FIRST IN HUMAN TRIALS OF STEM CELL DERIVED DOPAMINE NEURONS in PARKINSON'S DISEASE: DAWN OF A NEW ERA

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ABSTRACT

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 the rigidity and bradykinesia, which can be treated with great effect in early disease using dopaminergic drugs. However, these drugs do not replace dopamine only at the site of greatest loss nor do they mimick the normal release of dopamine at these sites. As a result their use results in side effects such as dyskinesias and behavioural problems. In contrast, targeted dopamine 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 (hfVM) can release dopamine 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 hfVM over the last 2 and a half years.

However, the use of fetal tissue is problematic, both in terms of the ethics and practical issues linked to its acquisition and broader use, as well as the inability to standardise 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 centres worldwide, and in 2014, major academic networks in Europe, US and Japan that share common therapeutic ambitions regarding hPSC-derived dopaminergic neurons for PD decided to join forces. This new initiative, GForce-PD (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 centred 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 (NCT03119636).

The roadmap to a clinical trial

The starting material for developing dopamine cells for the clinical trials planned within GForce-PD has been defined by whether it will be iPS or ES cell derived (Figure 1). Some groups are choosing to use and evaluate non-matched, HLA-matched, or autologous iPS cells, while others will use ES cells (Table 1). The use of autologous or HLA matched cells is thought 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 dopamine cells from hPSC sources, along with cryopreservation and QC assays. These protocols involve differentiation to committed dopamine neuroblasts, which generates the best results in animal models of PD. The 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 tumourogenic 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 ES and iPS cells 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 tumour 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 publicised stem cell trials in 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 production, 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 dopamine 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 v MHRA v PMDA), with the results made publically available similar to what have done previously (Grealish et al., 2014; Kikuchi et al., 2017; Kriks et al., 2011; Steinbeck et al., 2015).

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 4 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 3 main purposes: 1) it critically appraises the pre-clinical evidence from all groups supporting the adoption of the derived cells as a dopamine replacement therapy, 2) it openly discusses the important and challenging aspects of clinical translation and trial design, and 3) it finally seeks to harmonise the work being done, and design all the planned work and trials so that they can be compared to maximise what can be learnt from them. This year we concentrated on 4 key issues:

-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 post grafting as outlined in Table 1. This is based in part on the current regimes being used in patients in receipt of 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 post grafting results in better graft dopaminergic cell survival compared with no immunosuppression or monotherapy with CyA for only 6 months post grafting.

-Patient selection The choice of patients for any first in human study with hPSC derived dopamine cells is not straightforward. They need to demonstrate a clear response to oral dopamine 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: NCT01898390). 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 also generates a clinically matched comparator arm by which to analyse 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 dopamine content of the transplant using PET or an equivalent. MRI safety monitoring is likely to occur at least 3 monthly for the first year after grafting, then 6 monthly for 3 years, and annually thereafter. PET imaging is likely to employ ^{18}F-dopa and/or ^{11}CPE2i, 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 phase1/2a studies, with 2 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 post 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 Parkinson's disease using new, manufactured dopamine 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 fetal human VM 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 pre-clinical 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.

TABLE 1- Main feature summary of GFORCE PD partners' clinical trials

	EUROPEAN STEM-PD*	**NYSTEM-PD**	**CiRA TRIAL**	**SUMMIT FOR PD TRIAL**
CELL SOURCE	ES	ES	Allogeneic iPS	Autologous iPS
CRYOPRESERVED CELL PRODUCT?	YES	YES	NO	YES
GENETIC TESTING OF CELL PRODUCT	TBD	Karyotype / + TBD	Sequencing for certain genes	Full genome seq
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
IMMUNO-SUPPRESSIVE 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** **Disease Duration** **Significant LIDs?** **L-dopa response?**	 <70 years old <12 years No >30%	 40-70 years old 5-12 years No >50%	 50-70 years old >5 years No >30%	 45-70 years >5 years No >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	2020

* the outcome of NeuroStemCellRepair and TRANSEURO

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References

Andrews, P.W., Ben-David, U., Benvenisty, N., Coffey, P., Eggan, K., Knowles, B.B., Nagy, A., Pera, M., Reubinoff, B., Rugg-Gunn, P.J.*, et al.* (2017). Assessing the Safety of Human Pluripotent Stem Cells and Their Derivatives for Clinical Applications. Stem Cell Reports *9*, 1-4.

Barker, R.A., Drouin-Ouellet, J., and Parmar, M. (2015). Cell-based therapies for Parkinson disease-past insights and future potential. Nat Rev Neurol *11*, 492-503.

Barker, R.A., Parmar, M., Kirkeby, A., Bjorklund, A., Thompson, L., and Brundin, P. (2016). Are Stem Cell-Based Therapies for Parkinson's Disease Ready for the Clinic in 2016? J Parkinsons Dis *6*, 57-63.

Cyranoski, D. (2017). Trials of embryonic stem cells to launch in China. Nature *546*, 15-16.

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

Kikuchi, T., Morizane, A., Doi, D., Magotani, H., Onoe, H., Hayashi, H., Mizuma, H., Takara, S., Takahashi, R., Inoue, H.*, et al.* (2017). Human iPSC-derived dopaminergic neurons function in primate Parkinson's disease models. Nature, In press.

Kirkeby, A., Grealish, S., Wolf, D.A., Nelander, J., Wood, J., Lundblad, M., Lindvall, O., and Parmar, M. (2012). Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep *1*, 703-714.

Kriks, S., Shim, J.W., Piao, J., Ganat, Y.M., Wakeman, D.R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A.*, et al.* (2011). Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature *480*, 547-551.

Li, W., Englund, E., Widner, H., Mattsson, B., van Westen, D., Latt, J., Rehncrona, S., Brundin, P., Bjorklund, A., Lindvall, O.*, et al.* (2016). Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. Proc Natl Acad Sci U S A *113*, 6544-6549.

Steinbeck, J.A., Choi, S.J., Mrejeru, A., Ganat, Y., Deisseroth, K., Sulzer, D., Mosharov, E.V., and Studer, L. (2015). Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. Nat Biotechnol *33*, 204-209.

Figure Legend:

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 (iPS cells) from non-matched or HLA-matched donors (left side) as well as patient specific iPS cells (right side).