Regenerative Therapies for Parkinson's Disease: An Update

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Abstract

Parkinson's disease is the second most common neurodegenerative disorder. It is characterised by a typical movement disorder which occurs in part due to the selective degeneration of the dopaminergic neurons of the substantia nigra pars compacta. Current treatment for the motor disorder of Parkinson's disease consists of dopaminergic medications, but these come with significant adverse effects, themselves an important part of the clinical course of Parkinson's disease, particularly in the advanced stages. There is therefore a need for a treatment that is able to restore dopaminergic tone in the striatum in a physiological and targeted manner, such that these side effects are averted. A number of potential regenerative treatments have been developed with a view to achieving this. Following decades of optimisation and development, clinical trials of stem cell-based treatments and viral gene-delivery are on the horizon. In order for these treatments to be widely useful they must be clinically effective, cost-efficient, safe, and a number of practical aspects regarding storage and delivery of treatment, must be optimised. Whilst there have been many barriers to overcome, the field of regenerative medicine for Parkinson's disease is now increasingly focussed on how these treatments will be delivered, demonstrating the significant progress that has been made, and the optimism surrounding these approaches.

Key Points

- Current treatments for Parkinson's disease result in significant adverse effects due to non-targeted, non-physiological delivery of dopamine
- Cell-based and gene-delivery treatments offer a means of restoring dopamine to the striatum in Parkinson's disease patients in a targeted manner
- Several clinical trials of regenerative therapies are due to commence within the next two years

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease [1]. It results in a typical movement disorder consisting of bradykinesia, rigidity, rest tremor, and as disease progresses, postural instability [1]. Additionally, there are a number of non-motor features such as cognitive impairment and dementia, neuropsychiatric symptoms (e.g. depression and anxiety), fatigue, anosmia and rapid-eye movement (REM)-sleep behaviour disorder [2]. The natural course of PD is one of gradual progression, with functional decline occurring over years [3].

The neuropathological hallmark of PD is the presence of Lewy bodies and Lewy neurites – intra-neuronal protein aggregates consisting largely of abnormal alpha-synuclein [4]. Whilst the non-motor features of PD occur due to neurodegeneration in the cerebral cortex and a number of brainstem nuclei, the movement disorder of PD primarily relates to the relatively selective degeneration of the neurons of the substantia nigra pars compacta [1, 5, 6]. These neurons produce the neurotransmitter dopamine, and deliver it to the striatum where it plays a crucial role in control of motor activity, as well as some cognitive processes. Treatment of the motor features of PD therefore, involves restoration of dopamine activity in the striatum. Current treatment options however, result in significant adverse effects which themselves constitute an important part of the illness that is experienced by the patient, particularly in the advanced stages of disease [1]. There is therefore much interest in development of novel therapies that are able to restore dopamine activity without development of these unwanted side effects. In this review we discuss progress towards these therapies, and their future prospects for the management of PD.

2. Rationale for Regenerative Approaches in Parkinson's Disease

Current treatment of PD motor features generally involves the use of dopaminergic drugs. Most commonly, this involves administration of the dopamine precursor, levodopa, in combination with a peripheral dopa-decarboxylase inhibitor to minimise peripheral side effects. Dopamine agonists or monoamine oxidase B inhibitors may be used in some patients, though as disease progresses, the majority require levodopa therapy. In the initial stages of levodopa treatment, most patients experience significant improvement in their motor problems and an improvement in function. However, with prolonged treatment, problematic adverse effects can have a significant impact on quality of life. These can include neuropsychiatric features such as hallucinations, thought to occur due to delivery of dopamine to extra-striatal brain regions (off-target effects). Patients may also develop disabling levodopa-induced dyskinesias – continuous involuntary movements which may affect the limbs, trunk or face. These motor effects are thought to occur due to the manner in which dopamine reaches the striatum following levodopa administration. Rather than occurring in a physiological pulsatile fashion, it is delivered continuously [7, 8]. Additionally, severe motor fluctuations may occur due to variations in the plasma concentration of levodopa and its transit across the blood-brain-barrier [9]. Sudden "off" spells can be particularly disabling for patients. There is therefore a currently unmet need to deliver dopamine specifically to the striatum, in a physiological manner, which is not achievable with current pharmacological agents.

3. Cell Grafting for Parkinson's Disease

The movement disorder of PD largely results from a decline in the number and functional capacity of dopaminergic neurons in the substantia nigra. The onset of disease probably precedes the development of motor features by several years [2, 10, 11]. At the time of diagnosis therefore, significant neuronal loss has already occurred [12]. Restoration of a population of cells delivering dopamine to the striatum could theoretically allow for an improvement in motor abnormalities, without the development of the off-target and motor side effects associated with prolonged use of dopaminergic medication.

A number of cell sources have been considered as potential options for grafting in PD. The first reports involved the grafting of autologous adrenal medullary cells (which release small amounts of dopamine) into the striatum [13, 14]. The initial reported positive results in these patients, despite only a short-period of follow-up, led to a number of patients receiving this type of graft [15-20]. However, it became clear that the recipients in fact experienced little clinical benefit, with a high incidence of psychiatric complications [21]. Furthermore, when these patients came to post-mortem it was found that the grafted cells had not survived [22].

Around the same time there was interest in using foetal midbrains, which contain the developing nigral dopaminergic neurons, derived from terminated pregnancies as an alternative source of dopaminergic cells. After initial disappointing results in the first two patients [23], adjustments to the grafting procedure led to significant clinical benefits in a number of recipients, with some able to come off medication [24-27]. At post-mortem, these grafts have been shown to have survived for over two decades [28]. This suggested that cell-based approaches could indeed be useful for treating the movement disorder of PD, at least in some patients. However, enthusiasm was dampened following two sham-surgery controlled trials of foetal tissue grafts, in which little clinical benefit was observed, with several recipients developing graft-induced dyskinesias [29, 30]. Whilst these trials had several flaws in design, they highlighted the need for optimisation of the approach, in terms of the delivery method, the age and number of foetal midbrains used, the immunosuppressive regime, and the identification of a suitable recipient population, for example. A further trial was set up in Europe after analysis of the outcomes of the preceding foetal grafts, in which an optimised approach has been employed, with the aim of showing that cell-based therapies for PD can be effective in appropriately selected patients (https://clinicaltrials.gov/ct2/show/NCT01898390). Eleven participants in this trial have now received grafts and are undergoing follow-up. However, even if shown to be effective, it has become clear that it will not be feasible to use this approach as a widespread treatment for PD, predominantly due to an inadequate supply of foetal tissue.

A number of other cell sources have been investigated for grafting in PD, including porcine midbrain tissue, autologous carotid body cells, and retinal pigment epithelial (RPE) cells bound to microcarriers (Spheramine), with disappointing results (figure 1) [31-34]. Carotid body cells were investigated as a means of delivering glial-derived neurotrophic factor (GDNF) to the striatum, which had appeared to be effective in animal models of PD [35]. Clinical effects however, were modest in human trials [32, 33]. RPE cells release small amounts of levodopa so have been investigated as a source of cells that could replenish striatal dopamine. However, in a sham-surgery controlled phase II trial, no benefit was demonstrated [31]. These approaches therefore did not offer any advantages over the use of foetal tissue, with which experience was building by this time.

3.1. Stem Cell Treatments for Parkinson's Disease

Though the previous grafting trials have produced mixed results, the significant clinical benefit observed in some of the recipients of human foetal ventral mesencephalon grafts, has offered proof-of-concept that dopamine cell-based therapies can be useful in PD. However, particularly given that PD is a common condition, a reliable source of dopaminergic cells is required for this approach to be widely useful – a condition that cannot be met by the unpredictable supply of foetal tissue. In contrast, this is potentially achievable through the use of stem cells to generate dopaminergic neural progenitor cells for grafting. Though other stem cell types have been purported as potential treatment options, there are two stem cell approaches in particular that might offer an effective treatment for the dopaminergic deficits of PD – embryonic stem cell (ESC)-derived and induced pluripotent stem cell (iPSC)-derived dopaminergic neural progenitor cells (figure 2) [36].

Human ESCs were first derived in 1998, opening up possibilities for development of regenerative therapies for a number of conditions, including PD [37]. ESCs are pluripotent cells derived from the inner cell mass of the early blastocyst, harvested from surplus human embryos from *in vitro* fertilisation procedures [36]. Generation of authentic dopaminergic neurons or their precursors however, has been challenging, and progress has been slow. In particular, whilst it was clear that ESCs could produce cells expressing tyroxine hydroxylase (the rate-limiting enzyme in dopamine synthesis) and that these could survive transplantation into rodents, the yield was highly variable [38-41]. Subsequent refinements of the differentiation protocols led to reports of ESC products that could not only survive grafting and integrate into the host, but could also produce a degree of functional recovery in pre-clinical models [38-42].

A retrospective analysis of over 500 ESC-derived neural progenitor grafts into rats sought to identify factors that determined favourable graft outcome [43]. In this study it was found that high content of tyroxine hydroxylase-positive cells were obtained in grafts enriched with neural progenitors expressing caudal midbrain markers, such as *EN1* and *CNPY1*. However, the markers that had traditionally been used to signify dopaminergic fate (*LMX1A*, *FOXA2*, and *OTX2*), were found to not only be expressed in midbrain nigral progenitors, but also in the rostral midbrain subthalamic neuron progenitors, explaining the heterogeneity in graft outcome seen in prior studies [43]. Exposure to FGF8b at the later stages of the reprogramming protocol resulted in a high purity of neuronal

progenitors expressing markers of caudal midbrain patterning, which when grafted yielded a high content of dopaminergic neurons, and functional recovery [43]. This unexpected development in understanding means that it is now possible to generate ESC-derived neural progenitors which yield consistently high numbers of tyroxine hydroxylase-positive neurons, which could serve as the basis of a cell-replacement therapy.

In 2007 conversion of human somatic cells to pluripotent stem cells was first reported, offering an alternative renewable source of cells that could serve as the basis of a regenerative therapy for PD [44, 45]. These iPSCs can also be differentiated into dopaminergic neural progenitors through similar differentiation protocols to those used with ESCs, potentially offering an alternative source for a cell-based therapy for PD, as has been shown recently in a primate model [46, 47].

Each of these stem cell approaches has its own merits and drawbacks. Generation of ESC lines results in destruction of a viable human embryo, which of course results in ethical issues, though in most cultures the use of embryonic tissue that would otherwise be discarded is probably ethically favourable in comparison to the use of foetal tissue [48]. ESC-derived grafts would be allogeneic in nature, so would require a period of immunosuppression, and the associated risk of infection, malignancy and other adverse effects. The use of iPSCs does not carry these ethical implications, and can potentially be used to generate autologous grafts, derived from fibroblasts of the recipient, possibly circumventing the need for immunosuppressive treatment. However, heterogeneity in the response to a reprogramming protocol between individuals would mean that the cell product would vary between patients, with each product potentially being subject to regulatory approval and safety testing [36]. The more likely scenario is that investigators will need to prove that their reprogramming and differentiation processes are robust, yielding similar results for each patient, before an iPSC-derived product could be approved for clinical use. Generation of autologous iPSC-derived grafts is probably therefore prohibitively expensive for widespread use, at least in most current regulatory environments. Finally, with respect to autologous grafts, one should also consider the fact that the grafted cells will contain any PD genetic susceptibility factors carried by the patient, so may be at an increased risk of themselves succumbing to PD pathology – a situation that has been seen in some long-term surviving human foetal mesencephalon transplants [28].

One option that has been proposed is the generation of haplobanks, in which iPSC-lines could be derived from a number of individuals with specific homozygous HLA patterns, in order to provide coverage of a whole population. Whilst an appealing prospect, coverage of a national population through a haplobank would still require a large number of iPSC lines to be generated, all of which would be subject to safety testing and protocol optimisation, and the associated costs [36, 49]. Additionally, grafts delivered from a haplobanks would probably still warrant a period of immunosuppression, nullifying one of the main advantages of the iPSC approach over ESCs [36]. Whilst the use of haplobanks may not be a major advantage for transplantation into immune-privileged sites such as the brain, they may however confer a significant advantage in achieving transplantation of cell products to other organs.

With both iPSC- and ESC-derived grafts, there is a theoretical risk of tumour formation, through graft overgrowth, aberrant differentiation of the grafted progenitors, or the presence of residual pluripotent cells in the graft, as is discussed further below. Though tumour formation occurred in some of the early pre-clinical *in vivo* studies [40, 41, 50], developments in differentiation protocols have allowed for generation of refined cell products in which there are no residual pluripotent cells, and at least in animals, no tumour formation.

In addition, bone marrow-derived mesenchymal stem cells have been investigated as a potential therapeutic option for PD. Whilst tyroxine hydroxylase-positive cells have been generated from mesenchymal stem cells [51], generation of midbrain dopaminergic neurons has not been achieved, so they are unlikely to be useful as a cell-replacement therapy for PD[52]. Alternatively, it has been suggested that these cells may convey a neuroprotective effect through paracrine and anti-inflammatory properties [53, 54]. An open-label trial in a small group of PD patients demonstrated short-term safety of the use of these cells, but clinical effectiveness could not be demonstrated [55].

4. Gene Delivery Therapies for Parkinson's Disease

An alternative approach towards a regenerative treatment for PD involves the use of viral vectors to deliver genes into the striatum. A number of virus-based gene-delivery therapies have now be investigated, some aiming to increase striatal dopamine [56-59], with potentially regenerative potential, and some aiming to convey a neuroprotective, potentially disease-modifying effect [60].

Two phase I trials have investigated the safety of adeno-associated virus (AAV) vectors delivering aromatic amino decarboxylase (AADC – the enzyme responsible for the conversion of levodopa to dopamine) into the putamina of PD patients [56, 57]. Both of these trials reported improvements in clinical and imaging parameters at six months. These procedures were reported to be well tolerated, though 30% of the subjects in one of the trials developed intracranial haemorrhages along the injection tract [56].

Following these initial trials, a phase I/II trial in France and the United Kingdom investigated the safety of a lentivirus gene therapy (ProSavin, Oxford Biomedica, United Kingdom) [58]. Lentiviruses have a capacity for a larger genetic cargo in comparison to AAV vectors, meaning that it was possible to deliver the genes encoding the other two rate-limiting enzymes in dopamine synthesis, tyrosine hydroxylase and cyclohydrolase-1, in addition to AADC. Whilst generally well-tolerated, 73% of recipients experienced an increase in on-medication dyskinesias in the first twelve months, which improved with a reduction in the levodopa dose. All patients demonstrated a significant improvement in the off-medication Unified Parkinson's Disease Rating Scale (UPDRS) motor score at six months, with continued improvement over 48 months [58].

As well as targeting the dopamine synthesis pathway, AAVs have been used to deliver other genes to PD patients. A phase I trial in which the GDNF-like growth factor, neurturin, was delivered in an AAV vector, demonstrated that this approach was well-tolerated [60], though clinical outcomes in a follow-up sham-surgery controlled double-blind trial showed no benefit [61]. The glutamic acid decarboxylase gene has also been delivered using an AAV vector, with improvement in the UPDRS motor score being reported at six months compared to a sham-surgery group [62], which persisted at 12 months [63].

5. Requirements for a Regenerative Therapy

As has been discussed, the past few decades have seen significant progress towards a clinically useful regenerative therapy for PD. Some of the experimental techniques have shown initial promise in pre-clinical and early clinical trials, but have failed to live up to their expectations when tested in larger studies. Others, such as foetal midbrain grafts, appear to be effective in appropriately selected patients, but are not likely to be clinically useful for logistical and ethical reasons. A number of criteria must therefore be met if a regenerative therapy is to be useful going forward.

Firstly, any potential future regenerative therapy must produce significant clinical benefit in the patient. In the case of the cell-based treatments that have been discussed, this relies on delivery of an adequate number of dopaminergic neuron precursors into the striatum. The graft must have a high purity of dopaminergic cells, and must receive signals from the host brain, so that dopamine is released in a physiological manner. Grafted cells must extend axons across sufficient distances to innervate the recipient striatum. Additionally, the grafted cells must survive for years in order for the patient to gain maximal benefit during the course of their disease. This includes a need to avoid rejection from the host immune system, and to avoid acquisition of pathology following grafting. Gene-delivery techniques must result in acquisition of dopamine-producing potential in a sufficient number of striatal cells to restore dopaminergic tone. As with potential cell-grafting therapies, the target cells of gene-delivery treatments must avoid acquisition of PD pathology, at least for a number of years, in order to provide significant clinical benefit. It is also important that any of these therapies does not result in significant extra-striatal dopamine release, in order to retain the theoretical advantage over levodopa therapy. Finally the cell transplants must be free of a significant number of cells that could mediate adverse effects, such as the serotonergic neurons that may have been responsible for the graft-induced dyskinesias seen in some of the recipients of human foetal mesencephalon transplants [64, 65].

The second important consideration with regard to the utility of future regenerative therapies, is safety. These experimental treatments not only involve a neurosurgical procedure, which itself conveys a risk of intracranial haemorrhage, infection, and the risks associated with hospital admission, but are also associated with specific

safety concerns that must be addressed before they approach the clinic. With regard to stem cell-based treatments, the most significant concern is of potential tumour formation. Though tumour formation has not generally been seen in pre-clinical studies involving optimised reprogramming protocols, the risk of tumour formation after grafting into humans, in whom the graft will potentially be *in situ* for decades instead of the one or two years that it is present in a rodent or non-human primate, is not known. Unpublished spike-in experiments in which pluripotent cells were intentionally included in the graft have shown that tumour formation from residual pluripotent cells occurs early and at high frequency, so one would perhaps expect that if tumours are not observed over a period of a year in a rodent, that the risk of tumour formation from residual pluripotent cells will be non-existent.

In addition to the theoretical risk of tumour formation from residual stem cells, there is also potential for increased tumour risk due to acquisition of mutations in the grafted cells during cell culture, in which mutations that favour replication, or prevent apoptosis for example, provide a survival advantage over wild-type cells. For instance, it is clear that mutations in the tumour suppressor molecule P53 occur during cell culture, albeit at low frequency [66]. The risk that this poses in terms of *de novo* tumour formation following grafting is not known, particularly in view of the fact that mutations in these tumour suppressor genes may require a second-hit to herald neoplastic transformation. There is also doubt about the relevance of acquisition of oncogenic mutations that are associated with non-neuronal cancers, for example *BRCA1* mutations, which are strongly associated with breast cancer, but are not seen in intracranial tumours. A planned Japanese clinical trial of iPSC-derived RPE cell transplants for age-related macular degeneration was halted after identification of a cancer-associated mutation in the cell product of the second patient [67]. Though there was no evidence of tumour formation with this cell product in animals, the risk that it posed to the potential recipient was difficult to interpret [68]. In view of this significant uncertainty about the implications of finding genetic abnormalities, there is variability in how upcoming clinical trials will screen for genetic aberrations in graft products, where some may perform whole genome sequencing, some may test for specific oncogenic mutations and some may test for karyotype anomalies only [69].

Delivery of genes using viral vectors is also associated with specific risks, and safety must be proven before they can be adopted in the clinic. The use of integrating vectors such as lentiviruses and gammaretroviruses can lead to insertional mutagenesis, with risk of transformation in the transfected cell [70]. Given that lentivirus gene therapies, such as ProSavin, target post-mitotic cells, the risk of malignant transformation is thought to be low, with no evidence of insertional mutagenesis in pre-clinical studies [58]. Lentiviral vectors are of low immunogenicity, so the impact of an intracranial host inflammatory response against the virus or transfected cells, is unlikely to be of clinical significance.

Thirdly, any regenerative therapy must be ethically acceptable if it is to be employed widely [48]. Whilst the use of foetal tissue is particularly ethically contentious, the potential future therapies that have been discussed are generally considered to be less so, and are probably acceptable to most societies. Ethical barriers are unlikely therefore, to be a considerable challenge going forwards, although issues still exist with the use of human ESC-derived products in some countries.

Additionally, if these regenerative therapies will be widely used, it will be necessary for them to compete economically with current and other future treatments. In particular, these treatments will need to be comparable both in terms of efficacy and cost, with deep brain stimulation, and levodopa intestinal gel (Duodopa), both of which target the motor features of PD whilst minimising motor adverse effects [71, 72]. Furthermore, trials of novel multimodal drugs such as safinamide have demonstrated positive effects on motor function, with no increase in dyskinesia, providing another potential alternative to the regenerative therapies that have been discussed [73]. Whilst the cost of future regenerative therapies will be dependent on as yet undetermined manufacturing and marketing influences, the ultimate cost is likely to be similar to that of levodopa therapy and deep brain stimulation, and less than the current cost of Duodopa treatment, given the highly efficient differentiation protocols that now exist for making midbrain dopamine neuroblasts.

Finally, a number of technical aspects must be addressed for any of these regenerative therapies to become widely useful. Procedures for freezing, storing, and thawing cell-based and virus-based products must be optimised and robust, and must not alter the properties of the treatment. Production of biological products must adhere to Good Manufacturing Practice regulations, which may require adjustments to the neurosurgical theatres in which these treatments will be delivered. However, whilst there are many issues still to be resolved, the focus of the field is

now increasingly shifting towards answering questions about the practicalities of how these treatments can be delivered, which highlights the progress that has been made over the past couple of decades.

6. Characteristics of Suitable Patient Populations for Regenerative Therapies

Clinical efficacy will depend on defining an appropriate patient population to target with regenerative therapies. It is becoming increasingly clear that PD encompasses a variety of clinical patterns, with some patients experiencing exclusively motor features, whilst others are at high risk of developing cognitive decline and dementia [3]. Some patients may be predisposed to the development of dyskinesias in comparison to others. The rate of functional decline differs considerably within the PD population. Regenerative therapies designed to increase striatal dopamine, and essentially replace the function of the nigral neurons that have already been lost, will theoretically be effective in patients whose predominant features are bradykinesia and rigidity. However, in patients with or at high risk of developing dementia, in whom the pathological correlate is more widespread Lewy body pathology and loss of extra-nigral neuronal populations [6], replenishing striatal dopamine will have limited effect on the clinical features that they have. Whilst there are emerging clinical markers of dementia risk including tau haplotype, and performance on specific cognitive tasks, success of regenerative therapies will in part be determined by the ability to accurately determine which patients are likely to benefit from these approaches [74].

One of the safety issues that arose in the randomised clinical trials of human foetal mesencephalon grafting was the high incidence of disabling graft-induced dyskinesias [29, 30]. However, graft recipients in these trials with pre-existing drug-induced dyskinesias were not excluded, and it is felt that appropriate selection of patients for future cell-based therapy trials, along with the use of cell products of increased purity and optimised grafting protocols, will circumvent this risk. However, exclusion of those with established pre-existing drug-induced dyskinesias will prevent the use of these approaches in a significant proportion of PD patients, and further work will be necessary to determine the population in which regenerative therapies can be used safely. An increase in dyskinesias was also observed in the ProSavin trial which highlights the need to determine the optimal approach for striatal dopamine delivery before these techniques are used widely [58].

7. Future Perspectives

Since the first reports of the isolation and culturing of human ESCs two decades ago there have been steady progress towards a stem cell-based therapy that could be trialled in PD. This has warranted extensive *in vitro* optimisation of reprogramming protocols, as well as *in vivo* pre-clinical studies, which are ongoing. Many challenges have been overcome, including unexpected findings in neurodevelopmental biology, and the first in-human trials of stem cell-derived dopaminergic neuron precursors are now on the horizon.

Trials of ESC-derived dopaminergic neuron precursors are due to commence in the USA and in Europe over the next two years [64]. Additionally, a study of ESC-derived neural precursor cells is currently in the recruitment phase, sponsored by the Chinese Academy of Sciences (https://clinicaltrials.gov/ct2/show/NCT03119636). In Japan, trials of allogenic iPSC-derived neurons and autologous iPSC-derived neurons are expected to commence next year [69]. These trials will provide feasibility and tolerability data for stem-cell derived treatments, as well as early data about their clinical effectiveness. A follow-up trial of ProSavin in which an optimised gene-therapy product is to be investigated, is also due to commence this year.

Viral-mediated direct conversion of somatic cells (e.g. fibroblasts) to generate induced neurons (iNs) also offers a potential source of dopaminergic neurons which could serve as a platform for a cell-based treatment for PD. This is a relatively novel technique, first described in 2010, which has a potential advantage over stem-cell based therapies in that there is no stem cell phase during reprogramming, theoretically reducing the risk of tumour formation [75]. However, it has not yet been possible to generate high purities of dopaminergic neurons, and the potential number of neurons produced is limited by the number of available somatic cells, which is in contrast to the renewable supply of neurons that can be derived from ESCs and iPSCs. iNs therefore do not currently offer a realistic approach to a useful cell-based therapy. One interesting concept associated with this direct conversion approach is the ability to generate dopaminergic neurons through *in vivo* reprogramming of host astrocytes [76]. Whilst this technique is a long way from being thought of as a potential therapeutic approach for PD, if safety can

be demonstrated and pure dopaminergic neuronal yields can be achieved, it may be considered for further investigation in the future.

In addition to the experimental regenerative approaches that have been discussed, there is also much interest in development of therapies that could potentially slow or arrest disease progression. These putative treatments are designed to reduce the presence of aggregated alpha-synuclein, for example with novel immunological agents or repurposed drugs that enhance protein clearance mechanisms [71]. With a number of potential treatments currently in trials, the question is where will regenerative therapies fit into the future therapeutic approach to PD? Whilst it is possible that these alpha-synuclein reducing therapies may slow the progression of the motor and even cognitive aspects of PD, they will not restore the function of neurons that have already been lost. Given that significant neuronal loss occurs before the clinical diagnosis of PD, even with disease-modifying treatments many patients will still experience functional impairments due to the neuronal degeneration that has occurred in the prodromal and pre-diagnosis stage of disease. The regenerative therapies that have been discussed in this review aim to replace the function of the lost dopaminergic neurons, potentially restoring motor function to the pre-morbid level, without the problematic adverse effects seen with current dopaminergic drugs. One could therefore envisage a landscape in which regenerative therapies are used to restore motor function in patients that have already accrued motor disability, in combination with alpha-synuclein reduction treatments to prevent disease progression, particularly in individuals at higher risk of cognitive symptoms.

8. Concluding Remarks

Since the early grafting trials for PD in the 1980s, significant progress has now been made towards effective and deliverable cell-based and gene delivery therapies for the motor disorder of PD. Over the coming decade these treatments will be investigated in the clinic, and it now seems likely that regenerative therapies will have a role in the management of PD in the medium-term future. Since the introduction of levodopa in the 1960s there have been few significant advances in the treatment of PD, but the regenerative approaches that have been discussed in this review, in combination with the emerging potentially disease-modifying approaches, provide optimism with regard to the future of regenerative treatments for PD.

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Figure Legends

Fig. 1 - Sources of potential and previously investigated regenerative treatments for Parkinson's disease. Bold arrows indicate approaches that may offer deliverable treatment options, taking into account ethical, logistical, and regulatory factors. Abbreviations: ESC = embryonic stem cell, iPSC = induced pluripotent stem cell

Fig. 2 – Stem cell therapies for Parkinson's disease. Abbreviations: ESCs = embryonic stem cells, iPSCs = induced pluripotent stem cells