

# Stem cells and regenerative therapies for Parkinson's disease

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**Abstract:** Currently the mainstay of Parkinson's disease (PD) therapy is the pharmacological replacement of the loss of the dopaminergic nigrostriatal pathway using drugs such as dopamine agonists and levodopa. Whilst these drugs effectively ameliorate some of the motor features of PD, they do not improve many of the nonmotor features that arise secondary to pathology outside of this system, nor do they slow the progressive neurodegeneration that is a characteristic of the disease. Regenerative therapies for PD seek to fill this therapeutic gap, with cell transplantation being the most explored approach to date. A number of different cell sources have been used in this therapeutic approach, but to date, the most successful has been the use of fetal ventral mesencephalic (VM) tissue that contains within it the developing nigral dopaminergic cells. Cell transplantation for PD was pioneered in the 1980–1990s, with several successful open-label trials of fetal VM transplantation in patients with relatively advanced PD. Whilst these findings were not replicated in two subsequent double-blind sham-surgery controlled trials, there were reasons to explain this outside of the one drawn at the time that these therapies are ineffective. Indeed all these studies have provided evidence that following the transplantation of fetal VM tissue, dopaminergic cells can survive long term, produce dopamine, and bring about clinical improvements in younger patients over many years. The use of fetal tissue, irrespective of its true efficacy, will never become a widely available therapy for PD for a host of practical and ethical reasons, and thus much work has been put in recently to exploring the utility of stem cells as a source of nigral dopaminergic neurons. In this respect, the advent of embryonic stem cell and induced pluripotent cells has heralded a new era in cell therapy for PD, and several groups have now demonstrated that these cells can form dopaminergic neurons which improve functional deficits in animal models of PD. Whilst encouraging, problems with respect to the immunogenicity and tumorigenicity of these cells means that they will need to be used in the clinic cautiously. Other regenerative therapies in PD have been tried over the years and include the use of trophic factors. This has primarily involved glial cell line-derived neurotrophic factor (GDNF) and again has produced mixed clinical effects, and in order to try and resolve this, a new trial of intraputamenal GDNF is now being planned. In addition, a new trial for platelet derived growth factor as a treatment for PD has just completed recruitment, and PYM50028 (Cogane) an oral agent shown in animal models to reduce the effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) lesioning by the induction of growth factors is currently under investigation in a multicentre Phase II trial. Overall, there are a number of promising new regenerative therapies being developed and tested in PD, although the true long-term efficacy of any of these in large numbers of patients is still not known.

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## Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder affecting 1% of the population over the age of 65.<sup>1,2</sup> The disease is classically defined by its motor features – tremor, bradykinesia, rigidity, and postural instability; but cognitive impairments, disturbances of mood, and autonomic dysfunction also contribute significantly to the morbidity of the disease.<sup>3,4</sup> The underlying pathology of PD involves the degeneration of the dopaminergic (DA) neurons that project from the substantia nigra into the striatum, with the formation of alpha synuclein-containing Lewy bodies.<sup>5,6</sup> Currently, the mainstay of PD therapy is the replacement of this dopamine deficiency using drugs such as dopamine agonists and levodopa. Whilst these drugs effectively ameliorate some of the motor features of PD in the early stages of the disease, they do not treat all of them equally well, nor do they improve most of the nonmotor features that arise secondary to pathology outside of the nigrostriatal pathway. Furthermore, they do not have a disease-modifying effect; that is, they do not modulate the inexorable neurodegeneration that underlies PD, and their long-term use brings about motor complications such as on–off fluctuations and drug-induced dyskinesias.<sup>7</sup> As a result, there is a need for the development of regenerative therapies for PD, with the purpose of slowing down, if not stopping, the pathology, with a concomitant slowing of the progression of symptoms and signs.

## Overview of studies conducted to date using regenerative therapies and stem cells

Regenerative therapies for PD can be broadly divided into two categories: (1) those that seek to replace the cells that have been lost; and (2) those that induce the regeneration of the existing neuronal network. In terms of cell replacement, a number of different cell types have been tried, of which the most successful to date involves the transplantation of fetal ventral mesencephalic (VM) tissue into the striatum of patients with PD.<sup>8–10</sup> Studies (reviewed below) have clearly shown that VM DA neurons can survive, long term, in the PD brain. There, they can produce significant functional benefits for patients, implying that nigral DA neurons derived from stem cell sources should, theoretically, have the same capabilities. In this respect, studies have been done showing that this is in fact possible using DA cell transplants derived from embryonic stem (ES) cells and induced pluripotent stem cells, and indeed studies are ongoing to establish whether direct transdifferentiation of skin fibroblasts to DA cells

also have this capability, abrogating the need to use stem cells at all.<sup>11</sup>

In terms of restorative approaches, to date the most encouraging results have been around the delivery of glial cell line-derived neurotrophic factor (GDNF) or related trophic factors. The direct delivery of trophic factors into the brain parenchyma seems to be the most successful, but the use of viral vectors and the administration of drugs that induce the local production of neurotrophic factors have also been tried with some degree of success.<sup>12</sup>

## Fetal transplants

The main aim of fetal VM transplantation is to physiologically replace the DA input to the striatum. In pioneering work in the 1980s, it was shown that DA neuroblasts from the fetal VM could survive long term in the adult brain and reinnervate the striatum of 6-hydroxydopamine (6-OHDA)-lesioned animals, with a consequent improvement in most but not all of their behavioral deficits.<sup>13–18</sup> As a result of this work, clinical trials were started in the late 1980s to assess the safety and efficacy of this approach in patients with PD.

The initial open-label trials were undertaken in Mexico and Sweden,<sup>10,19</sup> with the latter center going on to systematically explore the utility of this approach. In the original Lindvall et al study in Sweden, two PD patients received fetal VM tissue of gestational age 8–10 weeks. These patients showed minimal clinical improvement, and this failure to see a more dramatic response was felt, at least in part, to be due to issues of tissue preparation and implantation. The surgical technique was subsequently modified, and with this, it was shown that fetal VM transplants could survive better in the human brain and produce marked improvement in the motor features of PD. The transplants were initially placed on one side, with improvements being seen bilaterally, but greatest contralateral to the transplanted side.<sup>20</sup> Objective evidence that the clinical improvement observed was likely due to the graft, was obtained through positron emission tomography (PET) studies showing increased F-dopa signal at the site of the tissue implantation.<sup>21</sup>

Subsequent to the initial Swedish studies, other groups, most notably in North America and France undertook similar studies with results that were not dissimilar. In some of these cases, postmortem material became available as patients died from causes unrelated to their disease or transplant, and this demonstrated that DA neurons could be found in the grafts with innervation of the surrounding striatum, the extent of which correlated with improvements seen clinically and on F-dopa PET scans.<sup>22–24</sup>

As a result of the success of the open-label trials, two randomized sham-surgery controlled trials were undertaken to further evaluate the efficacy of fetal grafting.

The first trial, undertaken by Freed et al, randomized 40 patients with moderately severe PD to receive either transplants of human VM tissue or sham/imitation surgery.<sup>8</sup> Strands of VM tissue were transplanted within 4 weeks of dissection, bilaterally into the putamen using two needle tracks via a transfrontal approach. Patients undergoing sham surgery received burr holes that did not breach the dura, and none of the patients were immunosuppressed. Assessments were undertaken at baseline, 4, 8, and 12 months post-transplantation. The primary outcome was a subjective global rating in the severity of the disease 12 months post-transplantation, with the nongrafted arm being offered a VM transplant after this primary endpoint. The authors found no statistically significant improvement in their primary outcome measure across the entire group, though for younger patients (<60), there was an improvement in the Unified Parkinson's Disease Rating Scale (UPDRS), and PET scanning demonstrated increased fluorodopa uptake in 17/20 patients. Worryingly, for the first time, significant side effects were reported. With 15% of the patients developing graft-induced dyskinesias (GIDs) that persisted even after the cessation of their levodopa therapy, and in some cases the dyskinesias were so severe that further neurosurgery was required to treat them. These patients did, however, have significant L-dopa-induced dyskinesias prior to transplantation.<sup>8</sup>

Overall, 33 patients in this study were eventually grafted, with 29 being followed up at 2 years, and 15 of these had documented assessments at 4 years, the results of which were rather different to the original 1-year data. In particular, the UPDRS motor scores declined over time after transplantation ( $P < 0.001$ ), and this effect was sustained up to 4 years post-transplantation in those that were still being followed up. They also reported significant increases in putamenal (18)F-Fdopa (1-3,4-dihydroxy-6-18F-fluoro-phenyl-alanine) uptake at all post-transplantation time points ( $P < 0.001$ ), and this correlated with clinical improvement.<sup>25</sup>

In a subsequent trial, Olanow et al reported similar outcomes. Their trial randomized 34 patients to receive either imitation surgery or a graft derived from either one or four donor embryos. In contrast to the Freed study, patients were immunosuppressed with cyclosporine for 6 months post-grafting, and the primary outcome was the change in UPDRS motor subscore in the "off" state at 24 months. The overall effect was that transplants were ineffective,

although there was a clear trend to improvement in the grafted patients, particularly in the four donor groups at the earlier time points prior to the cessation of immunotherapy. Interestingly, patients who underwent sham surgery did not improve (ie, there was no placebo effect), and those with milder disease had greatest benefit, though again GIDs were seen, this time in 56% of patients.<sup>9,26</sup>

The negative results coupled to the development of GIDs, caused many to doubt the usefulness of this approach.<sup>9</sup> However, the long-term benefits seen in some grafted PD patients means that, whilst this approach can work, it is not clear why it does so in only some patients. This variable outcome post VM transplantation may be due to issues of patient selection, transplantation techniques (including the method of storage and preparation of the tissue to be grafted), the site of transplantation, the type and duration of the immunosuppressive regime adopted, and the actual design of the trial.<sup>27,28</sup> These issues have all been vigorously debated, and of late, a new project to further probe these problems has been funded by the EU (TransEuro). (<http://www.transeuro.org.uk>).

## Carotid body grafts

Cells of the carotid body secrete dopamine in response to hypoxia, and as a result, transplantation of carotid body aggregates in primate models of PD were undertaken with the grafted animals showing a degree of functional recovery.<sup>29-31</sup> These findings led to a single open-label trial, which showed a modest (between 13% and 52%) improvement in the patients' UPDRS motor "off" scores.<sup>32</sup> There was, however, no objective evidence to suggest increased striatal dopamine synthesis on F-dopa PET, and a postmortem study of one patient showed only low numbers of surviving DA neurons.<sup>33</sup> Based on work done in rodents demonstrating the release of GDNF from carotid body grafts, the authors proposed that neurotrophic factor release might be the cause for the clinical improvement seen. However, if this was the case, one might have anticipated improvements in F-dopa PET scans, similar to that seen in the GDNF infusion studies.<sup>34</sup>

## Retinal pigment epithelium (RPE)

RPE cells are known to produce dopamine as well as a range of neurotrophic factors, such as GDNF, brain-derived neurotrophic factor (BDNF), and pigment epithelium-derived factor (PEDF), which may help explain how such cells could restore/protect the nigrostriatal DA pathway following experimental lesioning.<sup>35-37</sup> These observations led to an open-label clinical trial in which human RPE cells were bound to a microcarrier (Spheramine<sup>®</sup>, Titan Pharmaceuticals, Inc,

South San Francisco, CA) and transplanted into the striatum of six patients with advanced PD.<sup>38</sup> The implants were well tolerated, and no major adverse effects, including GIDs, were seen. Patients demonstrated, on average, a 48% improvement in their off-state UPDRS scores. This led on to a randomized double-blind sham-surgery controlled trial in 71 advanced PD patients (Hoehn and Yahr stages 3–4), where it was shown that these cells did not have any major beneficial effects. At the end of the trial, the patients were unmasked and followed up for a further 4 years without any evidence of benefit emerging.<sup>39</sup> The failure to find any difference between the groups was likely due to inadequate cell survival, as a postmortem case reported of a patient who had received such a transplant showed that only 0.03% of the grafted cells had survived.<sup>40</sup>

## Growth factors

The rationale for using neurotrophic factor therapy in PD is to slow or halt the degenerative process by encouraging innate repair and regeneration within the nigrostriatal pathway.

## GDNF

GDNF has been shown *in vitro* to enhance the differentiation and survival of DA neurons.<sup>41</sup> This effect has also been demonstrated in a number of animal models of PD, and as a result GDNF has been the subject of a number of clinical trials in PD.<sup>42–44</sup>

GDNF is unable to cross the blood–brain barrier, and therefore, for it to have an effect, it must be delivered directly to the central nervous system.<sup>45</sup> The first randomized controlled trial using GDNF in PD involved the delivery of GDNF at monthly intervals and at varying doses into the ventricular space of 50 moderately advanced levodopa-responsive patients, with the primary outcome being a change in the motor UPDRS “off” state.<sup>46</sup> The outcome of this trial was negative at both the neurological and pathological level, almost certainly because GDNF was unable to penetrate the brain parenchyma and therefore unable to act on the DA neurons and their axonal projections.

Following the failure of the intraventricular GDNF infusions, direct intraparenchymal infusions of GDNF were undertaken whereby GDNF was delivered directly and continuously into the postcommisural putamen.<sup>34</sup> The initial open-label trial involved bilateral infusions in five patients and reported good results, with the authors finding a mean reduction in UPDRS “off” motor scores of 48% at 3 months. At this point, all the patients were enrolled into a 12-month follow-up study, and these improvements in motor function were shown to be sustained. These improvements

were also observed at the level of imaging, with F-dopa PET scans showing increased uptake at the site of GDNF delivery. Furthermore, in a single postmortem case, there was evidence of DA fiber sprouting around the GDNF infusion site.<sup>47</sup> Similar findings were reported in another open-label trial by Slevin et al, who demonstrated an improvement in bilateral motor function following unilateral intraputamenal infusion of GDNF in ten patients with advanced PD.<sup>48</sup> These authors used a dose-escalation regime and reported a 34% and 33% improvement in UPDRS in the “on” and “off” states, respectively, at 24 weeks compared with baseline scores, and these improvements were sustained in the washout period.

The findings of the open-label trial, however, were not replicated in a subsequent double-blind placebo controlled trial.<sup>49</sup> This trial enrolled 34 patients who were randomized 1:1 to receive either intraputamenal GDNF or placebo for 6 months. There was no statistically significant difference between the treatment and placebo groups, with the percentage change in UPDRS “off” motor scores being –10% and –4.5%, respectively ( $P = 0.53$ ).<sup>50</sup> The reasons for this are debated and have centered around the use of pulsatile versus continuous trophic factor delivery, variation in catheter size and port number, and the use of convection-enhanced delivery systems. In this respect, San Sebastian et al reported in nonhuman primates that magnetic resonance imaging (MRI)-guided convection-enhanced delivery, using gadolinium in the infusate improved tissue distribution of AAV2-hAADC without any specific side effects. Though trophic factors were not used in this study, this methodology could be applied in future GDNF trials to increase the volume of distribution.<sup>51</sup>

In addition to the failure of the above trial to show efficacy, other anxieties to do with its toxicity also placed the use of GDNF on hold. In a preclinical study of primates receiving chronic intraputamenal infusions of GDNF, some of the monkeys receiving the highest dose (100 µg/d) developed multifocal cerebellar Purkinje cell loss associated with atrophy of the molecular layer and, in some cases, granule cell loss, the cause of which has yet to be established.<sup>52</sup> Additionally, Slevin et al reported the development of anti-GDNF antibodies in some patients, albeit without any clinical sequelae.<sup>53</sup> Nevertheless, there are plans to resume studies with GDNF, and in addition, allied studies with the related neurotrophic factor neurturin (NTN) are ongoing (Ceregene studies – see below).

## BDNF

BDNF is a member of a large family of neurotrophins originally identified in the pig and shown to promote survival

of a number of neuronal populations including nigral DA neurons.<sup>54</sup> This experimental evidence taken together with the demonstration of reduced expression of BDNF in Parkinsonian brains led to further exploration of a restorative role for this trophic factor in PD.<sup>55-57</sup>

BDNF has been trialed in several animal models of PD, including the transplantation of BDNF-releasing astrocytes into the striatum of 6-OHDA-lesioned rats. BDNF was shown to have an effect on improving drug-induced rotational behavior, although there was no increase in tyrosine hydroxylase-positive neuronal density in the substantia nigra, nor detectable BDNF expression after 42 days. There was, however, reduced neuronal loss following 6-OHDA lesioning, but this only occurred in close proximity to the injection site. Other strategies utilizing viral delivery of BDNF have also demonstrated some degree of behavioral improvement, but have again failed to demonstrate evidence of increased neuroprotection in the DA neuronal network.<sup>58,59</sup> All of these results taken together have not been sufficiently encouraging to warrant clinical trials, and thus no such efforts have been undertaken to date.

## PDGF

PDGF is a dimeric glycoprotein composed of two A (-AA) or two B (-BB) chains or a combination of the two (-AB). PDGF is a potent mitogen for cells of mesenchymal origin, including smooth muscle cells, glial cells, and neural precursor cells (NPCs),<sup>60,61</sup> and is upregulated following injury to the adult DA neurons.<sup>62</sup> Pietz et al have shown that neuronal death was significantly decreased (from 75% to about 25%) following the administration of PDGF-AA and PDGF-BB in 6-OHDA-lesioned rats when it was administered prior to the insult.<sup>63</sup> Mohapel et al have also found that intracerebroventricular (ICV) infusions of PDGF and BDNF into the brains of 6-OHDA-lesioned rats resulted in a 1.9-fold increase in bromodeoxyuridine (BrdU)-labeled cells in both the striatum and substantia nigra when compared with controls.<sup>64</sup> Some of these cells expressed markers of immature and mature neurons, although these newborn neurons did not differentiate into DA neurons.

These preclinical findings led to a Phase I/II trial of the intracerebroventricular infusion of PDGF led by Sven Pahagen, which has just completed recruitment. In this study, PD patients between the ages of 30 and 75 receive a 2-week infusion of either PDGF at one of three doses or placebo. They will be followed up for 20 weeks following the administration of the PDGF to assess safety and tolerability with a view to future evaluation of efficacy. Safety and tolerability will

be assessed using standard methods, including MRI and cerebrospinal fluid (CSF) sampling. Secondary outcome measures will be the assessment of motor function using the UPDRS, MADRS (Montgomery Asberg Depression Rating Scale), and manual muscle testing (MMT) rating scales at multiple time points over 3 months, with measurement of dopamine turnover using PET scanning at baseline and 3 months (<http://www.clinicaltrials.gov>).

## Gene therapies

Viral vectors offer the distinct advantage of allowing for the delivery of a specific agent, to a discrete anatomical locus, with the potential for long-term expression and therefore long-term benefits for patients.<sup>65</sup> To date, most gene-therapy clinical trials have utilized recombinant adeno-associated virus-2 (rAAV-2) due to its high transduction efficiency and low immunogenicity.<sup>66,67</sup> Gene therapy strategies for PD to date include those designed to: (1) increase the amount of dopamine in the striatum,<sup>68</sup> (2) reduce the overactivity of the globus pallidus internus and the STN – a gene therapy version of deep brain stimulation (DBS),<sup>69,70</sup> and (3) deliver trophic factors into the striatum.<sup>71</sup> The first two approaches being symptomatic treatments will not be discussed here; however, the third approach involves neurturin (NTT), a member of the GDNF family of trophic factors, and is a potentially disease-modifying therapy.

NTN has been shown to have a neuroprotective effect in animal models of PD and to promote DA fiber sprouting in monkeys.<sup>72,73</sup> As a result, a Phase I open-label clinical trial investigated the safety and tolerability of CERE-120 (adeno-associated virus serotype 2-neurturin) in patients with idiopathic PD. Patients received bilateral, intraputamenal injections of CERE 120 at two different doses and showed a 40% improvement in UPDRS for the low and high dose group, and was well tolerated.<sup>74</sup>

As a result, a Phase II randomized controlled trial using sham/imitation surgery was undertaken.<sup>71</sup> This study randomized 58 patients in a 2:1 ratio to receive either AAV2-NTN or sham surgery. The primary endpoint was the change in the motor subscore of the UPDRS at 12 months, and no significant difference was noted. However, a subset of the patients were followed up for a further 6 months, at which point there appeared to be a modest but significant benefit of AAV2-NTN over sham surgery.

The failure to replicate the findings of the open-label trial at 12 months, but with some benefit at 18 months of follow up, might suggest that a longer period is required for NTN to be transported to the substantia nigra and to exert its trophic effects. In addition, the modest effects in this

study may be due to the dose of NTN used and the stage of disease, as low doses in advanced patients lacking significant numbers of surviving striatal DA fibers will limit the efficacy of this agent. As a result, Ceregene are currently recruiting for a further Phase II study which will involve injections of AAV2-NTN into both the substantia nigra and the putamen (<http://www.clinicaltrials.gov>).

## Oral regenerative therapies

At present, the only oral regenerative therapy under investigation in PD is PYM50028. PYM50028 (Cogane™, Phytopharm Plc, Cambridgeshire, UK), has been shown to restore DA function after 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>)-induced damage to mesencephalic neurons *in vitro* and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mice.<sup>75</sup> PYM50028, when administered orally at a dose of 10 mg/kg/d for 60 days to MPTP-lesioned mice, resulted in a significant elevation of striatal GDNF (297%) and BDNF (511%), and reduced the loss of DA fibers and neurons in the striatum and substantia nigra respectively. These preclinical data have led onto four short (~1 month duration) Phase I trials of Cogane in healthy adults and PD patients, following which, the drug was reported to be well tolerated (<http://www.phytopharm.com>).

Cogane is currently being evaluated in a 400-patient multinational Phase II, randomized, double-blind, placebo-controlled, dose-ranging study (CONFIDENT-PD) (<http://www.clinicaltrials.gov.uk>). Phytopharm, the makers of Cogane, report that there is evidence that Cogane may prevent cognitive decline, making this the first disease-modifying PD drug therapy to potentially have an effect on nonmotor features of PD (<http://www.phytopharm.com>).

## Advantages and disadvantages of stem cell and other regenerative therapies in current use over symptomatic treatments

Most of the regenerative therapies trialed to date require neurosurgical interventions, the risks of which are arguably outweighed by the potential for a single procedure to preclude or reduce the need for lifelong oral therapies. Also, the success of growth factor gene therapies, and in particular their evaluation in the context of a clinical trial, is contingent upon the identification of patients early in the course of their disease, before the underlying pathology has advanced beyond the point at which meaningful

rescue can be achieved. This can be challenging and is certainly not without its ethical concerns. Cell transplants, however, that seek to replace lost cells are perhaps the preserve of more advanced patients where some of these concerns are less of an issue, although even this area is not without its problems, and indeed the data available to date suggest that younger, earlier stage patients do best with this therapy.<sup>76</sup>

## The problems with cell transplantation and its implications for stem cell grafts in PD

It is unlikely that fetal VM transplantation itself will ever become a widespread treatment for PD, given the ethical concerns related to its use and the limited supply of suitable aborted fetal tissue. However, the development of a method demonstrating a consistent efficacious use may be a stepping stone for further work using stem cells. There has previously been marked variability in outcome for patients receiving fetal VM grafts, and this may be due in part to difficulties in standardizing the treatment, as each patient will be the recipient of their own unique cell transplant.

Indeed, the majority of cells grafted in VM transplants are not nigral DA neurons, which are the ones that mediate graft efficacy, and some of these other cell types may underlie some of the side-effects reported (eg, 5-hydroxytryptamine neurons may contribute to GIDs).<sup>77,78</sup> Furthermore, the grafted DA cells do not innervate the entire striatum, as local sprouting of axons from the transplant only reach areas in close proximity to the actual implantation site, which might limit their efficiency and could also cause problems such as GIDs.<sup>79</sup>

Another problem with cell transplantation has recently been demonstrated in grafted patients who have come to postmortem. It has been shown that grafted cells can acquire PD pathology, with some of them containing alpha-synuclein and ubiquitin positive Lewy bodies.<sup>80,81</sup> The reason for this is debated, but a popular view is that this may be because the disease has spread into the transplant. If so, it is unlikely that stem cell grafts will be immune to this process, although the extent to which this pathology adversely affects the graft in the short term is not clear.

As a consequence of the problems relating to the availability of VM tissue and its assorted cellular composition, a more readily available, well defined implant of cells may have distinct advantages. Stem cell-derived cells may be especially useful in this regard, although it must be recognized that their efficacy will be limited if they only target the

loss of DA neurons in the nigrostriatal pathway, as this forms only one part of a more distributed network pathology.

## The problems with growth factor delivery

Intraventricular infusions of GDNF have not only failed to show efficacy, but also have resulted in nausea, reduced appetite, paresthesias, hallucinations, and weight loss. These side effects, however, were not observed with intra-putamenal infusions (presumably because the agent did not enter the CSF in sufficient concentration), although other problems were reported including the development of anti-GDNF antibodies.<sup>46</sup> The need for prolonged infusion via a catheter also carries the risk of mechanical failures and infection. However, these potential complications could be circumvented by using viral vectors as a mode of delivery for trophic factors.

## Gene therapy

Whilst there are now many safe viral vector delivery systems in existence, issues about safety will naturally arise.<sup>82</sup> These risks are considered to be so small that future trials utilizing gene therapy are planned, although which agent is best, the optimal viral system, alongside issues to do with patient selection and target site of injection, remain largely unresolved.

With respect to the viral delivery of trophic agents, problems relate to the dose of agent delivered and its volume of distribution and the fact that once delivered, the agent cannot be retrieved.<sup>83,84</sup> As a result, regulatable systems are being developed along with the introduction of drug-activated suicide genes that will allow for the modulation of these therapies once delivered.<sup>85–87</sup>

The precision targeting that gene therapy allows is in some respects advantageous, but this precision limits the scope for treatment for the nonmotor features of PD that result from pathology in a range of other CNS networks.<sup>88,89</sup> As with direct growth factor therapy, gene therapy will require the identification of patients who are early in the course of their disease, which in the case of PD is somewhat challenging given the absence of any robust biomarkers of disease.

## Oral regenerative therapies (Cogane)

There is an obvious advantage of any oral therapy over those outlined above including ease of administration. The widespread induction of growth factors throughout the brain parenchyma shows greater potential for rectification of the nonmotor aspects of PD, although it remains to be seen whether this will occur to any meaningful extent in patients with PD.

## Pluripotent cells

Despite the many drawbacks of the therapies outlined above, the field of regenerative medicine is continuously moving forward, and the future may lie in a combination of these strategies. The ideal therapy for any neurodegenerative disease is that it will either halt the actual neurodegenerative process and/or replace the full complement of functions of the cells lost. The treatment should ideally be minimally invasive, ethically neutral, practical, well tolerated, and highly efficacious. As neuroprotective strategies are beyond the scope of this review, the treatment with perhaps the best potential to do this is the use of patients own induced pluripotent stem cells (iPSCs) or induced neuronal cells (iN cells).

As much of what is known about these cells has been derived from our knowledge of the development of (ES) cells, these different cell types and their role in the future treatment of PD will be considered together.

## Embryonic stem cells

As ES cells are derived from blastocysts,<sup>90</sup> requiring the destruction of an embryo, there are a number of ethical issues with regards to their use.<sup>91</sup> Nevertheless, these pluripotent cells have the potential to be used as a source of DA neurons in PD, and this has been done with mouse ES cells<sup>92</sup> (both in vitro and in animal models of PD) with some success.<sup>93</sup>

Human ES cells, exhibit rapid proliferation rates, a lack of contact inhibition,<sup>90</sup> genomic instability, high telomerase activity,<sup>94</sup> and expression of oncogenes such as myc and KLF4.<sup>95</sup> These are properties of cancer cells, and as a consequence, the use of human ES cells as a source for cells for grafting carries with it the risk of teratoma formation in transplanted animals.<sup>96,97</sup> However in vitro differentiation of human ES cells can largely eliminate this risk, as can sorting of cells pre-transplantation using methods such as fluorescent-activated cell sorting and magnetic-activated cell sorting.<sup>98,99</sup>

The differentiation of human ES cells into DA neurons using standard protocols is inefficient.<sup>100</sup> However, by adopting combinations of factors known to be involved in normal DA neuronal development, the yield of DA cells from such sources can be dramatically improved.<sup>101–104</sup> Indeed, this ability to make large numbers of functional nigral DA cells from ES sources without teratoma formation has been shown in a number of in-vivo transplant studies, although the results until very recently were less impressive than older studies using fetal VM tissue.<sup>105,106</sup>

## Neural Precursor Cells (NPCs)

NPCs are stem cells committed to a neural lineage, and they are able to form neurons, oligodendrocytes, and astrocytes.<sup>107</sup> These can be derived from ES cell sources, and they have also been shown to exist in the adult brain. As with ES cells, DA neurons can be generated from NPCs, especially when the fetal VM is the source of those cells,<sup>108</sup> and these cells have also been successfully grafted into animal models of PD.<sup>109</sup>

One advantage of NPCs over ES cells is that they have yet to be shown to form the same type of tumors, although they do share the same ethical concerns given their common origin. Also, there are problems with regards to the efficiency with which DA cells can be derived from them.

NPCs have also been shown to exist in the adult brain in two sites in particular – the subventricular zone (SVZ) and the subgranular zone of the hippocampal dentate gyrus.<sup>110,111</sup> These cells generated in the SVZ migrate to the olfactory bulb along the rostral migratory stream,<sup>112–114</sup> and their production is influenced by the nigral DA innervation.<sup>115</sup> This may explain why anosmia is a feature of PD and why anecdotally it has been reported to improve following intrastriatal GDNF infusions.

Whether these SVZ NPCs could be recruited for innate repair in the PD brain has been the subject of much interest, and recently a study by Deleidi et al reported the formation of DA neurons from adult SVZ NPCs.<sup>116</sup> In this study, the authors undertook epigenetic modifications using chromatin-modifying agents to reactivate pluripotency genes such as *oct4* and *klf4*. *Oct4* overexpression in these cells, in the presence of the chromatin-modifying agent valproic acid, induced SVZ NSCs to be reprogrammed into iPSCs, which could then be differentiated into DA neurons. This paper demonstrated that the production of DA neuron from adult NPCs required the formation of pluripotent cells and epigenetic modifications.

It has previously been suggested that adult NPCs could be extracted, expanded, and transplanted back into the donor, but a pluripotent phase in their production is likely to increase their tumorigenicity, and the practical limitations of their extraction are likely to limit their use, especially in the context of the burgeoning field of iPSCs, where the initial cell source is more readily accessible. Furthermore, recent questions have arisen as to the extent to which the SVZ neurogenic process is truly active in the adult human brain.<sup>117</sup>

## Induced pluripotent stem cells (iPSCs)

In 2006, somatic cells were first successfully transformed into pluripotent stem cells by the retroviral expression of a set of four genes in mouse fibroblasts. *Oct4*, *Sox2*, *Klf4*, and *c-Myc*

were shown to convert somatic cells into pluripotent cells, albeit at a very low efficiency.<sup>119</sup> These cells demonstrated properties similar to those of ES cells in terms of their ability to proliferate and differentiate into all three primary germ cell layers, and also with respect to their gene expression patterns and epigenetic status. The following year, similar results were obtained using human cells, ushering in a new era in the field of regenerative medicine.<sup>119,120</sup>

iPSCs have the potential to play a major role in neurodegenerative disease, as they could be used to: (1) model diseases in vitro, allowing for the elucidation of pathological processes and the screening of potential drug therapies; and (2) generate cells from the patient themselves to be used for transplantation.

## Disease modeling

The ability of these cells to form any tissue type in the body will theoretically allow for in vitro modeling of a wide range of diseases using donor derived cells. Though PD arises sporadically in over 85% of cases, there are several Mendelian forms of Parkinsonism that exist,<sup>121</sup> and iPSCs have now started to be grown from such cases. For example, Nguyen et al have described their findings in iPSCs and neurons derived from a patient with the common mutation in the leucine-rich repeat kinase 2 gene (*LRRK2*). They showed that whilst there were no major basal abnormalities in these cells, when exposed to oxidative stress they behaved differently and showed features of cellular dysfunction when stressed.<sup>122</sup> Devine et al have also recently shown that iPSCs derived from a patient with a triplication of *SNCA* – the gene coding for alpha synuclein – produced twice as much alpha synuclein as cells derived from an unaffected first-degree relative.<sup>123</sup> Whilst another study that generated DA neurons from iPSCs derived from patients with sporadic Parkinson's disease demonstrated no such abnormalities,<sup>124</sup> highlighting the possibility that different forms of Parkinsonism may have slightly different pathogenic pathways underlying their cell loss. Of course it is still unknown whether disease pathogenesis can truly be studied in IPS-derived neurons, given the timeframe of disease in vivo compared with in-vitro cell culture studies.

## Cell transplantation

The advantages of producing patient-derived cells for the treatment of neurodegenerative disease are myriad. Firstly, there should be no need for immunosuppression if they are grafted back (although see Fairchild<sup>152</sup>), as the cells are derived from the patients themselves. Secondly, the ethical obstacles that exist with the use of ES cells and fetal tissue

grafts no longer apply.<sup>125</sup> Finally, assuming that a cell line can be expanded adequately, issues of supply will no longer apply. The disadvantage is that if the cells so derived are grafted back, then there is a risk that they could develop the very disease pathology they were designed to treat.

Furthermore for iPSCs to be used in this way, they must be produced efficiently, in sufficient quantity, in a process that meets Good Manufacturing Practice standards. The cells must also:

- be able to fully differentiate *in vivo* to form the correct nigral DA neurons and to retain that phenotype, and form appropriate synaptic connections with existing neural networks;
- not proliferate to form tumors;
- not migrate away from the site of grafting and become abnormally integrated into other neuronal networks within the host brain.

These problems and possibilities will be discussed next, given that there is a growing expectation that these cells are likely to enter the clinical arena in the not too distant future.

## The differentiation of iPSCs to nigral DA neurons

Fully differentiated IPS-derived NPCs have been successfully transplanted into animal models of PD, utilizing a number of different techniques with functional benefits.<sup>127–129</sup>

Most recently, Sanchez-Danes et al showed that using lentiviral vectors to drive the controlled expression of LMX1A efficiently produced human A9-subtype ventral midbrain DA neurons, and Jeager et al showed that fibroblast growth factors signaling is necessary for the efficient production of functional, midbrain-specific DA neurons in both human- and mouse-derived iPSCs.<sup>101,129</sup> Chang et al demonstrated that docosahexanoic acid (DHA)-treated iPSCs differentiated into tyrosine hydroxylase-positive neurons *in vitro* and *in vivo*.<sup>130</sup> Interestingly, DHA also upregulated the endogenous expression levels of genes such as BDNF and GDNF and protected against MPTP-induced apoptosis. These cells when transplanted, significantly improved the behavior of 6-OHDA-lesioned rats compared with control or eicosapentaenoic acid-treated cells.

## Efficiency of cell production

At present, the efficiency of producing iPSCs remains low, with 4–125 ES cell-like colonies being produced from 800,000 mouse fibroblasts. These rates are even lower

when adult human fibroblasts are used, and so several approaches have been employed to increase the efficiency of this derivation process. Genetic modification using viral vectors, alteration in the combinations of transcription factors employed,<sup>131,132</sup> and RNA inhibition of transcription factors and DNA methyltransferase inhibitors might all increase the efficiency of iPSC production.<sup>133</sup> The aforementioned methods, however, will preclude the use of such cells in a clinical setting due to the fact that they have undergone genetic manipulation, and this is compounded by the fact that they are often grown using animal-derived culture reagents. There have, however, been improvements in this regard with a reduction in the number of genes required for reprogramming, the use of nonintegrating approaches, and the introduction of temperature-sensitive mutations into vectors, allowing for the easy removal of the viral vectors at the appropriate temperatures.<sup>134</sup> More recently, as opposed to using fibroblasts as a cell source, attempts using keratinocytes have been shown to be both faster and more efficient.<sup>135,136</sup>

## Tumorigenicity

Human iPSCs share with human ES cells basic properties of self renewal and pluripotency. There are, however, differences between the two cell types, including epigenetic profiles,<sup>137</sup> global gene expression,<sup>138</sup> and genomic imprinting,<sup>139,140</sup> although the exact extent to which these differences truly occur remains a topic of debate.

The production of iPSCs involves the use of oncogenes. The initial studies involved Myc, a well established oncogene, and oct 4, sox2, and KLF4 – all of which are variably expressed in different types of cancers.<sup>141</sup> Though the reprogramming process itself has been refined in recent years, with some protocols allowing for the exclusion of myc for example, the remaining factors still represent a potential risk for tumorigenesis.

Genomic instability, leading to tumor formation can arise as a result of the use of integrating vectors, and as result there have been several developments in the reprogramming protocols that allow for the use of nonintegrating adenoviruses,<sup>142,143</sup> expression plasmids, episomal vectors,<sup>144</sup> excisable vectors,<sup>145</sup> and the direct delivery of reprogramming factors,<sup>146</sup> thereby reducing the risk of genomic instability.

In addition, iPSCs produced from somatic cells, have already undergone a number of cell divisions, and therefore they may have acquired genetic mutations that predispose to tumor development.<sup>147</sup> Human ES cells cultured *in vitro*

are susceptible to developing chromosomal abnormalities with chromosomes 12, 20, 17, and X being the sites of the most common aberrations, and similarly, iPSCs have demonstrated duplication and trisomy of chromosome 12.<sup>148,149</sup> Epigenetic factors may also contribute to the increased tumorigenicity of iPSCs. Micro RNA (miRNA) expression in two human iPSCs demonstrated an overexpression of ten cancer-related miRNAs in human IPS cells, and other studies have reported altered DNA methylation in cancer-specific gene promoter regions.<sup>150</sup> iPSCs also pose a risk that does not exist with human ES cells, ie, the potential to form somatic tumors, and many of the mouse chimaeras formed from the four gene iPSCs died within the first months of life from such tumors.<sup>97</sup> Similarly, when human ES cells and iPSCs are compared, iPSCs were shown to form tumors, especially highly malignant ones, more readily.<sup>150</sup>

In the context of the above, the problem of tumorigenicity of iPSCs could be reduced by using cells that have been terminally differentiated, and the use of suicide genes may be required. Finally, the silencing of onco-fetal genes using pharmacological methods may be of use and would in theory target those genes that are necessary for teratoma formation but dispensable in fully differentiated cells; though, such targets are yet to be identified.<sup>151</sup>

## Immunogenicity

iPSCs, unlike ES cells, should not induce immune alloreactivity in theory. However, in practice it may not be that simple because the altered gene products that arise in the derivation of the cells may not be recognized as self.<sup>152</sup> Evidence that this may in fact be the case comes from a recent study undertaken by Zhao et al. They demonstrated that iPSC teratomas derived from C57BL/6 mice using both a retroviral and an episomal approach were rejected when transplanted into C57BL/6 mice. This, however, was not the case when ES cells were transplanted into the same mice. The authors went on to show that this was at least in part due to the overexpression of genes by the iPSCs, the products of which were directly immunogenic.<sup>153</sup>

## Is pluripotency really necessary?

As outlined above, there are many obstacles to the clinical application of iPSCs. Relatively undifferentiated cells result in the best graft survival and greatest functional recovery but also demonstrate a propensity for the development of tumors. An argument can therefore be put forward for the direct production of neurons from somatic cells. This would

circumvent both the ethical problems of using ES cells as well as issues of tumor formation.

Vierbuchen et al were the first to demonstrate that the expression of three transcription factors could directly convert mouse fibroblasts into functional neurons (iN cells).<sup>154</sup> This has now been replicated in a number of different studies, and it has now been shown that it is possible to make DA neurons directly from fibroblasts. Pfisterer et al have shown that by combining the expression of these three factors with the expression of two genes known to be involved in the production of DA neurons – Lmx1a and FoxA2 with ASCL1, Brn2, and Myt1l – DA neurons could be produced.<sup>155</sup> Subsequently, Caiazzao et al have produced DA neurons, only with the expression of Mash1(Ascl1), Nurr1, and Lmx1a,<sup>156</sup> and have shown that these cells release dopamine and have spontaneous electrical activity consistent with the pacemaker activity typical of nigral DA neurons. Induced DA neurons have also recently been shown to have some functional capacity following transplantation into animal models of PD.<sup>157</sup>

At present, the experimental evidence for the use of induced neurons is limited, and a robust effect in animal models of PD would need to be clearly demonstrated prior to its consideration for clinical use.

## Future directions/conclusion

Here we have summarized the results of several open-label trials that have not gone on to show any benefit in two small randomized controlled trials. Though it could be argued that these studies failed for a whole host of reasons, it does highlight that we are still some way from knowing how best to test experimental biological, cell-based therapies in the clinic in PD.<sup>158–161</sup>

In the next few years the results of several key trials of growth factors and cell transplants will become available, and these will no doubt shape the future of this field. Whilst an emphasis must be placed on developing effective strategies for the replacement of cells lost in PD, one must not ignore neuroprotective strategies that may work alongside regenerative therapies as ultimately, combination therapies might be the optimal therapeutic approach. Additionally adequate assessment of any new intervention is contingent upon a good understanding of the natural history of the disease and access to objective measures of disease progression. These areas of PD research must therefore develop in parallel with regenerative medicine so as to ensure that future trials can be undertaken with greater confidence.

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