 150 patients recruited to the observational study. Originally, the number to be grafted was 20 patients, but because of major issues of tissue supply (see above), 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 is already recruited and being followed up long term, and then applying the same assessment protocol to the grafted patients seems logical in early stage trials as it: (i) helps to ensure there are good pre-intervention clinical data defining the disease trajectory for individual patients (i.e., they act as their own controls); (ii) 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; (iii) guarantees a good contemporaneous comparator cohort by which 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 (iv) 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 above). 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, that have been followed up for at least 12-24 months prior to any planned intervention would be ideal. Finally patients should be tested on an assessment protocol that can be used not only to follow disease course but can also serve to detect a signal of graft efficacy and ideally should involve scoring from video recordings of patients wearing caps to minimise investigator bias in these assessments.

Imaging in TRANSEURO

We have confirmed that imaging markers assessing the dopaminergic system correlated well with clinical measures such as the MDS-UPDRS motor scores, both of which tracked disease progression

in the observational cohort. In particular, the dopamine transporter (DAT) ligand ^{11}C-PE2I seemed to have a greater predictive value and sensitivity for detecting differences in motor impairment than 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[24].

Implications:

Ideally one would want to be able to image everyone in the observational cohort as well as post grafting but this is not financially viable using PET imaging because of the costs of the scans (typically >£5000 per scan in the UK). Thus, we elected to scan only patients chosen for grafting 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 5HT neurons. As for the need for fMRI studies, this is debatable for first in-human studies, but structural MRI scans will be needed to monitor graft placement and growth as well as 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 gave good dopamine cell survival and innervation across the grafted striatum (e.g. Li et al 2016). However, what did emerge as a major consideration was the instrument to be used for tissue delivery, given the original studies using cell suspension approaches employed a non-CE marked device developed in house in Lund by Stig Rehncrona and Janos Legradi [25].

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 in other sites. Thus, at the surgical centre in the UK, a new device had to be made in house 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 is 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: (i) easy to use, disposable and safe; (ii) available at any site (thus CE marked for European use and FDA approved for use in the US) and (iii) shown not to adversely affect the viability of cells delivered to the striatum. Work to better define this instrument is now being undertaken.

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 for delivery. Evidence must be provided that such an instrument is not only compatible with most neurosurgical centres' practice but also with the survival of implanted cells and their capacity to reinnervate the denervated striatum.

The immunosuppressive regime

The regime for preventing rejection of the transplant is still debated as evident from previous hfVM trials in which treatment has varied from no immunosuppression [6], monotherapy with ciclosporin A (CyA) for 6 months[7], or triple therapy with CyA, azathioprine and steroids for at least 12 months

post-grafting[26]. 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 regime 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; (i) human fetal tissue expresses low levels of MHC antigens, which are upregulated in the presence of inflammatory cytokines (as is found at the graft site)[27]; (ii) post mortem studies in grafted patients from the early post implantation period has revealed that there is an inflammatory infiltrate around the grafts[17] and (iii) long term graft survival can be obtained in the absence of long term immunosuppression[18,20], 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 regime are relatively benign and also many of the long-term complications associated with these drugs (e.g. increased risk of solid tumour development with CyA) are avoided. In our trial we only had three major problems with this regime- 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 (AST) values), that completely resolved once the azathioprine was discontinued and replaced by mycophenolate.

Implications:

For future stem cell trials, there would seem to be a need for immunosuppressive treatment to be continued for 12 months post grafting[28,29]. The optimal regime is unclear, and it also cannot be assumed that the graft composition and the presentation of antigens are the same in fetal as in stem cell-derived grafts. Some form of combination therapy, as is used in solid organ transplant programmes, would be best, although improved ways to measure intracerebral graft rejection are still urgently needed.

Follow up, timing of primary end points and choice of comparator

The selection of the outcome measure must include some read out around the dopamine responsive aspects of PD, given this is what the therapy is designed to treat. Motor measures in the “OFF” state are optimal for doing this although changes in ON scores 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.

As to what constitutes a major quantitative improvement is unclear, but we estimate that at least a 30-50% improvement is needed in the MDS-UPDRS part 3 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[30]. 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 based dopamine 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 be in receipt of 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 cell will need to address tolerability and feasibility, with an emphasis on safety rather than efficacy. However, we would recommend that all patients recruited into cell therapy trials are followed up long term, ideally indefinitely, with declarations of intent for 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 end points are chosen that primarily focus on the dopaminergic aspects of PD, given that this is what one is treating, but that this also includes cognitive, motor and quality of life (QoL) 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 FDA would recommend that this is part of 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 cell to the right patient.

Conclusion

In this Perspective, we have summarised the rationale and structure of the clinical trial design for both the observational natural history and hfVM transplant arms of TRANSEURO and laid out the lessons we have learnt en route, and how all this can be used to optimize stem cell-based dopamine replacement trials entering the clinic. Importantly, the stem cell-derived neurons have the potential to provide solutions to two of the major problems highlighted by the TRANSEURO trial and hindering the further development of hfVM transplantation towards 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 numbers of patients that can be transplanted. Second, compared to the grafts comprising hfVM tissue from several donors of different ages used in the TRANSEURO trial, the variability in outcome will conceivably be much less by 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 leading 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 long-term for the PD patients if the motor improvements outweigh the worsening of non-motor symptoms.

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Conflict of interest:

RAB advises Living Cell Technologies, FujiFilm Cellular Dynamics Inc; BlueRock Therapeutics; Novo Nordisk; Sana Therapeutics and Cellino Biotech on their cell based therapies for Parkinson's disease as well as UCB, Roche and Lundbeck on other aspects of neurodegenerative disorders of the brain.

Tobias Piroth: Not related to this research: I received honorary fees from Boston Scientific for peer-to-peer workshops (related to DBS) in 2018 and 2019

TF 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.

Anders Bjorklund is a consultant for Novo Nordisk

Malin Parmar 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.

Stéphane Palfi advises Oxford Biomedica and Axovant

TEXT BOX 1

INCLUSION and EXCLUSION CRITERIA for PATIENTS IN THE OBSERVATIONAL TRANSEURO STUDY- IN RED ARE THOSE ADDITIONAL ONES FOR THE TRANSPLANT COHORT

Inclusion criteria

- PD as defined using Queen Square Brain Bank criteria
- Disease duration **≥ 2 year and ≤ 13 years**
- Aged ≥ 30 years and **≤ 68 years at the time of grafting**
- Hoehn & Yahr stage 2 or better when "on"
- On no therapy or only receiving standard anti PD treatment
- No significant L-Dopa induced dyskinesia
- **Significant, ≥ 33% response in UPDRS part III score in response to L-DOPA**
- **Preserved ^{18}F-dopa signal in ventral striatum**

Exclusion criteria

- Atypical parkinsonism incl. F-DOPA PET patterns consistent with this
- MMSE score of < 24 **(<26)** or evidence for dementia using DSM-IV criteria
- Unable to do normal copying of interlocking pentagons and semantic fluency score <20 over 90 secs
- Ongoing major medical or psychiatric disorder incl. depression and psychosis
- Other concomitant treatment with neuroleptics
- Significant drug induced dyskinesias (>2 for any body part on the AIMS scale)
- Previous neurosurgery
- Unable to be imaged using MRI
- **Clinically insignificant response to Levodopa**
- **Any contraindication to immunosuppression therapy.**
- **Patients on anticoagulants**
- **Patients who are left handed**

TEXT 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" state at 36 months post transplantation. "OFF" being defined as receiving no dopamine (DA) therapy for 12 hours prior to assessment or 36 hours in the case of long acting dopamine agonists (e.g. Ropinirole slow release).

Secondary Endpoints:

• Change in timed motor tasks at 36 months post transplantation

• The number of patients with dyskinesias (including L-dopa and graft induced dyskinesias) 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.

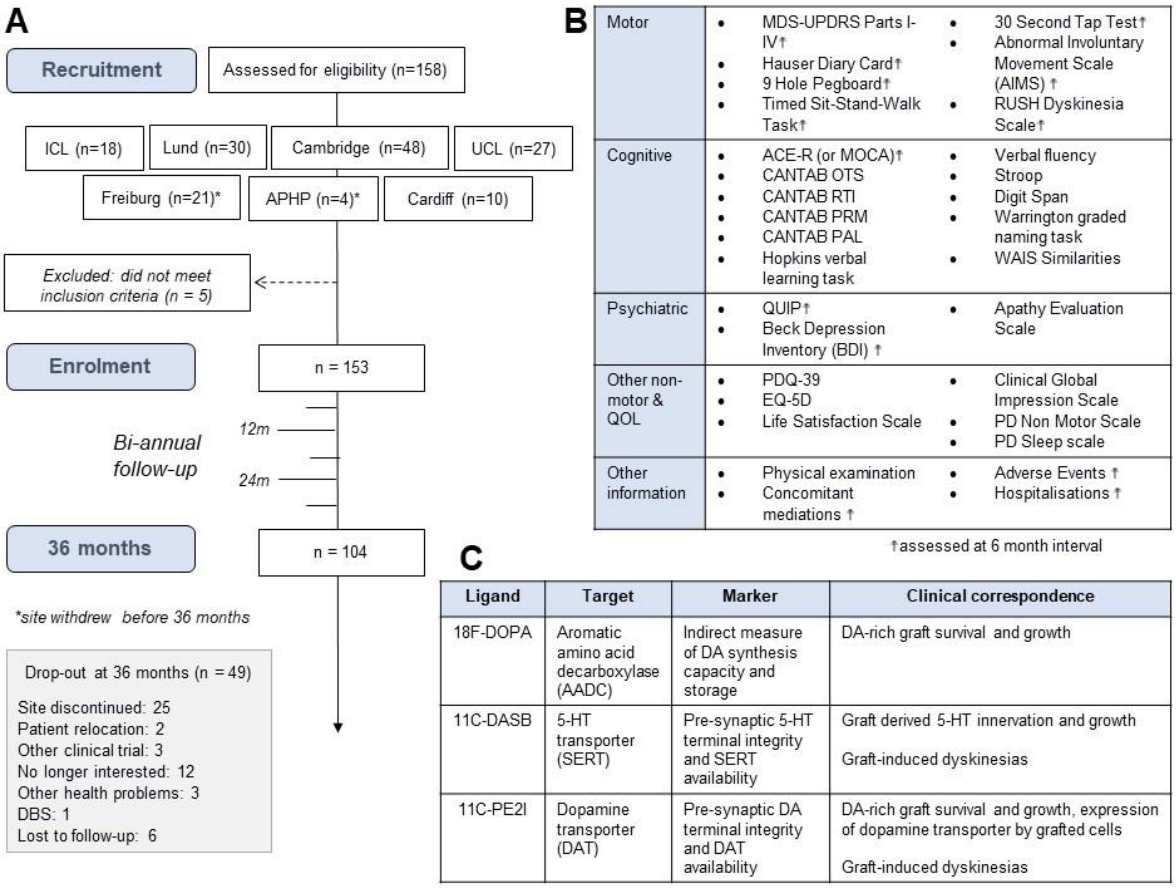
• Changes in F-DOPA PET in transplanted patients 36 months post transplantation.

Safety Endpoints:

• The number of adverse events and serious adverse events associated with the neural transplant.

• Laboratory Parameters – any reported changes in haematology, biochemistry or urinalysis measures outside the normal range.

• Other Safety parameters – vital signs, Physical Exam – new abnormalities will be recorded as an adverse event.



B

Motor	• MDS-UPDRS Parts I-IV† • Hauser Diary Card† • 9 Hole Pegboard† • Timed Sit-Stand-Walk Task†	• 30 Second Tap Test† • Abnormal Involuntary Movement Scale (AIMS) † • 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 Similarities
Psychiatric	• QUIP† • Beck Depression Inventory (BDI) †	• 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 † • Hospitalisations †

†assessed at 6 month interval

C

Ligand	Target	Marker	Clinical correspondence
18F-DOPA	Aromatic amino acid decarboxylase (AADC)	Indirect measure of DA synthesis capacity and storage	DA-rich graft survival and growth
11C-DASB	5-HT transporter (SERT)	Pre-synaptic 5-HT terminal integrity and SERT availability	Graft derived 5-HT innervation and growth Graft-induced dyskinesias
11C-PE2I	Dopamine transporter (DAT)	Pre-synaptic DA terminal integrity and DAT availability	DA-rich graft survival and growth, expression of dopamine transporter by grafted cells Graft-induced dyskinesias

Figure 1: Overview of the TRANSEURO protocol. A: recruitment by centre, enrolment and follow-up of patients in the observational arm including reasons for drop-out at 36 months. B: complete list of assessments undertaken by all patients every 12 months. At 6 months a more limited set of assessments were undertaken. C: PET-imaging schedule adopted and the reasons for this.

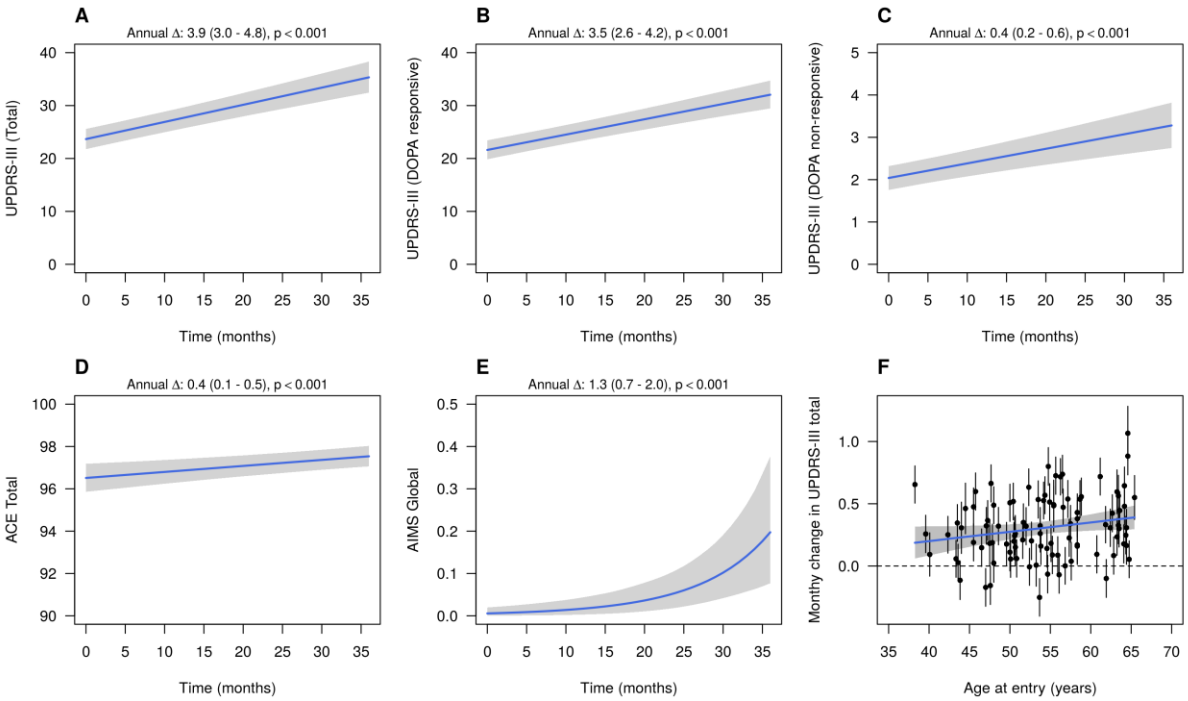


FIGURE 2: 36 month data from the observational cohort. Graphs A-E show the average change over time across all patients and 95% CIs (shaded regions) for key clinical outcomes. The annual rate of change (± 95% CI) is reported above each graph. Age at entry predicts the rate of decline, with older patients deteriorating faster (p = 0.023).

TABLE 1. THE TIMETABLE OF TRANSPLANTS AND THE REASONS WHY PLANNED SURGERIES WERE CANCELLED. 21 transplant surgeries were completed across the 2 sites. This included 10 bilateral grafts that were done sequentially (i.e. at 2 different surgical operations) and one patient elected not to have a second transplant after their unilateral surgery.

	2015	2016	2017	2018*	Total
Theatre 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
Localisation queries		2			2
Oncology case		1			1

*final procedure March 2018

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