

Human Trials of Stem Cell-Derived Dopamine Neurons for Parkinson's Disease: Dawn of a New Era

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Stem cell-based therapies for Parkinson's disease are moving into a new and exciting era, with several groups pursuing clinical trials with pluripotent stem cell (PSC)-derived dopamine neurons. As many groups have ongoing or completed GMP-level cell manufacturing, we highlight key clinical translation considerations from our recent fourth GForce-PD meeting.

Parkinson's disease (PD) is particularly attractive for stem cell-based therapies, since its core pathology involves the loss of highly specialized dopamine (DA) neurons in the substantia nigra. DA neuronal loss is responsible for many of the pathophysiological features of the disease, such as rigidity and bradykinesia, which can be treated with great effect in early disease using dopaminergic drugs. However, these drugs do not replace DA only at the site of greatest loss nor do they mimic the normal release of DA at these sites. As a result, their use results in side effects such as dyskinesias and behavioral problems. In contrast, targeted DA neuronal replacement therapies have the potential to address these shortcomings of the dopaminergic drugs.

Proof-of-principle studies supporting this therapeutic strategy have used early fetal brain tissue. In particular, fetal ventral mesencephalic allografts (hfVMs) can release DA and have shown long-term efficacy and survival, as well as improvements in quality of life and some non-motor features of PD (reviewed in [Barker et al., 2015](#)). However, such transplants have not always worked and have even generated side effects (e.g., graft-induced dyskinesias) with signs of disease-related pathology in the transplanted cells years after being implanted ([Barker et al., 2015](#)). A number of tractable issues may explain this variability in clinical response, and efforts to resolve these issues led to TRANSEURO, a new trial in Europe that has now grafted 11 patients with hfVMs over the last 2.5 years.

However, the use of fetal tissue is problematic, in terms of both the ethics and practical issues linked to its acquisition and broader use and the inability to standardize it for clinical application. For example, in TRANSEURO, only 20 out of a planned 90 or more surgeries have taken place because of tissue supply. Thus, there is a need for an alternative tissue source, ideally one that can be readily manufactured to a defined specification at the scale needed to treat the large number of PD patients.

One source that has gained prominence in recent years is the use of human pluripotent stem cells (hPSCs). hPSCs are derived from early pre-implantation embryos (ESCs) or reprogrammed adult somatic cells (iPSCs), and they can be robustly differentiated into authentic midbrain dopaminergic neurons using recently developed protocols ([Kirkeby et al., 2012](#); [Kriks et al., 2011](#)). This work has been concentrated in a number of centers worldwide, and in 2014, major academic networks in Europe, the US, and Japan that share common therapeutic ambitions regarding hPSC-derived dopaminergic neurons for PD decided to join forces. This new initiative, GForce-PD (<http://www.gforce-pd.com>), recently had its fourth annual meeting in Kyoto. During this meeting, it became clear that many of the teams have advanced to the point where GMP manufacturing is now in progress/completed, and the discussions therefore centered around how to use these cells in first-in-human clinical trials while being compliant with each

region's national guidelines. The meeting revealed that all teams were planning trials with start dates in the next couple of years (see [Table 1](#)). However, some clinical trials using stem cells for PD outside of GForce-PD have already started, involving commercial groups (ISCO) or academically led studies, such as a new Chinese HLA matched hESC trial (clinical trial ID: NCT03119636; <https://clinicaltrials.gov/>).

The Roadmap to a Clinical Trial

The starting material for developing DA cells for the clinical trials planned within GForce-PD has been defined by whether it will be iPSC or ESC derived ([Figure 1](#)). Some groups are choosing to use and evaluate non-matched, HLA-matched, or autologous iPSCs, while others will use ESCs ([Table 1](#)). The use of autologous or HLA-matched cells is thought to be desirable as it reduces the need for immunosuppression in the recipient, although debate remains on whether ongoing immune rejection of allogeneic intracerebrally transplanted developing neural tissue occurs, since PD patients who had stopped taking immunosuppression for many years have had long-term survival of fetal allografts ([Li et al., 2016](#)).

Each team within GForce-PD has now developed GMP protocols for deriving authentic and functional midbrain DA cells from hPSC sources, along with cryopreservation and QC assays. These protocols involve differentiation to committed DA neuroblasts, which generates the best results in animal models of PD. The

Table 1. Main Feature Summary of GForce-PD Partners' Clinical Trials

	EUROPEAN STEM-PD ^a	NYSTEM-PD	CiRA Trial	Summit for PD Trial
Cell source	ESCs	ESCs	allogeneic iPSCs	autologous iPSCs
Cryopreserved cell product?	yes	yes	no	yes
Genetic testing of cell product	TBD	karyotype / + TBD	sequencing for certain genes	full genome sequencing
Cell delivery method	"Rehncrona" instrument previously used in fetal VM trials	MRI/Clearpoint system	purpose made needle	MRI/Clearpoint system
Dosing?	low dose, high dose	low dose, high dose	one dose	low dose, high dose
Immunosuppressive regime	yes, at least 12 months; probably CiclosporinA; Azathioprine; steroids	yes, 12 months; FK506; Basiliximab; TBD ± mycophenolate	yes, 1–2 years; FK506	none
Patient characteristics				
Age	<70 years old	40–70 years old	50–70 years old	45–70 years
Disease duration	<12 years	5–12 years	>5 years	>5 years
Significant LIDs?	no	no	no	no
L-dopa response?	>30%	>50%	>30%	>20%
Pre-transplant run-in period	>1 year	>1 year	TBD	>1 year
Follow-up period	indefinitely	at least 2 years	at least 2 years	at least 1 year
PET imaging	F-dopa; PE2i	F-dopa; PE2i; DPA 713	F-dopa; DAT-SPECT; FLT GE180	F-dopa; DAT-SPECT FLT GE180
Primary end point	adverse events	adverse events	adverse events	adverse events
Secondary clinical end points (changes in)	UPDRS motor 3 in defined "off"; PDQ39; Addenbrooke's Cognitive Examination (Revised)	UPDRS motor 3 in defined "off"; PDQ39; Montreal Cognitive Assessment	UPDRS motor 3 in defined "off"; "off" time period PDQ39; Mini Mental State Examination score	UPDRS motor 3 in defined "off"; PDQ39; Mini Mental State Examination Score
Date for planned first-in-human study	2019–2020	2018	2018	2019

^aThe outcome of NeuroStemCellRepair and TRANSEURO.

protocols are reproducible and scalable, although issues still exist around the most appropriate genetic testing of the starting material and/or final product. Questions under discussion, for example, include whether karyotyping and exclusion of tumorigenic mutations is sufficient, or if more in-depth analysis, such as next generation sequencing, is required. If the latter is needed, then what constitutes a significant genetic variant and what is non-consequential, and who should make this decision? Should standards be the same for ESCs and iPSCs as well as for cell banks where the final product can be tested extensively for safety in large numbers of animals, in contrast to autologous cell lines where this may not be feasible in every single cell line and patient? At the moment, there are no definitive answers, and groups have clearly pursued different strategies in the absence of any scientifically conclusive

data or consensus from different national regulators, although international efforts may help resolve some of these issues in the next few years (Andrews et al., 2017).

Although not the topic of this Forum, it is critical to mention that the protocols employed in all the studies within GForce-PD have worked well in a number of *in vivo* studies with no tumor formation or uncontrolled growth (Grealish et al., 2014; Kikuchi et al., 2017; Kirkeby et al., 2012; Kriks et al., 2011; Steinbeck et al., 2015). It is also important to note the rigorous documentation of this level of safety along with consistent efficacy and reproducibility, since in its absence, anxieties about both issues arise, which occurred in two recent highly publicized stem cell trials for PD (Barker et al., 2016; Cyranoski, 2017).

The protocols developed by the members of GForce-PD are now close to or completed at the level of GMP produc-

tion, with the definitive preclinical efficacy and safety studies ongoing or planned to be completed over the next 6–36 months. Recruitment to an observational arm of PD patients is ongoing with the aim of selecting patients from this cohort for transplantation in the first-in-human studies.

The work presented at this year's GForce-PD meeting also included for the first time the ongoing preclinical work by Summit for Stem Cell. Most of these groups (Table 1) are looking to manufacture large batches of cryopreserved vials of DA precursors. The final clinical cell product will then be tested for stability, tumorigenesis, biodistribution, and toxicology in accordance with relevant national regulatory agencies (e.g., FDA versus MHRA versus PMDA), with the results made publically available, similar to what has previously been done (Grealish et al., 2014; Kikuchi et al., 2017; Kriks et al., 2011; Steinbeck et al., 2015).

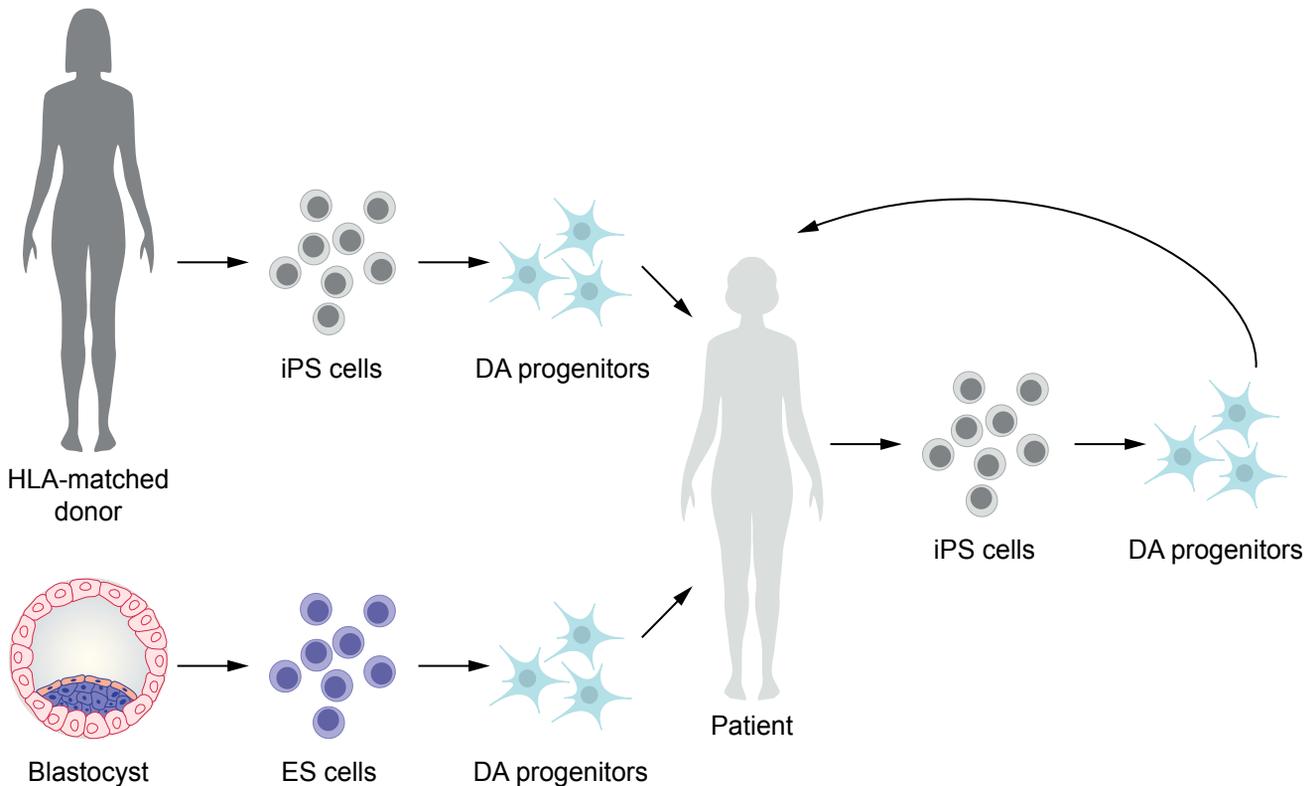


Figure 1. Overview of Cells for Clinical Trials

Possible donor cells explored within GForce-PD include human embryonic stem cells (ES cells) and induced pluripotent stem cells (iPSCs) from non-matched or HLA-matched donors (left side) as well as patient-specific iPSCs (right side).

This GMP-level manufacturing of the cell product required for human trials is not a trivial exercise. DA cell production faces many of the same issues as other fields attempting to use cell-based therapies. However, the efficiency of the protocols coupled to the relatively small number of cells needed to treat individual patients means we can still use small-scale manual manufacturing processes that employ standard culture flasks and incubators to make cells for hundreds of patients, which is more than sufficient for the planned trials. Nevertheless, if the therapy moves on to a phase III/market authorization phase, it will be necessary to further scale up the procedure and/or develop automated manufacturing systems.

Goals of GForce-PD

The four teams currently represented at this last GForce-PD meeting are all moving forward with the aim of undertaking their own clinical trials within the next 1–3 years. As progress is pursued, GForce-PD serves three main purposes:

(1) it critically appraises the preclinical evidence from all groups supporting the adoption of the derived cells as a DA replacement therapy, (2) it openly discusses the important and challenging aspects of clinical translation and trial design, and (3) it finally seeks to harmonize the work being done and design all the planned work and trials so that they can be compared to maximize what can be learned from them. This year we concentrated on four key issues, as follows.

Immunosuppressive Regime

The immunogenicity of dopaminergic neurons derived from hPSC sources is unknown, and it is thus unclear what the optimal immunosuppressive regime would look like in any clinical trial using these cells. The general consensus is that a period of immunosuppression is needed and will involve using a least one immunosuppressive agent, such as FK506, for 1–2 years after grafting as outlined in Table 1. This is based in part on the current regimes being used in patients receiving hfVM transplants, where long-

term graft survival has been seen in some PD patients without the need for lifetime immunosuppression. In addition, it has been shown that triple immunotherapy for a year after grafting results in better graft dopaminergic cell survival compared with no immunosuppression or monotherapy with CyA for only 6 months after grafting.

Patient Selection

The choice of patients for any first-in-human study with hPSC-derived DA cells is not straightforward. They need to demonstrate a clear response to oral DA medications, but when in the disease course should they be treated with this new experimental therapy? Some argue that the ideal cohort should capture patients that are most likely to get maximal benefit from their transplant, similar to the ones enrolled for the TRANSEURO study, namely, younger patients with less advanced disease and no significant L-dopa induced dyskinesias (LIDs), as well as no cognitive deficits predictive of early dementia and a good response to dopaminergic medications (Barker et al.,

2015). However, others will argue that subjecting patients to an unproven stem cell therapy at this stage of their illness is unethical and that instead the treatment should be trialled in those with more advanced disease and motor fluctuations, given that they are at a stage of their illness where a more invasive therapeutic approach is needed (e.g., DBS or apomorphine/DuoDopa). In addition, it will be easier to monitor efficacy in this latter group of patients compared to patients with milder disease where responses to drug therapies are often excellent and sustained, although this clearly creates a therapeutic conundrum as to whether clinicians/patients should opt for an established therapy such as DBS or more experimental, unproven, cell-based approaches. At the moment, most groups are erring on the side of choosing patients with slightly more advanced disease compared to TRANSEURO, but not so advanced that they have significant LIDs.

Patient Assessment

The protocol for assessing patients will include a comprehensive set of standard motor, cognitive, psychiatric, non-motor, and quality of life assessments as outlined in Table 1, all of which exist for PD and are well validated (TRANSEURO: clinical trial ID NCT01898390; <https://clinicaltrials.gov/>). Indeed, several groups have already started an observational study using these assessments in new cohorts of PD patients with the aim of randomly recruiting some of them into the planned trials. This not only facilitates the clinical trial once regulatory approval has been granted, but it also generates a clinically matched comparator arm by which to analyze any early signs of clinical efficacy with the new therapy.

In addition to these clinical tests, imaging will be required for two purposes: ensuring safety using MRI and monitoring the DA content of the transplant using PET or its equivalent. MRI safety monitoring is likely to occur at least every 3 months for the first year after grafting, then every 6 months for 3 years, and annually thereafter. PET imaging is likely to employ ^{18}F -dopa and/or $^{11}\text{CPE}2i$, although some groups may pursue additional measures to look not only at the dopaminergic cells in the graft but also at cell proliferation and microglial activation as outlined in Table 1.

Trial Design

The first-in-human studies will be open label and also involve a dose-finding element with small numbers of patients. Most groups are thinking of recruiting no more than 12 patients for these phase I/IIa studies, with two different doses of cells being given across this group (see Table 1). None of the groups plan for sham surgery in these initial dose-finding trials, and the use of sham/imitation surgery at later stages is an active area of discussion as is the need to show that this therapy has therapeutic equivalence or superiority to that which already exists for PD, including DBS and advanced forms of DA delivery. This can be studied in part by using a nested trial design with patients recruited for the new intervention coming from a well-matched larger cohort of patients, all of whom are assessed in identical ways.

The primary end point for all these trials will be tolerability and feasibility, as they will not be sufficiently powered to show safety and/or efficacy. In addition, as with any such cell therapy, any signs of clinical efficacy may take up to 3–5 years to be maximally evident based on what is observed with hfVM transplants, and thus cannot be a primary end point in these early trials, especially given the absence of any sham surgery control arm. Thus, most groups will wait at least 2 years after grafting before publishing their results so that better measures of tolerability can be reported as well as any signs of clinical potency, although it should be stated that patients should ideally be followed up indefinitely until death given the irreversible nature of intracerebral neural grafting.

The Dawn of a New Era?

Treating PD using new, manufactured DA cells has been a goal since the first pioneering clinical transplantation studies using fetal cells more than 25 years ago. The limitation of using fetal tissue was already recognized at this time, and so began the long journey to find a scalable, ethically acceptable, safe cell source. En route, other neuronal alternatives have been considered including *ex vivo* expanded hfVM DA neuroblasts and xenotransplants of pig VM tissue, but these sources have met with only limited success.

The derivation of the first hESCs in 1998 brought with it a new hope that

this could be the source from which authentic human midbrain DA neurons could be derived. However, this achievement took longer than expected, with two groups reporting protocols in 2011–2012 with long-term survival and functional efficacy in animal models of PD (Kirkeby et al., 2012; Kriks et al., 2011). These studies were a turning point in the field and catalyzed the development of new protocols with a realistic expectation that this approach can now be considered for clinical trials. However, only in the last year has this goal become a reality, with the GMP cell manufacturing either already completed or in progress. Thus, we are entering the last phase of preclinical work with clinical trials planned in 2018 and in the years thereafter, and as such the use of stem cells for PD has entered a new era.

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