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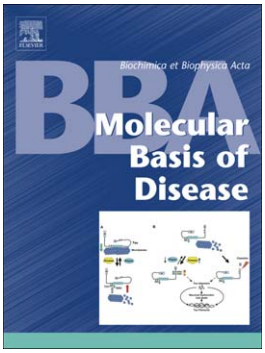

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Cell Replacement Therapy for Parkinson's Disease

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SUMMARY

Parkinson's disease (PD) is a progressive neurodegenerative disorder in which the degeneration of dopaminergic neurons projecting from the substantia nigra to the striatum is a key pathological feature of the disease. Although pharmacological dopamine replacement is generally very effective in early disease, it is only a symptomatic therapy and can have significant side effects with long term use. One of the key strategies in a more restorative approach to PD therapy involves replacement of this degenerating nigro-striatal dopaminergic network with cells and several possible cell sources are being explored. While much experience and some success has been gained with fetal ventral mesencephalic (FVM) tissue transplants, the rapidly advancing stem cell field is providing attractive alternative options which circumvent many of the ethical and practical problems inherent in trials with FVM tissue. Of these embryonic stem cells and induced pluripotent stem cells seem the most promising. However further development and optimisation of the safety and efficacy of the techniques involved in generating and manipulating these, as well as other, cell sources will be essential before any further clinical trials are carried out.

KEY WORDS –

Parkinson's disease; Cell replacement; Transplantation; Fetal Ventral Mesencephalon; Stem cell

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1.INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease affecting approximately 1% of people over 65 years of age. It is characterised clinically by the development of bradykinesia, rigidity and a resting tremor, which has been attributed in part to the progressive degeneration of the dopaminergic input from the substantia nigra to the striatum. The cause for this progressive loss of nigrostriatal dopaminergic neurones is not entirely understood, but it is associated with the presence of alpha-synuclein positive Lewy bodies, in the remaining substantia nigra pars compacta neurones (SNc) at post-mortem. However, Lewy bodies are also found in many other parts of the brain (including other subcortical and brainstem nuclei as well as the cortex) and this diffuse pathology underlies the other non-motor features of PD such as cognitive, sleep and affective problems as well as autonomic dysfunction, which are found in many PD patients. Indeed it has even been proposed that this non-nigral pathology dominates the very early and late stages of disease [1-3].

Nevertheless, despite this non-nigral, non-dopaminergic pathology, the principal feature of the disease remains the progressive loss of dopamine in the striatum, which can be treated successfully with a range of symptomatic dopaminergic drug therapies. Thus both levodopa and dopamine agonists can significantly help many of the features of PD, particularly in the early stages of the disease. However with time, the disease progresses and this coupled to the long-term use of dopaminergic drug therapies produces a range of problems including the development of drug-induced motor complications such as "on-off" fluctuations and levodopa-induced dyskinesias (LID). At this stage of the disease simple drug therapies become increasingly disappointing in terms of a reliable therapeutic benefit and thus other approaches are sought

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including more invasive ways of delivering more continuous dopaminergic therapy such as apomorphine pumps and DuoDopa®, as well as neurosurgical interventions such as deep brain stimulation which typically, but not exclusively, targets the subthalamic nucleus.

These latter therapies can be very effective, but only ever treat the symptoms without any attempt to repair the underlying diseased network. Thus these treatments also start to fail, in part because of the progressive nature of the non-nigral, non-motor aspects of PD and in part because of the continued loss of nigral dopaminergic neurones. Therefore, whilst a more complete understanding of disease pathogenesis may enable us to better treat all aspects of PD, more restorative approaches that involve repairing the dopaminergic nigrostriatal tract (including cell replacement, neurotrophic support and pharmacological and gene therapies) may also prove very useful (see Table 1). It is this latter area that we will be concentrating on in this review, as we discuss the different approaches using cell therapies in PD and their clinical efficacy to date.

Cell therapies in PD have been a major research interest for the last 30 years with the main focus being on using them for replacement of the degenerating, and lost, dopaminergic neuronal innervation of the striatum from the nigra. This review will discuss the different sources for dopaminergic cell replacement therapy in PD, with a particular emphasis on fetal ventral mesencephalic tissue and stem cell sources for transplantation and replacement of the dopaminergic innervation of the striatum rather than reconstruction of the nigrostriatal pathway. Obviously, cell therapies can be used to repair/ restore function through mechanisms other than simple cell replacement and whilst this is not the subject of this review, it should be stressed that cell therapies for PD may work by more than one pathway which are all complementary in mediating repair (Table 1).

A number of different types of ‘dopaminergic-like’ cell have been considered for grafting in PD (see Figure 2) and in this review we will be concentrating on those which have shown the greatest

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clinical benefit (e.g Fetal ventral mesencephalon (FVM)) and those with the greatest promise (e.g.-: Embryonic Stem Cells (ESCs) and Induced Pluripotent Stem (iPS) Cells).

2. AUTOGRAFTS

The use of autografts has always been attractive for the treatment of any disorder including Parkinson's disease because of the ease of tissue collection without the ethical and immunological problems inherent in allogenic and xenogenic tissue. It is therefore not surprising that much of the early work on cell therapies for PD concentrated on autologous cellular sources of dopamine, although overall the results to date have been disappointing using tissue of this type.

2.1 Adrenal Medulla

Initial attempts at autologous cell transplantation in PD included the use of adrenal medullary (AM) tissue, which secretes some dopamine among other catecholamines. Early studies in animal models of PD were very variable, but provided some evidence for survival and functional efficacy [4-6]. Despite uncertainties about the mode of action of the AM grafts and the limited functional benefits experimentally, small open label clinical trials started in the early 1980s with all the inherent problems that exist when no placebo group is used for comparison - a problem that has emerged many times in this field of brain repair. Nevertheless these early studies with AM transplants produced some slight transient improvement for the patients [7, 8], while another group at around the same time reported significant motor and cognitive improvements using an open neurosurgical technique [9, 10]. However, the interpretation of the data from this study has been queried [11].

Subsequently, other studies showed a modest effect (an increase, from 47.6% to 75% in time spent in the 'on' phase of L-dopa treatment) for a limited period of time only, namely ~18 months

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[12, 13]. Furthermore, post-mortem studies showed poor survival of grafted tissue [14, 15] with a recent neuropathological study done 16 years after an autologous adrenal medullary transplantation showing few surviving chromogranin A positive cells and a complete absence of cells staining for tyrosine hydroxylase (TH) (the key enzyme in dopamine synthesis) at the graft site [16]. This patient had derived some clinical benefit from the graft for ~4 years post transplantation.

In order to improve the survival of these AM transplants, co-grafts of tissue secreting Nerve Growth Factor (NGF) were investigated. The animal studies underpinning this approach involved co-grafting AM with pre-transected peripheral nerve (a source of NGF) and showed improvement in graft survival and functional recovery [17, 18]. However clinical studies using a similar approach have reported only modest improvements of Unified Parkinson's Disease Rating Scale (UPDRS) scores in both 'on' and 'off' phases with an increased proportion of time when the dopaminergic medication is working, namely the 'on' phase [19, 20], with a corresponding reduction in the "off" phase. Clinical trials of direct NGF infusion to support AM autografts have also been tried with some benefit [21]. However the high level of morbidity and mortality associated with the techniques involved in the obtainment and transplantation of AM tissue are significant and currently outweigh the (at best) modest benefits of these grafts. As a result there is little justification to continue with AM transplants for PD.

2.2 Sympathetic Ganglion Neurones

Sympathetic ganglion neurones have also been considered as a source of cell replacement for PD as they express AADC and VMAT2, (proteins involved in the production and transport of dopamine) in culture [22] as well as following transplantation in a rat model of PD [23]. Their action is thought to be mediated by their ability to convert and store dopamine found in the

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extracellular space. In clinical studies sympathetic ganglion tissue has been harvested from the cervical [24-26] or thoracic [22, 23] sympathetic trunk and transplanted into the striatum of patients with PD. Approximately half the patients having such grafts experienced reduced 'off' time and increased 'on' time with an overall improvement in bradykinesia and gait in some, but no significant change in UPDRS scores. These limited clinical benefits and the invasive techniques and associated morbidity of the procedure involved in obtaining the donor tissue make this a relatively impractical option for clinical use in patients with PD.

2.3 Carotid Body

The cells of the carotid body are known to be chemosensitive and secrete dopamine and divide in response to hypoxic stimuli [27, 28]. These properties have led to an exploration of their use in dopaminergic cell replacement for PD and animal studies in rodents and primate models of PD have demonstrated partial functional recovery following intra-striatal transplantation of carotid body cell aggregates [28-30]. This led to a clinical study, in which 13 patients aged 43-61 years with advanced PD underwent bilateral carotid body transplants into the striatum [31]. Improvement in the UPDRS motor score at 1 year post transplantation was the primary outcome measure, which most patients achieved with a reduction in their UPDRS 'off' score of between 13% - 52%. Of the 6 patients assessed at 3 years post transplant, the improvement was maintained in 3 and this appeared to correlate with milder baseline disease severity and the absence of fibrotic atrophy within the carotid body [32].

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There was, however, no increase in fluorodopa (F-dopa) uptake – a measure of presynaptic dopaminergic nerve terminals - in the transplanted striatum on Positron Emission Tomography (PET) imaging and thus the reason for the motor improvement was not clear. Surviving numbers of dopaminergic neurons in the transplants were only ~10% of that seen in the successful fetal ventral mesencephalic allograft recipients (see below) and so the authors suggest that the therapeutic benefit of carotid body cell transplants may relate more to the release of trophic factors rather than dopamine release. However, PET scanning data from successful trophic factor (GDNF) infusion studies in PD have shown increases in striatal F-dopa uptake in response to trophic factor infusion [33] so that if this were the explanation for the success of the carotid body transplants, one would still expect to see a change in PET F-dopa signal.

3.0 ALLOGRAFTS

3.1 Retinal Pigment Epithelium

The cells of the retinal pigmentary epithelium (RPE) act as support cells in the retina and produce L-dopa as part of their melanin synthetic pathway. They have also been shown to have neurotrophic effects on rat striatal and mesencephalic neurones in vitro [34] and have been investigated as a source of dopaminergic cells for transplantation in PD. The attachment of the RPE cells to microcarriers made of gelatin seems to increase their survival on grafting and studies in rat and primate PD models have shown improvements in motor symptoms and F-dopa PET imaging following their transplantation [35, 36]. This led to a clinical open label trial involving human RPE cells attached to microcarriers (Spheramine™) which were transplanted unilaterally into the putamen of 6 patients with advanced PD. This brought about an average improvement in

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the 'off' UPDRS motor score of ~48% at 1 year. Following this a double-blind placebo controlled study using Spheramine™ was commenced. Initial analyses of results, however, have not detected any significant differences between the Spheramine and sham surgery arms of the study after 12 months of follow up and the future potential of RPE cells as a source for cell replacement therapy in PD is still unclear. (http://www.titanpharm.com/products-trials.php).

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3.2 Fetal Ventral Mesencephalic Transplants

3.2.1 Animal Studies

The very first successful attempts at fetal dopaminergic cell transplantation were reported by two groups in 1979. The procedure involved the allogenic transplantation of fetal ventral mesencephalon (VM) tissue into the rat 6-OHDA model of PD. Perlow et al placed the grafts into the lateral ventricle [37], while a dorsal cortical cavity was used by Björklund and Stenevi for the placement of their grafts [38]. In both studies, functional recovery was described on simple motor tasks and subsequent studies confirmed this and showed that there was graft survival, dopaminergic fibre outgrowth and synapse formation seen on histological analysis [39]. While improvements were seen when the tissue was placed striatally, attempts to place grafts in the

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substantia nigra, the site of dopaminergic neuronal cell body degeneration, showed survival of cells, but they did not seem to extend axons to the striatum and thus produce functional improvements [40]. This dependence on the striatal location of the graft for functional benefits [41], which has become the main site of dopaminergic graft implantation, showed some topographical specificity with more ventrally placed grafts in the striatum having greater effects on more complex nonmotor tasks. [41, 42]. The benefits were also dependent on the age of the donor embryo, such that harvesting the tissue at the time of normal dopaminergic nigral development was critical (reviewed in [43]). These rodent studies were then extended to non-human primates [44-47], where it was shown that the same principles apply.

These initial allogenic animal studies led onto work with human fetal ventral mesencephalon (VM) transplants in rat PD models. These studies demonstrated that human fetal VM tissue transplanted into the 6-OHDA rat striatum could survive, integrate, form dopaminergic connections and produce functional improvement [48] and were best seen in immunosuppressed rats receiving grafts from younger, 6-8 week old, fetuses. These animal studies paved the way for the translation of this experimental therapy to patients with PD, although even at this stage it was clear that striatally placed transplants of fetal VM could not improve all the deficits seen in this simple animal model of PD. As such it would be naïve to expect such transplants to reverse all aspects of PD in the more complex situation of patients and this needs to be borne in mind when interpreting any clinical trials using this approach.

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3.2.2 Clinical Studies

Open-label trials

The first clinical trials were carried out in the 1980s in Mexico [49] and Sweden [50] with 2 PD patients in each trial. The studies used VM tissue from 12-14 week and 8-10 week old embryos respectively, which were transplanted into the striatum with minimal clinical improvement, at least in the patients from Sweden. However, by refining the techniques, several subsequent open-label clinical studies have shown significant improvements in a number of parameters, including the UPDRS, Activities of Daily Living (ADL) scale, health related quality of life and levodopa requirements. [51-57]. These clinical improvements have been sustained and associated with increased fluorodopa (F-dopa) uptake on PET scans, as well as regulated dopamine release and activation of motor cortical areas [58, 59]. Furthermore, early (18 month) post-mortem pathological studies also showed the survival of grafted fetal VM dopaminergic neurones (~ between 81,905 – 135,673 TH+ neurons), with graft-mediated striatal reinnervation [57, 60]. Therefore the initial open label clinical studies demonstrated that fetal VM allografts could survive in patients with PD, become functionally integrated and produce sustained clinical benefits. However, the results were variable and one of the challenges was to explain this variability and how it could be minimised - an anxiety that became more of an issue with the publication of 2 double blind, placebo-controlled trials of fetal VM transplantation in PD.

Double-blind Placebo Controlled Trials

With encouragement from the initial open label results and the argument for the need to properly support the actual efficacy of the procedure, two double-blind placebo controlled trials

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of fetal VM transplantation in PD were funded by the National Institutes of Health (NIH), USA in the 1990s. The first trial was published in 2001 by Freed et al. It involved 40 patients with PD aged between 34-75 years and mean disease duration of 14 years [61]. Patients were randomly assigned to receive a transplant or imitation, sham surgery. VM tissue was obtained from aborted 7-8 week old embryos, cultured in F12 medium containing 5% human placental serum for up to 4 weeks prior to transplantation and specially prepared into strands of tissue. The transplants took place under local anaesthetic with the patient awake and involved stereotactic neurosurgical techniques. Tissue from two embryos was transplanted into the putamen on each side and no immunosuppression was used. The control patients received a sham surgical procedure involving burr holes with no penetration of the dura.

The primary outcome, a subjective global rating of clinical improvement at 1 year post transplant, showed no significant improvement in the transplant compared to the placebo group. However significant improvements were seen in the 'off' medication UPDRS total and motor scores in transplant patients below 60 years of age. Subsequent analysis however suggested that the main determinant of this correlation was the preoperative levodopa responsiveness rather than the age of the patient, as even older patients with good preoperative levodopa responsiveness showed similar improvements [62]. The main motor improvements were in rigidity and in the younger patients, bradykinesia, while tremor showed no response. Despite the modest and variable clinical benefits, PET scanning showed significant increases in F-dopa uptake in the putamen of the transplant group compared to placebo and post-mortem examinations showed dopaminergic neuronal survival and fibre outgrowth in the grafts. However, the number of surviving cells was less than that reported by others in patients with good clinical responses to their transplants (eg- ~11,592- 20,188 at 7 months and ~2,060 – 22,760 at 3 years) [61]. However, of concern in this trial was the first clear description of the development of significant graft induced dyskinesias (GID) in 15% of transplanted patients more than 1 year post transplant -

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dyskinesias that occurred in the absence of medication, but presence of the graft. Several of these patients required further surgical intervention to relieve them of these GIDs [63].

The reason for this negative outcome and the induction of GIDs was, and is still, unknown, but various factors may be critical including the following. The amount of tissue grafted was less than other more successful transplant trials and this was borne out by the post-mortem data. The neurosurgical approach used a different trajectory to target the striatal complex, which may have led to problems with dopaminergic cell distribution; a finding that gains some credence from the follow up PET study of Ma et al showing dopaminergic hot spots in patients developing GID [64]. The absence of immunosuppressive therapy may have compromised dopaminergic cell survival in the graft as may the storage of the tissue for up to 1 month prior to implantation. For these reasons it could be argued that the trial produced negative results with side effects for methodological reasons. However the second study by Olanow et al, published in 2003 tended to support, rather than refute, this trial result.

This second NIH sponsored study [65] involved 34 patients with advanced PD, aged between 30-75 years. Patients were randomised to receive bilateral transplantation with either one or four donors per side or a 'placebo'/ sham transplant procedure. Solid VM tissue was obtained from 6-9 week fetuses, stored in a hibernation medium for 2 days prior to transplantation. The surgery was performed under general anaesthesia using a 2 stage procedure separated by a week, with the tissue from one or four embryos being transplanted into the putamen bilaterally. All patients received immunosuppression with cyclosporine 2 weeks preoperatively and up to 6 months postoperatively. Sham surgery consisted of partial burr holes only with no breach of the dura.

The primary outcome measure was a change in the motor component of the UPDRS in the practically defined 'off' state, between the baseline and the final 24 month visit. Once again, no significant overall treatment effect was observed, although there was a tendency towards an

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improved motor score in the 4 donor group. There was also no difference between groups in terms of the change in percentage 'on' time without dyskinesias and in required L- dopa dose equivalents. However, stratification based on disease severity displayed that four donor transplant patients with less severe disease (UPDRS </= 49 at baseline) did show significant improvement in their UPDRS motor scores. Also patients in the one- and four-donor transplant groups showed significant motor improvement compared to placebo at 6 and 9 months post transplant, but deteriorated thereafter, perhaps relating to discontinuation of the immunosuppressive therapy at this time point. This initial improvement was similar to that reported for previous open label studies [51, 66, 67].

PET scanning showed significant bilateral increases in striatal F-dopa uptake in both transplant groups compared to placebo with the four donor group showing the greatest increase. Post mortem examination also showed good survival of dopamine neurones with relatively seamless reinnervation of the striatum, again with the four donor group showing the better results. However, as in the Freed et al. study there was, worryingly, the development of significant 'off-medication' graft induced dyskinesias (GID) in 56.5% of the grafted patients 6-12 months after transplantation. These dyskinesias consisted of stereotypic, rhythmic movements of one or both lower extremities, with three patients requiring further surgical intervention to reduce their severity.

Thus, the results from these two double-blind placebo controlled trials raised serious concerns about the utility and safety of FVM transplants in patients with PD. The main concerns related to the modest (non-statistically significant) efficacy of the transplants compared to previous open-label trials and to the development, in a significant number of patients, of GID. The reasons for these negative outcomes and the development of GID remain unresolved, but may relate to

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patient selection, graft tissue preparation and placement and the extent of immunosuppression. We will now deal with each of these items in turn.

Patient selection: The evidence that this may be an important factor comes from the observation that the best results in the double blind placebo controlled trials were seen in patients with less severe disease (UPDRS < 49 at baseline) and best preoperative response to levodopa [62]. There is also some evidence that older patients do less well [61].

Tissue preparation and placement: Factors such as the number and age of embryos used, coupled to the methods of storage and preparation and the techniques used in graft placement, may all have affected the survival and functional integration of donor dopaminergic neurones. Thus in the Freed et al study the methods adopted involved less tissue, stored for longer times, delivered by a transfrontal approach, while Olanow et al used tissue pieces, stored for short periods of time. Many studies have shown that a variety of factors in the tissue preparation process are critical for optimisation of dopaminergic cell survival in transplants and some of the factors in the tissue preparation protocols adopted by the trials may have had a deleterious effect on dopaminergic cell survival post transplantation [68][69]. Furthermore there is recent evidence for the emergence of PD like pathology in long term transplants of FVM and how this relates to the preparation and engraftment of the tissue remains unresolved (see below).

Immunosuppression: Although the brain is considered to be an immunologically privileged site, the host immune system can respond to the grafts, especially if they are not well matched from an HLA perspective to the host. This may relate to the drainage of antigen from the grafted cells out of the central nervous system into the deep cervical lymph nodes, the presence of activated T cells patrolling the central nervous system and the breach of the blood-brain barrier (one of the main factors contributing to the immune privilege) by the grafting procedure [70]. In Olanow et al.'s placebo controlled trial which involved immunosuppression with cyclosporine for 6 months, there was a deterioration in clinical response 6-9 months after grafting, with post-

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mortem tissue evidence for activated microglia and immune reactivity (CD45) in and around the graft deposits [65]. All of this may have compromised dopaminergic cell survival in the transplant.

Graft induced dyskinesias (GID): The reasons for, and the mechanism behind, the development of GID post transplantation is not fully understood as in retrospect they had been seen in patients transplanted prior to the placebo controlled trials, albeit to a much less severe extent [71]. These GID are different in nature to the typical dyskinesias seen in drug treated PD [72, 73]. The severity of the GID does tend to correlate negatively with the preoperative putaminal F-dopa uptake but does not correlate with improvement in the 'off' UPDRS motor scores or daily dopaminergic medication level. Furthermore their development does not seem to be related to the severity of the pretransplant L-dopa induced dyskinesias (LID) or the change in LID post operatively. In fact some transplant patients showed an improvement in LID together with worsening GID [57, 71]. Several possible factors may relate to the mechanism of these GID. These include the relatively inhomogeneous graft mediated dopaminergic reinnervation of the host putamen [64], an effect from cells other than the A9 dopaminergic neurones within the graft [74, 75], in particular the 5HT neurons included in the transplants [76, 77] and the graft size and placement within the striatum [78, 79].

At the present time there is no clear consensus as to whether one, or all, of these factors contribute. As a consequence of all these possible confounds, a re-evaluation of the field of neural grafting in PD is being undertaken – a point that has perhaps been reinforced by the recent long term post-mortem studies of FVM transplant patients showing alpha-synuclein pathology in the graft [80-82].

In the first of these studies, Mendez et al. [80] reported on 5 patients (referred to as subjects 1, 2, 4, 5 and 6) aged 55-69, with a 11-19 year history of PD, who underwent unilateral (subject 5)

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or bilateral (subjects 1, 2, 4 and 6) transplantation of VM cell suspensions from between two and four 6-10 week old fetuses into the putamen (caudate and substantia nigra as well in subject 2). Immunosuppression with cyclosporine covered the period 2 weeks prior to and 6 months following transplantation. Subjects 1 and 2 showed striking clinical benefits up to 3 years post transplant, with PET imaging showing increased F-dopa uptake within the right striatum [83]. They died of unrelated causes 3-4 years post transplantation and post-mortem showed ~98,913 – 202,933 grafted TH+ cells per side [83]. Subjects 4, 5 and 6 were reported to show variable clinical benefit lasting anywhere between 2 months and 3 years post transplantation. Postmortem studies done 9-14 years post transplantation showed well integrated grafts with between 9861 and 21552 TH+ neurons per putamen, mainly in the graft periphery. TH, alpha-synuclein and ubiquitin staining for Lewy bodies showed their presence in the cell bodies and terminals of the substantia nigra, the upper raphe nucleus, neocortex and putamen, as one would expect in PD, but not in the grafted dopaminergic neurons in the putamen. In addition, surrounding the grafts, there was a slightly higher density of CD45+ microglia, compared to a control PD putamen, but they were mostly in a non-activated state.

In contrast, Kordower et al reported on a 61-year-old patient with a 22 year history of PD who had received bilateral solid FVM transplants to the putamen from four, 6.5-9 week old, embryos [81]. The patient improved clinically for a period of ~11 years and then gradually deteriorated. Post mortem analysis at death 14 years post transplant showed good graft survival with extensive TH innervation of the host striatum. Staining for other markers of dopaminergic neurons – vesicular monoamine transporter 2 (VMAT2) and dopamine transporter (DAT) showed good staining for the former, but very light to no staining for the latter, which diverges from previous findings [60]. There was, as expected, a considerable loss of TH neurons in the host nigra and those remaining contained both cytoplasmic and aggregated alpha-synuclein and ubiquitin. The unexpected finding was the presence of cytoplasmic, aggregated and neuritic alpha-synuclein in grafted neurons. In other words, ubiquinated aggregates, some with the appearance of Lewy

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bodies were seen in grafted neurons and the transplants were also filled with activated microglia to a level far greater than that seen in the host striatum. Two further brains were examined from patients who had undergone a similar fetal transplant procedure and died approximately 4 years post transplant and again showed cytoplasmic and nonaggregated alpha-synuclein staining in the grafts [81]. This is at variance with what you would expect, as transplanted neurons would developmentally be only 4-14 years old and studies in normal humans have shown that cytoplasmic (nonaggregated) alpha-synuclein is not seen in nigral neurons until at least middle age and that aggregated alpha-synuclein may even be absent in people over 100 years of age [84].

A further report by Li et al also tends to support these findings [82]. In their study two patients age 53 and 45, with a 16 and 7 year history of PD underwent bilateral sequential transplantation of dissociated FVM tissue from 6-8 week old aborted embryos into either the putamen only or both caudate and putamen. The two patients received immunosuppression with prednisolone and cyclosporine for 24 and 18 months and azathioprine for 20 and 6 months respectively. They showed minimal clinical improvement [85] and at post mortem 11-16 years post grafting, they had few surviving and integrated grafted TH neurons (12,100-29,500 per injection track). In both patients the grafted neurons again contained alpha-synuclein- and ubiquitin-positive Lewy bodies and Lewy neurites, morphologically indistinguishable from those seen in surviving neurons in the host substantia nigra pars compacta. In one patient who had received sequential grafts to the putamen, the proportion of TH neurons with detectable amounts of alpha-synuclein was 40% in the graft done 12 years ago, and 80% in the graft done 16 years ago, suggesting evolving pathology with time. Finally in this paper, it was shown that the Lewy bodies in the grafts also stained with an antibody to alpha-synuclein phosphorylated at Ser129, indicating disease-related, post-translationally modified and aggregated alpha-synuclein [86] and whilst microglia accumulated around the grafts, they did not show significant activation.

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These unexpected findings of Lewy body-like pathology in implanted neurons in some patients with PD suggests that the pathological process in Parkinson's disease can affect young dopaminergic cells, with implications to the field of neural grafting in PD. It remains unclear, though, how significant these findings are to the future use of cell therapies in PD as it would appear that not all cases have developed this pathology and in those where it has been seen, it is still only in a small proportion of cells and with an uncertain significance to the viability and efficacy of the graft.

4.0 XENOGRAFTS

4.1 Porcine Ventral Mesencephalic Cells

Xenografting involves the transplanting of tissue between species and embryonic pig neural tissue has long been considered the most suitable source for transplantation into human brains due to their ease of breeding, the similarity in brain size between the species and the potential for genetic modification of porcine tissue. Early work on xenotransplants demonstrated longer axonal and dendritic outgrowths from xenografted cells [87-90] and greater migration [91] than equivalent allografted cells, which suggested that xenografts may have some primary advantage over allografts in terms of their ability to target diffuse pathology and recreate neural circuits. However it is more likely that these differences relate to their different gestational periods and the size of axonal projection required in bigger brains.

Nevertheless the ability of xenografted VM tissue to survive and mediate functional benefits in animal models of PD led onto a small-scale clinical study. In this study by Schumacher et al. [92] 12 PD patients had unilateral striatal transplants of suspensions of porcine VM tissue (E25-E28 gestation). A significant decrease, on average 19%, in the UPDRS scores was reported, but there was no significant increase in F-dopa uptake at the transplant sites and a post-mortem study ~7.5

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months post transplantation showed that only 638 TH+ cells out of ~12 million transplanted porcine-derived cells survived at this stage, with some lymphocyte infiltration into and around the graft despite the patient being immunosuppressed with cyclosporine A [93].

A second placebo controlled clinical trial involving 18 patients has shown only modest improvements (20-25% on UPDRS) in both transplant and sham surgery groups (Genzyme press release 16th March 2001).

These studies have shown disappointing results and this limits the enthusiasm for their adoption clinically, especially as the use of xenogenic tissue for cell replacement therapy in PD will always need to deal with some additional issues compared to allografts. Firstly there are the problems of increased graft rejection and secondly there are major issues regarding the potential transmission of porcine endogenous retroviruses to the patient, which may yield some hitherto undescribed new disease (reviewed in [70]). Thus, further work will be required before a decision can be made as to whether this tissue has any clinical potential.

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5.0 STEM CELLS

Cells that can self renew and produce progeny as well as differentiate are known as stem cells and various types of these cells have been identified in a number of sites in organisms at different stages of development. They have been considered prime candidates for transplantation therapies for a range of diseases including PD due to this proliferative capacity and the ease with which they can be manipulated in vitro.

The principal goals for any stem cell-based cell replacement therapy in PD include - the development of xenogenic free culture protocols that can generate large numbers of relatively defined transplantable cells; adequate survival and functional efficacy from the grafted cells following transplantation; the avoidance of potential adverse effects of stem cell derived grafts, such as tumour formation and immune rejection [94].

Several types of stem cell have been investigated in this respect and include embryonic stem cells (ESCs), neural precursor cells (NPCs) and mesenchymal stem cells (MSCs), each of which will be discussed further in this review.

5.1 Stem Cell Derived Allografts

5.1.1 Embryonic Stem Cells

Embryonic stem cells (ESCs) are extracted from the blastocyst and have the potential to self renew and differentiate into all the cells of an organism. Following the initial isolation of ESCs from the inner cell mass of the mouse blastocyst in 1981 [95, 96], the first reported human ESC line was established by Thomson et al in 1998 [97].

In relation to PD, much work has been devoted to the potential use of ESCs as a source of dopaminergic cell replacement and several methods have been explored to try and persuade ESCs

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to adopt this phenotype. The development of dopaminergic neurons from embryonic stem cells is a complex process involving a number of stages both in vivo and in vitro, including the formation of neural stem cells, and the precise mechanisms involved are not fully understood [98][99-101].

Mouse ESCs have been cultured and transplanted directly into the mouse striatum, leading to some dopaminergic differentiation and evidence of functional recovery, but the formation of teratomas from remaining undifferentiated stem cells was also noted in a significant number of animals [102]. The differentiation of mouse ESCs into Neural Precursor Cells (NPCs) (neural stem cells that can only go on to differentiate into neurons, oligodendrocytes and astrocytes) has been achieved [103][104]. Mouse ESC derived neural stem cells cultured with fibroblast growth factor -2 (FGF-2) and epidermal growth factor (EGF) can also undergo prolonged expansion and still differentiate into neurons and astrocytes on sequential growth factor withdrawal in vitro and following transplantation into the mouse brain in vivo [105]. Human ESCs have now been shown to have similar properties in vitro. However, the number of tyrosine hydroxylase positive (TH+) neurons (which indicate a catecholaminergic - and so includes dopaminergic -] phenotype) resulting from these methods has, though, been disappointingly low (<1%) [106, 107].

Attempts to further increase the differentiation of dopaminergic neurons from ESCs in vitro have involved several different approaches. One method involves co-culturing ESCs with feeder cells that possess stromal cell derived inducing activity (SDIA). Mouse ESCs grown with mouse PA6 stromal cells have produced approximately 16% TH+ neurons in one week [108], while ~7% of the total cells in similar human ESC cultures have given rise to a TH+ phenotype [109, 110]. However, the molecules involved and the mechanism by which the stromal feeder cells produce this differentiation is not yet known.

Other empirical studies have tried a number of soluble growth factors and chemicals to induce the dopaminergic neuronal differentiation of mouse [111], primate [112] and human ESCs [99, 101, 113-115]. Some groups have combined this strategy with mouse stromal cell co-culture

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(mESCs – [116]; hESCs – [117], [118], [119]), while Roy et al have grown human ESCs with human fetal midbrain astrocytes in addition to the use of growth factors [120].. Genetic manipulation has also been used as a way to increase the formation of dopamine neurons from ESCs including transcription factors such as Nurr1, Lmx1a, Pitx3 which have roles in the normal differentiation of dopamine neurones during development [121-123]. In all cases one of the major challenges has been to produce appropriate dopaminergic cells from an ES cell source without using any non-human reagents, as clearly the use of such products will create problems, if and when, the treatment comes to clinical use. In addition as with all therapies derived from such a source, a stable karyotype needs to be shown within the cell lines and their progeny if they are to gain acceptance for clinical use.

These above methods have produced varying proportions of TH+ neurons, with the best results involving dopaminergic differentiation from mouse ESCs using a combination of feeder cell culture, growth factors and genetic manipulation. With this, up to 90% of the neuronally differentiated β-III tubulin positive (TuJ+) cells developed a TH+ phenotype [124]. With human ESC, the most successful differentiation protocols have, until now, generally involved feeder cell cultures with the addition of growth factors and this produces TH+/ neuronal TuJ+ proportions of between 60-75% [113, 117, 120]. Recently though, Cho et al have developed a protocol which involves the formation of embryoid bodies (aggregates of ESCs formed by using culture methods that prevent the adhesion of cells to a surface and thus formation of the typical colony growth), followed by neural precursor cell selection and culture producing ~86% TH+neurons/ TuJ+cells. Of these TH+ cells, the majority expressed the markers of true midbrain dopamine neurons [125]. This is important as it is becoming increasingly clear that the best dopaminergic cells for treating PD will be those that are truly of nigral origin.

Many of the studies above have gone on to examine ESC derived TH+ cell survival and functional efficacy following transplantation into animal models of PD. Dopaminergic rich transplants derived from mouse ESCs have generally survived and produced behavioural

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recovery following grafting into the 6-OHDA lesioned rat model of PD [108, 124]. Primate ESC derived dopaminergic neuronal rich grafts transplanted into the putamen of the MPTP treated primate PD model have also shown survival of grafted TH+ neurons with significant neurological recovery and increased F-dopa uptake on functional imaging [112]. However studies using dopaminergic cells derived from human ESC have been more disappointing with only limited functional recovery and then only in those animals with large numbers of surviving TH+ neurons in the graft. (~1300 per graft [115]; ~10,732 per graft [125]; 27,000 TH+ cells/mm^3 [120]).

Several factors may contribute to the reduced survival and efficacy of human ESC derived dopaminergic cell rich grafts in animal models of PD. There may be increased cell death due to the relative maturity of the neurons so derived from cells which have been grown in culture for relatively long periods of time. There may also be unstable TH expression in transplants [125][94, 126]. Additionally, there have been concerns about the mode of action of the grafts in producing functional benefits, with the possibility of non-specific mass lesion effects from implanting relatively large numbers of proliferating TH+ cells [120, 127].

Apart from the issues of differentiation, the future potential of ESCs as a source for cell replacement therapy in PD will critically depend on avoiding adverse effects such as tumour formation and immune rejection. ESC transplants have been known to give rise to tumours, including teratomas composed of cells from all 3 germ cell layers, following implantation into animal models of disease including PD [102, 128]. The in vitro differentiation of ESCs prior to transplantation has been shown to reduce the incidence of tumour formation following grafting [110], but extensive differentiation and maturation may also adversely affect the survival of grafted cells. In addition the recent study of Roy et al in 2006 suggested that pre-differentiating ESCs to a neuronal lineage still does not remove forebrain progenitor cells, which continue to proliferate post transplantation with possible adverse effects [120]. Thus, blocking cell proliferation and survival pathways by manipulation of relevant genes may also be an effective way of reducing the chance of tumour formation [129], but it is currently not known if this is

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possible. Other strategies have involved genetic manipulation to make undifferentiated, dividing ESCs more susceptible to antimicrobial substances (eg-: insertion of 'suicide genes' under the control of tetracycline or gancyclovir) [103, 130, 131]; improved sorting of ESCs using inserted fluorescent reporter genes and fluorescent activated cell sorting (FACS) [132]; the use of conjugated cell-surface antibodies to allow for magnetic activated cell sorting (MACS) [133]; and finally the use of pharmacological agents that target dividing cells [134].

Overall, although human ESCs have much potential for use as a source of dopaminergic cells for grafting in PD, more work is needed to ensure that it can be done efficiently and safely with the production and survival of large numbers of true nigral neurons. In addition, it will be essential to optimise the production of such cells and improve their survival and functional capacity following transplantation whilst avoiding potential adverse effects, before clinical trials are attempted.

5.1.2 Neural Precursor Cells

A neural precursor cell (NPC) is a type of stem cell which is committed to the neural lineage and can generally only form neurons, oligodendrocytes or astrocytes, although some have proposed that they may be more multipotent than this [135]. They can be found in embryonic and adult neural tissue and also derived from ESC, as described above.

Fetal-derived Neural Precursor Cells

Neural precursor cells (NPCs) have been isolated from a number of sites in the fetal animal and human brain including the mesencephalon [136-141]. In relation to PD, similar principles to those involved in developing ESC derived transplants have been behind the studies on NPCs as a cell source for transplantation [142, 143].

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In terms of proliferation and differentiation, fetal NPCs from all parts of the central nervous system have been found to proliferate in vitro in the presence of growth factors such as basic FGF and EGF [139, 144] and even following prolonged expansion in vitro they seem to retain some of their original regional properties. Thus, dopaminergic differentiation is increased in NPCs from ventral mesencephalon (VM) compared to NPCs derived from other regions of the brain [145, 146].

A number of studies have now explicitly explored the formation of dopamine neurons from NPCs beginning with the study in 1998 by Studer et al. This study demonstrated that NPCs from the rat VM could be grown in vitro, with expansion using bFGF and then differentiated to yield a significant number of TH+ neurons, with functional benefits on transplantation in rat PD models [142]. The proportion of TH+ neurons produced using this approach could be further increased by culturing the cells in low oxygen conditions [147]. Others have also demonstrated the formation of TH+ neurons from embryonic rodent mesencephalon-derived NPCs, with behavioural recovery on transplantation in rat models of PD [148, 149] [150].

Carvey et al have produced a proportion of 20-25% TH+ cells from rat embryonic mesencephalon neural progenitors using added soluble factors such as interleukin-1 (IL-1), IL-11, leukaemia inhibitory factor (LIF) and glial cell-line derived neurotrophic factor (GDNF). Further proliferation of these TH+ cells produced clones, but now with up to 98% of the cells showing a TH+ phenotype, and subsequent transplantation into rat models of PD resulted in significant functional recovery [151].

Genetic modification of rat forebrain NPCs to produce factors involved in dopaminergic specification and survival (Nurr1, sonic hedgehog (SHH) and Bcl-XL;Nurr1 and Mash1 or Pitx3) has been shown to result in dopaminergic differentiation and functional benefit on grafting in 6-OHDA rats [152, 153]. Overexpression of Nurr1 in rat NPCs resulted in ~5% TH+ expression [154], while co-expression of Nurr1 and Neurogenin2 (a transcription factor required for the correct development of mesencephalic dopamine neurons), resulted in the increased maturation of

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the midbrain dopaminergic phenotype [155]. In contrast, Roybon et al did not find an increased formation of dopaminergic neurons following retroviral vector-induced transduction of rat embryonic NPCs with the midbrain transcription factors Lmx1a, Msx1, neurogenin2 and Pitx3 [156, 157]. In all cases the survival of TH+ neurons following transplantation has been poor with rates of only 0.8- 4.3% [142, 150].

Initial studies involving NPCs obtained from the human fetal mesencephalon involved expansion in FGF and EGF but the number of dopaminergic neurons derived from such an approach was very low (<0.01%) and this was also seen following transplantation in rat PD models [158]. Subsequently, Storch et al showed expansion of mesencephalic human NPCs under low oxygen conditions with further induction of differentiation by interleukin-1α (IL-1α), LIF, IL-11 and GDNF resulting in ~1% TH+ neurons [159]. Others have used a different combination of factors with some possible functional benefits [160], and Wang et al confirmed increased functional efficacy of transplantation of pre-differentiated (26% TH+) versus non-differentiated (1.4% TH+) mesencephalon-derived NPCs [161]. Riaz et al managed to improve on this using a combination of brain derived neurotrophic factor (BDNF), dopamine and forskolin with either retinoic acid or GDNF [162], whilst Yang et al and Christopherson et al only managed to obtain 25% and 4-10% TH+ neurons using a combination of different factors. They also found very few surviving TH+ cells following transplantation into the striatum of 6-OHDA lesioned rats and it was unclear if this was due to loss of TH expression or preferential death of TH+ neurons [163, 164]. Liste et al have since shown enhanced generation of dopaminergic neurons from human NPCs by immortalisation and overexpression of the anti-apoptotic protein Bcl-XL in vitro and in vivo [165, 166].

Despite the presence of some continued cell division, the development of tumours from NPC derived grafts has not been reported and this decreased tumorigenic potential is of obvious advantage compared to the approach of generating dopaminergic cells from an ESC source. However ethical and practical limitations with NPC extraction and differentiation still remain as

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the cells need to be derived from aborted fetuses and it is still not clear when this should be done in terms of the optimal age for harvesting. Further work is also needed to produce an efficient protocol for the generation of large numbers of transplantable dopaminergic neurons before clinical trials with this source of cells can be considered.

5.2 Stem Cell Derived Autografts

5.2.1 Endogenous Adult Neural Precursor Cells

The discovery of evidence for the presence of dividing cells in the adult rat hippocampus by Altman and Das in 1965 suggested that endogenous neural stem cells and neurogenesis in the adult mammalian central nervous system may occur [167]. However it was not until 1992, when such cells were isolated from the adult mouse striatum with subsequent in vitro proliferation and differentiation into neurons and astrocytes, that the field really took off [168]. Subsequently, in vivo neurogenesis and the isolation of neural precursor cells have now been convincingly demonstrated in the adult subventricular zone (SVZ) [169, 170] and the subgranular zone of the hippocampal dentate gyrus [171-174]. However several other regions of the adult central nervous system have been shown to possess self-renewing cells which can be isolated, expanded and differentiated in vitro, including the cerebral cortex [175], hypothalamus [176], septum [172], optic nerve [175], retina [177] spinal cord [178] and more controversially the adult substantia nigra [179]. NPCs, including those with neurogenic and dopaminergic potential, have been isolated from the adult substantia nigra/ tegmentum by some groups [180, 181] and Zhao et al have even suggested that the presence of a stable number of dopaminergic neurons in the adult rodent substantia nigra despite continuing neurodegeneration indicates that there is a degree of neurogenesis throughout the lifetime of the animal. A process that can be up-regulated following

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dopaminergic (MPTP) lesions [182]. Others have confirmed this using nestin-Lac-Z transgenic mice [183], whilst some other studies have not shown this to occur [184].

Any neurogenesis present in the adult substantia nigra may be influenced by variables such as the level of local growth factors (e.g.GDNF, BDNF and Platelet derived growth factor (PDGF)) all of which may be low in the midbrain and especially in PD [185, 186], as well as physiological stimuli like exercise and environmental enrichment [187]. In addition, dopamine and dopamine agonists have been shown to influence neurogenesis in the subependymal and subgranular zones of the adult rodent brain [69, 188, 189] and further investigation of these factors may be of significance for PD.

The presence of NPCs in the adult brain has also generated the possibility of ex-vivo autologous transplantation for PD. Despite the limited evidence from animal studies, there has been only one recorded attempt of autologous transplantation of adult neural precursor cells in a patient with PD (in [70]). The patient had a cerebral cortical biopsy, from which NPCs were isolated. These were "expanded" in vitro for months with several 'epigenetic factors' resulting in the formation of neurons, of which 15% were dopaminergic. These cells were then transplanted into the patient's left putamen and functional assessment showed an ~88% improvement in the motor 'off' phase at 12 months, with F-dopa PET showing a 55.6% increase in left putaminal dopamine uptake. However, this case has never been fully published and much more work with animal models, along with a better understanding on the natural functions, development and differentiation of adult NPC is needed before future clinical trials using this approach can be considered.

5.2.2 Carotid Body Stem Cells

In 2007, Pardal et al reported on the discovery of a resident population of neural-crest-derived progenitors in the carotid body of adult mice [190]. These stem cells are activated by hypoxic

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conditions and divide and give rise to new neuron-like dopaminergic glomus cells. They also provided evidence that these progenitors are the glia-like type II sustentacular cells of the carotid body, which can proliferate and differentiate in vitro and produce GDNF.

Following the experience with carotid body tissue as a possible source for cell replacement therapy in PD (see above), the potential to now use these carotid body stem cells in a similar way may enable the ex vivo generation of large numbers of dopaminergic glomus cells for autologous transplantation.

5.2.3 Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are non-haematopoietic, multipotent cells which arise from the stromal structures of the bone marrow and are present in adults [191]. In vivo, they usually give rise to osteocytes, chondrocytes and adipocytes, but may have the potential for transdifferentiation to form other non-mesenchymal cell types, including neurons [192, 193]. MSCs with neurogenic potential have also been found in peripheral blood and thus they represent a potentially very accessible source of cells for autologous transplantation therapies in neurodegenerative disease, including PD.

MSCs have been isolated from both rat and human bone marrow using cell surface markers and efficiently differentiated into dopaminergic neurons using defined protocols involving gene transfection and growth factors such as bFGF, forskolin, ciliary neurotrophic factor, and GDNF [194]. Using this approach up to 41% of the 96.5% neuronal type cells so derived have a TH+ phenotype and on transplantation into a rat model of PD, they demonstrated migration, dopamine secretion and functional recovery. Despite these positive findings, there is some uncertainty

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regarding their true in vivo differentiation potential [195, 196] and the mechanism of action of these cells post implantation [197, 198], which will need to be resolved before they can be considered for clinical use.

5.2.4 Induced Pluripotent Stem Cells

Of all the types of stem cell considered above, ESCs have been regarded to possess the most potential as an efficient, standardised source for cell replacement therapies in the future treatment of PD. However, ethical and practical issues such as the need to use human embryos and the possibility of immune rejection will continue to limit development in this area. Therefore, the recent attempts to form pluripotent / ESC-like cells from adult somatic cells, involving the reprogramming of the somatic cell nucleus, have been very exciting with major implications given the benefits such autografts will offer.

In somatic cell nuclear transfer, an unfertilised oocyte has its nucleus removed and the nucleus of an adult somatic cell is inserted instead, resulting in a pluripotent cell which can develop into a cloned embryo and animal (the method used to produce Dolly the sheep) [199]. Another method involves the fusion of an adult somatic cell with an ESC to form a pluripotent cell [200, 201]. However, these methods still require the use of human embryos or oocytes, with the accompanying problems.

Thus, the ability to reprogram adult cells has been an active area of research and recently Takahashi and Yamanaka showed that ESC like cells could be derived from embryonic and adult mouse fibroblasts through the over expression of four transcription factors – Oct3/4, Sox2, c-Myc and Klf4, using retroviral vectors [202]. These cells were named induced pluripotent stem cells (iPS cells) and while they showed several properties of ESCs such as the formation of embryoid bodies in vitro and teratomas in vivo and differentiation into various distinct cell types, some

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features such as gene expression, epigenetic status and contribution to developmental stages of the organism were similar, but not identical to ESC [203]. Several subsequent studies have used different selection methods for the induced ESC-like state and produced mouse iPS cells which are more indistinguishable from ESC [204-207]. In addition, iPS cells have been formed from other forms of somatic cell such as mature B lymphocytes [208] as well as adult mouse hepatocytes and gastric epithelial cells [209].

Following their initial work on reprogramming mouse fibroblasts, Yamanaka's group then reported on the generation of iPS cells from adult human dermal fibroblasts using the same four factors as had been shown to work with mouse cells [210]. It involved introducing a mouse retroviral receptor to improve transduction efficiency of the retroviral vectors and culturing with bFGF under primate ESC culture conditions, with the morphological identification and mechanical isolation of human ESC-like colonies at day 30, followed by expansion on feeder cells. The similarity of these human iPS cells to human ESC was evident using cell surface-marker expression, epigenetic status, telomerase activity, in vitro embryoid body formation, directed differentiation into neural and cardiac cells and teratoma formation in vivo [210]. Other groups have similarly demonstrated the formation of iPS cells from human ESC-derived somatic cells or human neonatal fibroblasts using the same combination of Oct3/4, Sox2, c-Myc and Klf4 [211, 212], while Yu et al have used Oct4, Sox2, Nanog and Lin28 to reprogram their ESC-derived somatic cells [213].

Thus, once derived, iPS cells could be used in a similar manner to ESC in the development of cell replacement therapies for degenerative disease and initial steps in the use of this technology for PD have now been taken by Wernig et al. [214]. Mouse fibroblast derived iPS cells expanded on irradiated mouse embryonic fibroblast feeder cells formed spheroid embryoid bodies in nonadherent cultures and then clusters of neuroepithelial-like cells in serum-free media. These cells were isolated and propagated with FGF-2 and expressed the neural stem cell markers nestin, Sox2 and Brn2. On treatment with SHH and FGF8, followed by withdrawal of all factors, most

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cells developed a neuronal morphology and were β-III-tubulin+ (TuJ+), with up to 5% of these TuJ+ cells also showing TH+ immunoreactivity. A majority of the TH+ cells also expressed En1, Pitx3 and Nurr1, factors typically seen in midbrain dopamine neurons. They then took ~100,000-300,000 of the differentiated cells obtained following growth factor withdrawal and transplanted them into the striatum of 6-OHDA lesioned rats. This resulted in behavioural recovery of 4/5 rats at 4 weeks, with histological analysis showing ~29,000 TH+ cells in one of the recovered rats and only ~1500 TH+ cells in the centre of the graft in the non-responding animal, with little fibre extension into the adjacent striatum. Of concern, in the recovered animals the transplants continued to show evidence of cell proliferation and teratoma formation. However, using FACS to separate out the remaining pluripotent cells prior to grafting resulted in similar behavioural recovery with smaller grafts and importantly no evidence of tumour formation at 8 weeks [214].

Despite the obvious potential for iPS cell based therapies for PD and other diseases, there are several issues which will need to be resolved before their clinical use can be considered. In general, the efficiency of generation of iPS cells has been relatively poor, ~ 4 -125 ESC-like colonies from ~800,000 mouse embryonic fibroblasts [205] and ~10 iPS colonies from ~50,000 human adult fibroblasts [210]. Park et al [211]also found that postnatal or adult human fibroblasts displayed a much lower efficiency of reprogramming compared to fetal or ESC-derived fibroblasts (0 versus 39-118 ESC-like colonies from 100,000 somatic cells), even following supplementation with genes known to have a role in establishing human cells in culture – hTERT and SV40 large T [215, 216] - (7-21 versus ~250 ESC-like colonies from ~100,000 somatic cells). Thus, further methods are being explored to increase the efficiency of iPS cell formation.

In this respect, the observed enhancing activity of SV40 large T antigen, which has potent anti-apoptotic activity, has also been demonstrated by Mali et al (23-70 fold increase in reprogramming) [217]. In addition, combining the factors used in Takahashi et al. 2007 [210] and Yu et al. 2007 [213]has produced a ~10 fold increase in iPS cell formation from human foreskin

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fibroblasts [218] compared to using only the 4 factors of Yu et al's study. Furthermore, Mikkelsen et al have suggested that the incomplete repression of lineage-specifying transcription factors and inefficient DNA de-methylation may cause some cells to be trapped in a partially reprogrammed state and that RNA inhibition of transcription factors and DNA methyltransferase inhibitors can lead to more efficient reprogramming towards the iPS cell state [219]. .

The current protocols for the generation of iPS cells involve genetic manipulation, viral vectors and animal derived culture conditions, which would prevent them from being used clinically. However, Shi et al have recently reported on the formation of iPS cells from mouse ESC-derived neural progenitor cells with fewer genetic manipulations (viral transduction of only Oct3/4 and Klf4) but with the addition of other small molecules (BIX-01294, an inhibitor of the G9a histone methyltransferase). They found using this approach that the efficiency and characteristics of the iPS cells were similar to those generated with the four factors [220].

iPS cell derived grafts also pose an increased risk of tumour formation as with ESC derived grafts, which can be decreased by preselecting the more differentiated cells prior to grafting [214]. However the reactivation of the viral induced oncogene c-myc has been shown to increase tumorigenicity in germ-line competent chimeras formed using iPS cells [205]. So importantly, Nakagawa et al's study showed that iPS cells can be generated from human adult fibroblasts without c-myc, albeit at a slower rate, with no tumours developing in chimeric mice generated from these iPS cells in a follow up study period of ~ 3-4 months [221].

This rapidly developing field of stem cell biology has much potential for the development of patient specific cell replacement therapies for PD, without the ethical and practical problems found with stem cells of embryonic or fetal origin. However, many challenges will have to be overcome in terms of minimising the genetic and viral manipulation required to form iPS cell derived dopaminergic neurons, as well as avoiding animal derivatives in their culturing. In

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addition, improving the efficiency and quality of the induction and subsequent differentiation processes and reducing tumour formation are all necessary for this approach to be useful in clinical practice. Finally ensuring that stem cells derived from patients have not 'inherited' the problem of the disease itself will be an important issue, so as to ensure that any grafts derived from such a source do not succumb to the disease process in the short term.

6.0 CONCLUSION

The development of definitive treatments for Parkinson's disease will ultimately rely on further understanding the precise pathogenic mechanisms of the disease. However, until such disease modifying treatments are available, efforts will continue in the development of strategies which can help patients by relieving symptoms and possibly retarding the progression of the disease. In this respect, better defining disease heterogeneity will be important, as each novel treatment may be more suitable for only some types of PD patients.

Cell replacement therapies for Parkinson's disease have had a variable history with a number of different cell sources being explored in experimental studies using animal models of disease as well as in patients. The early clinical experience with FVM transplants demonstrated that some patients obtained significant benefits with this treatment, but this was not seen in the larger double blind placebo controlled trials possibly due to a combination of factors relating to patient selection, the amount and mode of tissue engraftment, the power and design of the studies and the level of immunosuppression. While many of these issues can potentially be addressed experimentally, there will always be practical and ethical issues with the use of FVM tissue as a transplant source.

Thus, the search for new cell sources for transplantation in PD has gained much momentum especially with the recent work in the stem cell field. While ESCs have shown the most potential, the recently developed induced pluripotent stem cells bring the very real prospect of patient

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specific cell replacement therapies. Still, there are many issues to be overcome before any such stem cell based therapy can be trialled clinically and this may only be possible once a further successful FVM trial has been completed. Indeed, at the present time FVM tissue still remains the best standard against which to test future sources of cells for dopaminergic cell replacement in PD. However, it should be stressed that the success of the approach using cell therapies in PD will be through incremental advances and even then it will only ever treat those problems in PD that relate to a loss of striatal dopamine. For cures, we are going to have to better understand pathogenesis and in that respect iPS cells may be very instructive in a completely different way, namely through their ability to model disease.

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Table 1

Potential disease-modifying or neuroprotective / neurorestorative strategies for PD

Pharmacological	•The use of drugs to slow down the disease process; neuroprotective agents. - eg. Possibly Dopamine agonists – Ropinirole (REAL-PET study [218]); Pramipexole (CALM-PD study[219, 220]); Rasagiline (TEMPO study [221]; ADAGIO study, press release 2008).
Direct Infusion of Neurotrophic factors	• GDNF to rescue dopaminergic neurons [222, 223]
Cell Therapies	• Cell replacement of lost dopaminergic neurons. -eg. Transplantation of Fetal Ventral Mesencephalon [48, 58, 62]. • Cells engineered to deliver growth factor support to help rescue dopaminergic neurons. -eg. Implanting GDNF secreting cell lines [224, 225]
Gene Therapy *	• Providing neurotrophic/ supportive factors to dopaminergic neurons - eg. Ceregene Neurturin trial [226]

*This approach has also been employed to replace neurotransmitters within the basal ganglia circuitry, which is not strictly a neuroprotective/neurorestorative approach. This includes -:

1) Providing better local delivery of dopamine. - Tricistronic viral vector-mediated transfer of genes involved in the dopaminergic synthetic pathway [231, 232].

2) Increasing synthesis of GABA within the subthalamic nucleus. – Transfer of adeno-associated virus (AAV) borne glutamic acid decarboxylase (GAD) gene into the subthalamic nucleus [233].

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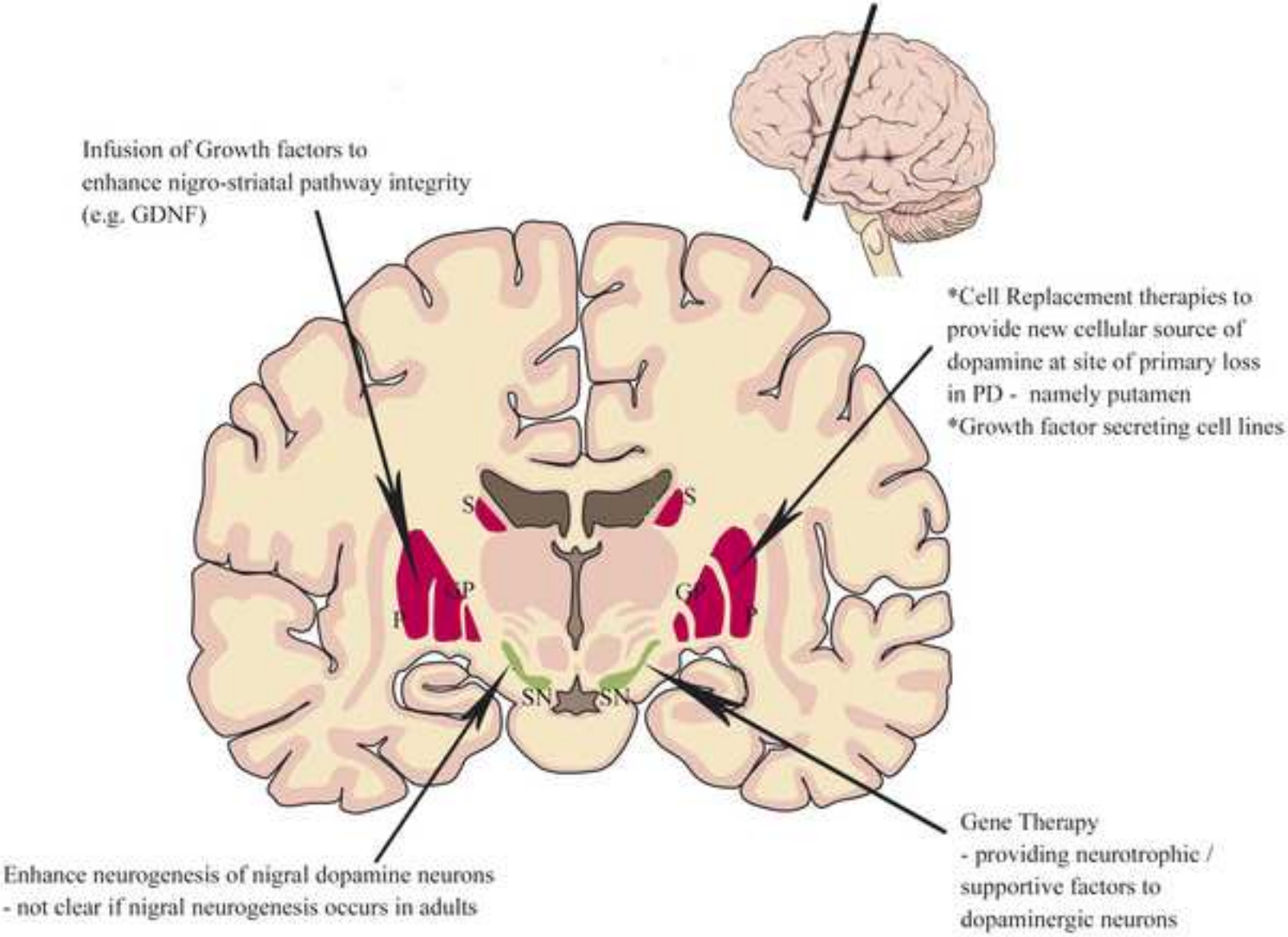

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Figure 1. Drawing illustrating some of the potential 'disease modifying' or neuroprotective/neurorestorative strategies for PD. The substantia nigra (SN) appears in green. The striatum (S), putamen (P) and globus pallidus (GP) appear in red. (Adopted from illustration by Mick Cafferkey).

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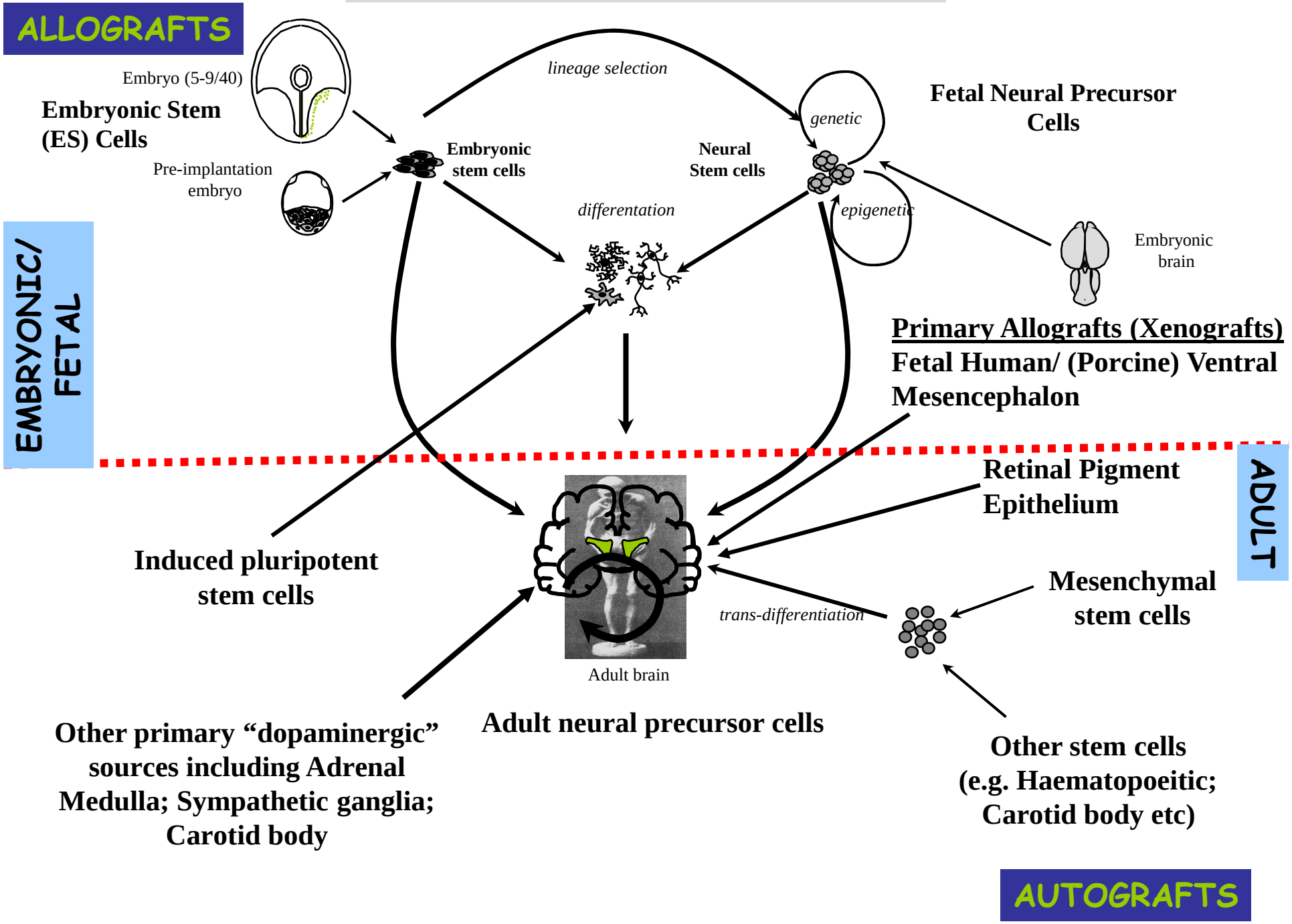

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Figure 2. Figure illustrating some of the cell sources considered for dopaminergic cell replacement in Parkinson's Disease.

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