Treating Parkinson's Disease in the 21st Century: Can Stem Cell Transplantation Compete?

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ABSTRACT

The characteristic and selective degeneration of a unique population of cells–the nigrostriatal dopamine (DA) neurons–that occurs in Parkinson's disease (PD) has made the condition an iconic target for cell replacement therapies. Indeed, transplantation of fetal ventral mesencephalic cells into the DA-deficient striatum was first trialled nearly 30 years ago, at a time when other treatments for the disease were less well developed. Over recent decades standard treatments for PD have advanced, and newer biological therapies are now emerging. In the 21st century, stem cell technology will have to compete alongside other sophisticated treatments, including deep brain stimulation and gene therapies. In this review we examine how stem cell–based transplantation therapies compare with these novel and emerging treatments in the management of this common condition. J. Comp. Neurol. 522:2802–2816, 2014.

INDEXING TERMS: dopamine; embryonic; pluripotent; gene therapy; growth factor; pharmaco-genetics

Treating Parkinson's disease (PD) requires choices, for patients and families, for physicians, and for societies. With the emergence of new drugs and technologies over the last few decades, treatment choices in PD have gradually expanded, but without any broad transformation of patient experience. Certainly, new treatments have emerged that have greatly improved quality of life for a minority of patients; however, no treatment has come forward as the hoped-for panacea that can slow the disease and transform lives.

At the societal level, PD is an increasing burden. Patients are living longer with the disease, and demographics has ensured its inevitable rise as a health issue for a world with an aging population (Lees et al., 2009). It is the second most common neurodegenerative disorder in those over the age of 60 and is also a major cause of dementia, as cognitive deficits emerge with disease progression in a significant proportion of patients (Burn and Barker, 2013; Hely et al., 2008; Parkinson'sUK, 2012; Williams-Gray et al., 2009).

Unsurprisingly, much hope has been invested in stem cell technologies for the treatment of diverse brain diseases. With its selective dopaminergic cell degeneration, PD has been at the forefront of attempts to use novel cell replacement strategies to restore a normal dopamine (DA) supply to the striatum. The recent emergence of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) has unveiled the real possibility of bringing the technology to the clinic in the foreseeable future, both in the form of disease modeling and as a viable therapy. This review examines where stem cells may fit, alongside other existent and proposed therapies, in the future management of this common disease (Fig. 1).

TREATING PARKINSON'S DISEASE: AN HISTORICAL PERSPECTIVE

Standard treatments

To treat PD is to treat a moving target. In the first years, motor symptoms often dominate, paralleling the degeneration of the nigrostriatal dopaminergic projections that defines the disease. However, its progressive nature

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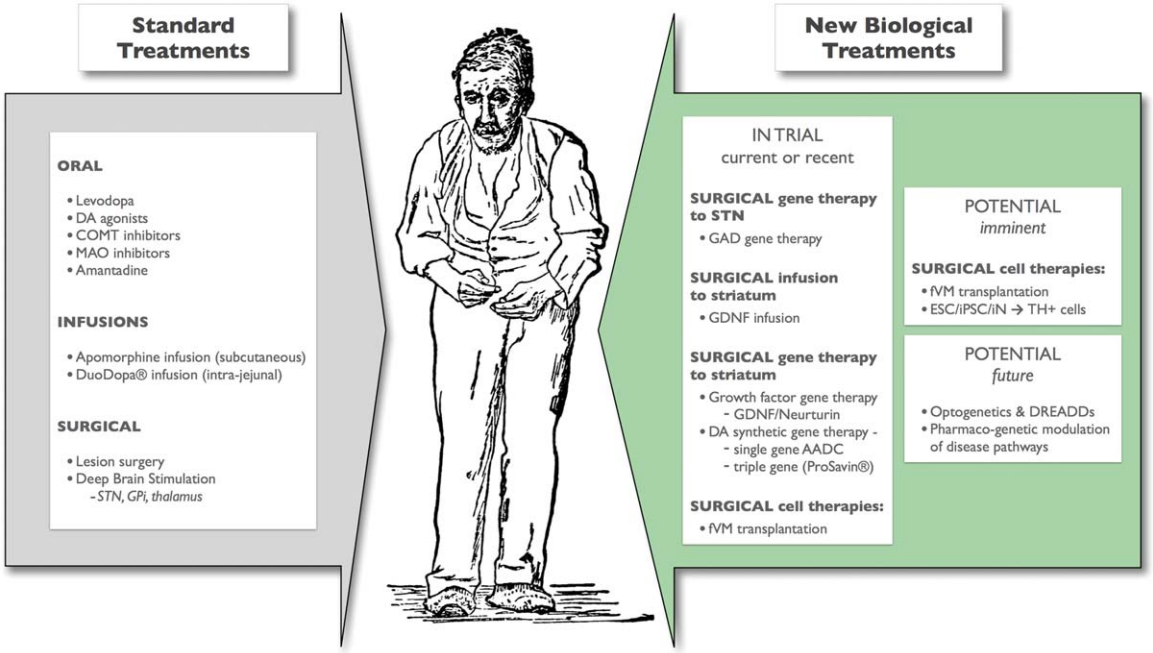

Figure 1. Summary of the standard and new biological treatments for Parkinson's disease. Standard treatments for PD include the DA precursor levodopa and DA agonists. Also used are inhibitors of DA breakdown (COMT and MAO inhibitors), which lengthen the duration of action of levodopa. Amantadine is an NMDA receptor antagonist that ameliorates dyskinesias in a proportion of patients. Apomorphine is a D1 and D2 DA receptor agonist that can be delivered by subcutaneous infusion. DuoDopa® is a gel formulation of levodopa that can be delivered by intrajejunal infusion. The new biological treatments have been under study recently using surgical delivery methods. They include delivery of inhibitory genes to STN (GAD); growth factors to the striatum (GDNF, Neurturin); dopamine synthetic pathway genes to the striatum (AADC only, or triple therapy with GCH1, TH, and AADC); also dopaminergic cells derived from fetal ventral mesencephalon. Potential future treatments include the surgical cell therapies, which will likely move on from fVM to use stem cell or iN-derived grafts; other technologies also in the pipeline include optogenetics, DREADD technology, and pharmaco-genetic modulation of disease pathways. See text for details and references. Abbreviations: AADC, aromatic amino acid decarboxylase; COMT, catechol-O-methyl transferase; DA, dopamine; DREADD, designer receptor(s) exclusively activated by designer drugs; ESC, embryonic stem cell; fVM, fetal ventral mesencephalon; GAD, glutamic acid decarboxylase; GCH1, GTP cyclohydrolase 1; GDNF, glial cell line derived neurotrophic factor; GPi, globus pallidus interna; iPSC, induced pluripotent stem cells; iN, induced neuronal; NMDA, N-methyl D-aspartate; MAO, monoamine oxidase; PD, Parkinson's disease; STN, subthalamic nucleus; TH, tyrosine hydroxylase. Image credit: Wikimedia Commons, William Richard Gowers.

dictates that new symptoms inevitably arise, both secondary to the treatments and related to progression of the degenerative changes within and beyond the nigrostriatal pathway. Thus fluctuations in motor performance, including ON/OFF phenomena and dyskinesias, were recognized soon after the introduction of treatment with the DA precursor L-3,4-dihydroxy-phenylalanine (levodopa) (Marsden and Parkes, 1976; Quinn, 1998), and have been the stimulus to the development of a modest armory of other drugs, including DA receptor agonists and inhibitors of DA breakdown (catechol-O-methyl transferase [COMT] inhibitors and monoamine oxidase [MAO] inhibitors), along with specific antidyskinetic agents such as amantadine (Fig. 1). These may help to delay or ameliorate motor fluctuations, but each also provokes a range of side effects, and none has managed either to abolish fluctuations or to slow disease progression.

More important, in terms of overall disease burden, are the multiplicity of nonmotor symptoms (Chaudhuri et al., 2006). These have their substrate in the widespread degenerative changes in systems outside the nigrostriatal pathway, and include impacts on mood, cognition, control of sleep, and autonomic function. They may be present throughout the disease, even preceding the motor features by some years (Langston, 2006). They are typically less helped by standard medications, worsen inexorably with disease duration, and have a considerable impact on quality of life and well-being in both patients and carers (Leroi et al., 2012; Simuni and Sethi, 2009).

Ventral mesencephalic cell transplantation and its usefulness in treating the dopaminergic aspects of PD

DA cell replacement therapy (CRT) first emerged as a potential treatment for PD in the 1980s, at a time when it

was clear that DA cell loss caused the motor deficits, but that treatment with oral DA drugs had its limitations. Initial studies were very wide ranging in the tissue used, including autografts of adrenal medulla, sympathetic ganglion, and carotid body–derived cells, as well as xenografts of fetal porcine ventral mesencephalon (Arjona et al., 2003; Backlund et al., 1985; Itakura et al., 1997; Schumacher et al., 2000). However, the most successful studies employed tissue from the human fetal ventral mesencephalon (fVM), with preclinical work in rodents showing success with both rodent and human fVM cells (Björklund et al., 1981; Brundin et al., 1986). On this background, human transplantation programs started in Mexico and Sweden in the late 1980s, and subsequently in other countries around the world (Lindvall et al., 1990; Madrazo et al., 1987; reviewed by Barker et al., 2013). Protocols differed considerably from center to center, and results were variable. Thus some patients experienced clear clinical improvement that correlated with changes on ^{18}Fluorodopa positron emission tomography (PET) scanning and at post-mortem examination (Hagell et al., 1999; Lindvall et al., 1994; Mendez et al., 2008; Piccini et al., 1999; Remy et al., 1995; Wenning et al., 1997; Widner et al., 1992). Other patients, however, showed minimal or modest gains, and the open-label nature of the trials always gave grounds for concern. Nevertheless, by the mid-1990s there were enough encouraging results for the U.S. National Institutes of Health (NIH) to put funding into two double-blind, placebo-controlled trials.

Ultimately, nearly 60 patients were enrolled into the two NIH studies, but the results, when they were published in 2001 and 2003, raised significant doubts about the merit of this whole approach (Freed et al., 2001; Olanow et al., 2003). Not only did both trials fail to meet their primary endpoints, but they also reported for the first time the phenomenon of graft-induced dyskinesias (GIDs), whereby patients experienced persistent involuntary movements even after complete withdrawal of DA medication. The trials were seen by some as conclusively showing that cell transplantation did not work in PD and actually made some patients worse. This conclusion has been intensely debated over the years since (e.g., Olanow et al., 2009 vs. Barker et al., 2013).

NEW PROMISE FROM NEW TECHNOLOGIES

Deep brain stimulation

Whereas the door seemed to be closing on transplantation at the turn of the millennium, stereotactic neurosurgery, long used as a treatment for difficult movement disorders, was seeing a renaissance. The early 1990s saw the introduction of implantable stimulator devices, able to create reversible lesions in selected nerve nuclei, and thus triggering a revolution in the treatment of the motor features of PD (Limousin et al., 1995a,b; Pollak et al., 1996).

Deep brain stimulation (DBS) has distinct benefits over conventional pharmacological treatments, as it is capable of producing marked improvements in the cardinal motor features of tremor, stiffness, and slowness, and corresponding improvements in quality of life, without any drug side effects. Indeed, stimulation to the subthalamic nucleus (STN-DBS) typically allows a concomitant reduction in medication doses (Deuschl et al., 2006). Its chief benefit, however, is to provide a consistent therapeutic effect over time, without the fluctuating motor response that is seen with medication in advanced disease (Benabid et al., 2009).

Although the benefits of surgery in many patients can be substantial, they are also restricted. Thus axial (e.g., balance) and gait symptoms are helped less initially, and typically progress despite surgery, so if these features are dominant, then STN-DBS is probably not indicated. Indeed, as gait and balance symptoms may relate to progressive cortical pathology and degeneration in brainstem cholinergic systems (Karachi et al., 2010; Yarnall et al., 2011), the brainstem pedunculopontine nucleus (PPN) has also been targeted in some studies. However, outcomes of PPN-DBS have been variable, and a future role remains uncertain (Ferraye et al., 2010; Mazzone et al., 2005; Plaha and Gill, 2005).

Nonmotor symptoms are also generally not helped by STN-DBS. Although some may improve alongside the motor benefits (Lhommée et al., 2012), cognitive and other nonmotor symptoms may be untouched or worsened, and disease progression is unaltered. Thus, although some evidence of disease modification does exist in animal models (Temel et al., 2006), most human studies suggest that DBS has little or no impact on the natural history of the underlying neurodegeneration (Aybek et al., 2007).

So above all, DBS remains a treatment for specific symptoms rather than overall disease. Although it has certainly widened the range of available treatments in PD, it is not an appropriate treatment choice for the majority of patients—in particular the more elderly and those in whom nonmotor manifestations provide the major impact on quality of life.

Gene therapy: inhibiting the STN

Attempts are currently being made to address at least some of the limitations of DBS using gene

therapy, which has now matured to the extent that long-term manipulations of neuronal function can be engineered with some ease. Three different strategies have been under examination in recent clinical trials, with different but overlapping aims, and with variable success. The first to reach a blinded trial phase came from a group at Weill Medical College of Cornell University in New York City, which sought to directly change the electrical activity of the STN (Kaplitt et al., 2007; Luo et al., 2002).

One of the achievements of DBS has been a better understanding of the network dynamics of movement control. The effect of stimulation is to interrupt a motor cortex–derived β-band (8–35 Hz) electrical oscillation within the cortico-striatal circuit loops that occurs with DA deprivation, and that is integral to the cardinal features of the disease (Kravitz et al., 2010; Kühn et al., 2006). The ability of DBS to revert this aberrant oscillatory activity back to a more "normal" pattern relies on electrode placement at a node in the motor circuitry (typically either the STN or the globus pallidus interna [GPi]), where the cortico-striatal loops converge (Ballanger et al., 2009; Boertien et al., 2011; Lin et al., 2008). Stimulation at other points in the circuitry has also been trialed, including the motor cortex, but with less convincing benefits (Canavero and Paolotti, 2000; Moro et al., 2011).

The New York group attempted to mimic this effect by using surgically mediated gene transfer of glutamic acid decarboxylase (GAD) to the STN. In essence, the GAD-STN approach was intended to normalize excessive STN activity, bringing activity within the movement circuit as a whole back to baseline, and thus achieving the same outcome as standard STN-DBS, but with the distinct advantage of leaving no wires or batteries behind. Preclinical studies showed clear benefits, and clinical studies then progressed to a blinded trial that was published in 2011 (LeWitt et al., 2011). However, the results were disappointing, with only a 23% improvement in Unified Parkinson's Disease Rating Scale (UPDRS) score at 6 months, compared with 12.7% in the sham arm. Such figures do not match up with the usual effectiveness of standard "electrical" STN-DBS, which may reflect an inadequate disruption of the β-band oscillation by this particular strategy (Gradinaru et al., 2009; Holgado et al., 2010). Either way, this specific form of the technology is now unlikely to progress in its current format, as the sponsoring company (Neurologix) filed for bankruptcy in early 2012. Even so, an ultimate molecular–genetic successor to DBS may yet emerge in due course (see below).

Gene therapy: biological dopamine replacement

The second class of gene therapy currently under assessment for PD is that of reconstructing DA synthesis in situ within the striatum, i.e., at the site where DA is most required. Thus two groups (based at the University of California and at Oxford, UK) are trialing two different forms of gene delivery based on this principle. In the first group, the enzyme aromatic L-amino acid decarboxylase (AADC) is supplied surgically, by means of an adeno-associated viral (AAV2) vector, to striatal neurons; here it is able to convert L-dopa (still supplied exogenously by tablets) into the DA necessary for neuromodulation (Bankiewicz et al., 2006). In contrast, the Oxford group has used a multi-cistronic (lentiviral) vector that incorporates genes for three enzymes (guanosine triphosphate [GTP] cyclohydrolase 1 [GCH1], tyrosine hydroxylase [TH], and AADC—marketed as ProSavin®, Oxford BioMedica, Oxford, UK), thereby supplying the entire molecular machinery for manufacturing DA (Azzouz et al., 2002; Jarraya et al., 2009). Both approaches have yielded encouraging results in early phase I studies, with the treatments being well tolerated over several years. However, interpretation of their effectiveness is currently unclear, owing to the small numbers treated and the likelihood of a significant placebo effect (Christine et al., 2009; Mittermeyer et al., 2012; S. Palfi et al., 2014). Thus larger, blinded trials are awaited.

Both of these DA-synthetic strategies ask cells that typically receive the nigral DA input (striatal medium spiny neurons [MSNs]) to instead make their own DA, and so autostimulate their own input. In principle the outcome of this tonic DA production should be similar to that of DBS— it should switch the steady-state circuit activity back to its DA-intact mode. Crucially, however, it should do this without the off-target effects of oral DA replacement, and without activating those molecular pathways in MSNs that may underlie some of the motor complications (Fasano et al., 2010).

In theory such gene therapies could avoid both the fluctuating effects of oral medication and the device-related side effects inherent to DBS. Conceptually, they compete with the "smooth delivery" infusion technologies of subcutaneous apomorphine and intra-jejunal levodopa (DuoDopa®, Abbott Laboratories, Green Oaks, IL) (Antonini et al., 2007; Olanow, 2008). The smooth delivery reinstates *tonic* DA receptor stimulation, and enables each medication to reduce motor fluctuations and improve some aspects of nonmotor symptoms (Honig et al., 2009; Jenner, 2008). Each of the technologies also has its potential drawbacks. For DuoDopa and apomorphine, they are expensive and sometimes

poorly tolerated—because of either device-related issues or drug side effects, including off-target (extrastriatal) effects on cognition and behavior. For the gene therapies, the surgical application of the vectors via intracranial injection still entails surgical risks, and the gene insertion itself is not reversible. So for the ProSavin gene therapy, the lack of control over DA production from the inserted gene could potentially lead to hyperdopaminergic side effects, including dyskinesia and behavioral problems, and there are also theoretical risks of inducing or potentiating neurodegeneration in striatal cells (Chen et al., 2008).

Gene therapy: biological disease modification

What is not achieved, either by DBS or by the DA gene therapies, is definitive *disease modification*. However, this is the clear goal for the growth factor (GF) gene therapies.

Glial cell line derived neurotrophic factor (GDNF) and neurturin are related GFs, both of which have been shown in cell culture and animal models to enhance survival and neurite outgrowth of dopaminergic neurons (Creedon et al., 1997; Gash et al., 1996; Horger et al., 1998; Kordower et al., 2000; Lin et al., 1993; Tomac et al., 1995). That the GFs engage known survival pathways for the relevant cell type makes their use a logical strategy for slowing disease progression in PD; however, GF-treated cells may additionally function better, so DA supply to the striatum may be secondarily enhanced. This at least has been the premise, but translating such promising preclinical studies to the clinic has proved problematic.

The use of neurturin has been pursued by a California group, in conjunction with the biotechnology company Ceregene (San Diego, Ca), using an AAV2 viral vector. However, although AAV2-neurturin (CERE-120) was well tolerated in phase I studies (Marks et al., 2008), a subsequent blinded study failed to meet significance at 12 months in the primary outcome measure of the UPDRS III (Marks et al., 2010). Postmortem data, and comparison with preclinical primate work, suggested a deficit in the transport of the growth factor from the striatal injection site back to the cell bodies in the substantia nigra (SN), which was specific to human PD subjects (Bartus et al., 2011). As the therapeutic effect probably requires this transport, the group took the logical step of evaluating dual injections to the SN and striatum (Bartus et al., 2013; Bartus et al., 2011). However, this strategy has now also failed in blinded studies, although analysis of subgroups showed significant improvements in UPDRS III OFF scores in those treated within 5 years of diagnosis (Ceregene, 2013). This failure may relate either to the relative denervation of the striatum in more advanced disease, or to problems of expression or activation of the GDNF/neurturin receptor Ret in the SN neurons. Thus a defect in Ret signaling, which may occur secondary to reduced expression of the orphan receptor Nurr1, is apparent in an α-synuclein animal model of PD, and is probably a feature of the human disease (Chu et al., 2006; Decressac et al., 2012; Kadkhodaei et al., 2013). Such observations suggest that future GF studies might not only focus on patients earlier in the disease, but also deploy expression of neurturin in combination with either Nurr1 overexpression, or the use of Nurr1 activators (Zhang et al., 2012).

Different problems have confounded attempts to bring the growth factor GDNF to the clinic. Here, initial open-label studies of intra-putamenal infusion of GDNF protein were encouraging (Gill et al., 2003; Slevin et al., 2007). However, problems were again encountered in subsequent phase II studies, which were interpreted either as being due to technical issues, or as evidence that this approach would not work (Barker, 2006; Lang et al., 2006). A poor volume of distribution of the GDNF protein beyond the catheter tip (shown subsequently in animal studies) may have been relevant (Salvatore et al., 2006). Although better delivery methods are being explored in a trial just started in Bristol (UK), including "convection-enhanced delivery" (CED) (Taylor et al., 2013), delivery of the GF by gene therapy, rather than implanted catheter, seems likely to be the eventual technology. Indeed, to this end, a phase I study sponsored by the U.S. National Institute of Neurological Disorders and Stroke (NINDS) has recently opened to recruitment, using AAV2-GDNF injected surgically via a CED system (ClinicalTrials.gov).

CELL TRANSPLANTATION REVISITED

A clinical niche for stem cells?

Although the NIH transplantation studies initially seemed to close the door on cell therapies, further re-evaluation suggests this conclusion may have been premature (Barker et al., 2013; Brundin et al., 2010; Evans et al., 2012; Politis and Lindvall, 2012). Primarily, it has been recognized that there were several methodological confounders to a clear-cut result in the blinded studies, including patient heterogeneity and small numbers. Other issues were a subjective endpoint in one of the trials (Freed et al., 2001); suboptimal preparation and surgical delivery of the donor tissue; and lack or inadequacy of immunosuppression. Long-term follow-up of patients was also lacking, particularly given the

relatively slow maturation of grafts that was apparent in retrospect. What is clear is that longer term data—from the blinded trials and the prior open-label studies—confirm that a proportion of subjects gained a very significant and lasting benefit from the grafts (Ma et al., 2010; Politis and Lindvall, 2012). Although this synthesis certainly leaves many unanswered questions, it also shows that, given the right cells in the right patient and enough time, cell-based therapy can radically improve motor symptom control over periods of years.

Recent advances in stem cell technologies are also likely to be key. Realistically, fVM transplantation was never more than an experimental therapy, and was unlikely ever to be widely available for use in PD, given the ethical and logistical problems of the use of fetal tissue. If new technologies now allow access to high-quality transplantable cells, in large numbers, this may trigger a paradigm shift in the use of cell-based therapies for PD.

Realistic options for cell transplantation

In essence, what a 12-year break from transplantation has provided is a change in question. It is no longer a question of feasibility, but rather of practicability and cost/benefit: can CRT offer sufficient advantages over other emerging and existent technologies to drive its expanded use? In this context, choosing the right patient and timing of treatment will be crucial.

The different potential cell therapies have different strengths and weaknesses. ESC lines offer greater potential for control and standardization of the production of patient-ready cells, and the technology has been used with success in animal models (Dezawa et al., 2004; Kim et al., 2002; Kriks et al., 2011; Takagi et al., 2005). However, concerns remain around the potential for uncontrolled cell proliferation and tumor formation (Brederlau et al., 2006; Roy et al., 2006), whereas the pluripotency of the cells also underlines that tight control must be maintained over their differentiation in culture, so as to produce the appropriate patient-ready cell type. This is important on several grounds, including the possibility that excess serotonergic cells in donor grafts may provoke the development of GIDs (Politis et al., 2010); also rodent studies suggest that different DA cell types may have very different abilities to reinnervate the denervated striatum (Brundin et al., 2010; Politis and Lindvall, 2012; Thompson and Björklund, 2012). Some of these issues may be addressed by optimizing the starting cell type, for example, using fetal mesencephalic neural precursors rather than ESCs, and other issues by engaging strategies that promote differentiation to TH+ cells (Parish et al., 2008). Either way, improved yields of appropriate cells may well be achieved, whereas the potential immunogenicity of ESC-derived grafts may end up as their biggest drawback.

Induced pluripotent stem cells (iPSCs) are felt by many to be the best option in the long term: they may be used autologously, thus avoiding immunosuppression, but can still be produced in large numbers for individual patients (Kiskinis and Eggan, 2010; Takahashi et al., 2007; Yu et al., 2007). As they are induced by reprogramming from patient-derived cells such as fibroblasts, using a combination of genetically encoded reprogramming factors (Takahashi and Yamanaka, 2006), there were initial concerns that residual (and potentially oncogenic) reprogramming factors might forestall the use of iPSCs in any clinical setting. However, such concerns have been steadily addressed over the last few years by the introduction of techniques that leave no reprogramming factors behind, and it now seems that clean and effective achievement of pluripotency is possible (Kaji et al., 2009; Okita et al., 2010; Soldner et al., 2009; Stadtfeld et al., 2008; Woltjen et al., 2009). As with ESCs, iPSCs can be used to derive DA neurons and these have been applied with success in animal models (Cai et al., 2010; Chambers et al., 2009; Hargus et al., 2010; Hu et al., 2010; Swistowski et al., 2010; Wernig et al., 2008). An outstanding concern is whether it is prudent to use a patient's own cells to derive dopaminergic neurons for therapy, in view of their presumed susceptibility to developing PD pathology, a concern that is particularly relevant given recent descriptions of a prion-like spread of α-synuclein, and the appearance of Lewy bodies (LBs) in fetal VM grafts (Desplats et al., 2009; Kordower et al., 2008; Li et al., 2008; Luk et al., 2009; Volpicelli-Daley et al., 2011). Transplanted ESCs and heterologous iPSCs might also be expected to succumb in small numbers to LB pathology, but may avoid a specific propensity to this, if they are not themselves derived from PD patients.

Most recently, direct conversion of fibroblasts (or iPSCs) into postmitotic neurons has also been demonstrated, again by overexpression of defined transcription factors (Pang et al., 2012; Vierbuchen et al., 2010); similarly, an alternative defined cocktail of dopaminergic transcription factors (Mash1, Nurr1, and Lmx1a) has been shown to drive direct conversion of fibroblasts to DA neurons (Caiazzo et al., 2011). These emerging techniques highlight the diversity of potential cellular sources for the preparation of patient-ready dopaminergic neurons for future CRT.

Cell replacement therapy versus gene therapy

So how does CRT compare alongside the other biological therapies? What CRT offers is a combination of

DA replacement and disease modification, with progressive improvement over time as the cells reinnervate the DA-denervated striatum. Viewed thus, there may be little to choose between CRT and its main competitors, the DA gene therapies. Both options offer reconstitution of the tonic supply of DA to the striatum, so ameliorating motor symptoms, while avoiding off-target effects of exposing the whole brain to pulsatile and supranormal dopaminergic drug levels. By dint of neurite outgrowth beyond the injected volume, CRT may allow a more physiologically complete delivery of DA to the striatum than the gene therapies, which may be important for better control of both motor and nonmotor symptoms, although comparative data here is lacking (reviewed in Lelos et al., 2012; Thompson and Björklund, 2012). However, the extent of reinnervation will also rely on multiple factors, including aspects of the host environment (patient age, extent of denervation, and other individual factors), and some of these may adversely affect engraftment more than they do the efficiency of viral gene transfer.

The benefits of restoration of tonic DA levels should probably not be underestimated, as this may not only dampen motor fluctuations, but also confer a form of neuroprotection at the level of individual synapses (Calabresi et al., 2006, 2007; Solis et al., 2007; Wang and Deutch, 2008). Prolonged loss of DA tone, with consequent impairment of synaptic plasticity (inadequately salvaged by oral therapies), may cause irreversible deleterious effects. This argues for early and sustained restoration of DA tone, and the efficiency with which the different therapies are able to achieve this may thus be important. For example, CRT could well establish more consistent DA delivery over time scales of decades compared with the gene therapies; however, again real data are lacking, and any differences will only become apparent with longer term studies.

Any other advantages of CRT are more uncertain still, and relate to integration of the engrafted cells with host circuitry. Thus there is the prospect that engrafted cells might exhibit some autoregulation of DA release, and might also be regulated by afferent (cortical) inputs within the striatum. The autoregulation could reduce the likelihood of excess tonic levels of DA, although if the cell type is present in the wrong ratio (serotonergic to dopaminergic neurons) then dyskinesias (GIDs) might still result (Politis et al., 2010). For afferent inputs, evidence in animals suggests that this integration does occur with graft maturation, although whether the extent will be sufficient to allow a useful re-emergence of phasic DA release (i.e., in response to cortical input) is still unclear (Clarke et al., 1988; Fisher et al., 1991). Restoration of phasic DA release may aid learning and behavior and, although relevant data are not available for human patients at present, a slow maturation of grafts is suggested by the observed longer term improvements in some patients (Grace, 2000, 2008; Piccini et al., 2000).

THE FUTURE OF BIOLOGICAL TREATMENTS IN PARKINSON'S DISEASE

Disrupting current treatment paradigms

Ultimately, these modern biological therapies, including CRT and the various gene therapies, will need to compete with established modalities—in particular DBS. The practical fallout is then that the *timing of treatment* may be the overriding issue for all of them (Fig. 2).

Current practice is usually to offer surgical treatments such as DBS (or DuoDopa) only as a last resort, when patients are failing on conventional pharmacological regimes. This strategy of *delayed treatment* is in part because of the large up-front cost, but also relates to the invasive (surgical) nature of the treatment, as well as issues of ongoing device management. However, this strategy may be inappropriate. Even for a nonbiological treatment like DBS, there is now increasing evidence that earlier treatment may benefit quality of life (Desouza et al., 2013; Deuschl et al., 2013). For biological treatments, with no ongoing device issues or battery replacements, and with a potential for an element of disease modification, the justification for early treatment may be stronger still. Indeed, for the future development of GF therapies it may be crucial, given the extent of pathological loss of TH fibers in the striatum in early disease, and the suggestion from trials that benefits may only be available if used early (Ceregene, 2013). For CRT, the slow nature of the maturation is also an incentive to a pre-emptive strategy.

So the advent of new biological treatments may trigger or enable changes in practice. For individuals, if motor complications are already present, then the short-term potency of DBS may still make it the treatment of choice—at least in those willing and able to undergo this sort of surgery. Its efficacy over short time scales—now well demonstrated in randomized trials—may be difficult to better by any of the biological methods (particularly if there is resistant tremor). So too, sticking to standard treatments for the first few years of the disease, with a view to DBS if difficult motor symptoms arise, will likely remain an attractive strategy for a proportion of patients. However, the risk of this strategy is that of "missing the boat": by the time motor symptoms deteriorate, the option of DBS may be precluded—either by advancing age or by the accumulation of nonmotor, particularly cognitive, symptoms (Desouza et al., 2013)—and it will also by then be too late

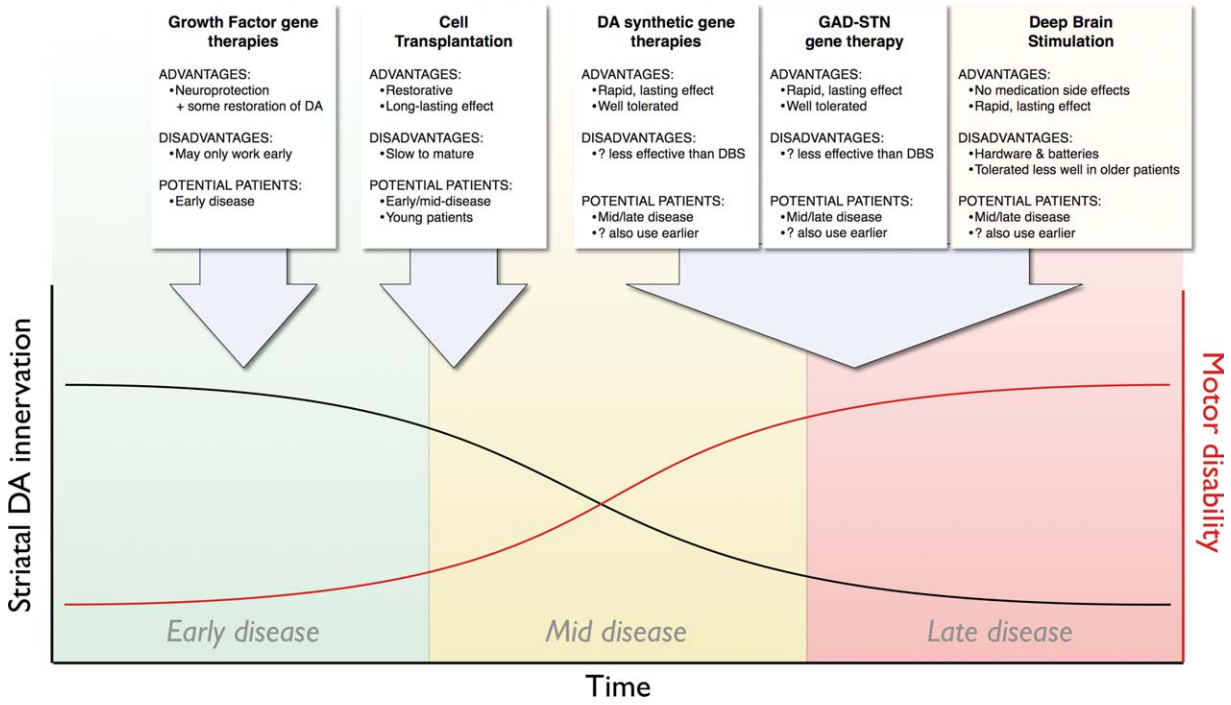

Figure 2. Summary of proposed timings for the new biological treatments for Parkinson's disease, compared with DBS. The growth factor gene therapies offer the best prospect for disease modification, but will probably need to be delivered early in the course of the disease, as they rely on sufficient sparing of existent nigrostriatal projections. Cell transplantation is able to deliver a reconstitution of the denervated striatum with new dopaminergic neurons, but as the transplant may mature slowly over years, it may be best delivered in early to mid-disease. The DA synthetic gene therapies (AADC only, or triple therapy [ProSavin®]), and also genetic STN inhibition (through GAD gene delivery), have initially been aimed at patients later in the disease course, with timings similar to DBS. In principle, as they are well tolerated and may have a lasting effect, these gene therapies could also be delivered earlier in the course of the disease. See text for references. Abbreviations: AADC, aromatic amino acid decarboxylase; DA, dopamine; GAD, glutamic acid decarboxylase; DBS, deep brain stimulation; STN, subthalamic nucleus.

to gain any useful disease modification from the biological therapies.

So with a little foresight, and playing to the advantages of the biological treatments, management paradigms may evolve. For all the biologicals, their key benefit may be the one-off nature of the treatment, with a promise of *sustained effect*. If this can be demonstrated in future studies, and used to justify earlier treatment, perhaps with a lower threshold to treat, then the dilemmas of delayed treatment may also be sidestepped. The quality of life impacts of motor fluctuations would be much reduced, and budgetary concerns might be mitigated by potential savings from reduced morbidity and social dependence. CRT and the GF gene therapies, with their potential for disease modification, may then find a specific niche in the treatment of younger patients, earlier in the disease.

Evolving technologies

All of these technologies have potential to evolve, improving and expanding their remit. For the gene therapies, there has been some recent enthusiasm for an optogenetic version of DBS, using light-driven switching of neuronal activity with designer (light-sensitive) G protein switches (Aston-Jones and Deisseroth, 2013; Gradinaru et al., 2009; Vazey and Aston-Jones, 2013). The advantages of such technology over conventional DBS remain theoretical at present, but it could be the technology of choice for "closed-loop" devices, which employ feedback regulation of stimulation, and which may have advantages over the conventional tonic stimulation used currently (Little and Brown, 2012; Rosin et al., 2011).

Perhaps a more widely applicable emergent genetic technology is that of designer receptors (Farrell and Roth, 2013). These DREADDs (Designer Receptor Exclusively Activated by Designer Drugs) are exclusively activated by the designer drug (such as clozapine N-oxide [CNO]), but are inert to endogenous signaling molecules. They can be virally inserted into selected neuronal populations and activated solely by systemic (oral) medication over periods from hours to months or years. Such a technology promises a form of biological DBS—

"DREADDed-DBS"—that may ultimately be the successor technology to GAD-STN gene therapy, pending a more complete unraveling of precisely how STN-DBS achieves its benefits (Gradinaru et al., 2009).

More exciting perhaps is the vision of smarter modulation of circuitry not amenable to DBS. Thus DREADD technology has the potential to target specific but distributed neuronal populations, or neuronal projections, by a tailored combination of local injection, retrograde transport, cell type–specific promotors and recombination strategies (Aston-Jones and Deisseroth, 2013; Farrell and Roth, 2013; Nair et al., 2013; Vazey and Aston-Jones, 2013). In this way, not only nigrostriatal dopaminergic, but also other catecholaminergic and cholinergic projections, could in principle be treated by using vectors targeted to these neurons or their associated glia (Drinkut et al., 2012). The clinical implications here are not immediately clear, although an initially attractive clinical target could be the treatment of STN-DBS–resistant features such as gait disorders, through targeting of the PPN and its connections. Beyond this, given the disseminated nature of the pathology in PD, an ability to modulate circuit activity outside the cortico-striatal motor loops is tantalizing.

CRT is also likely to evolve, though within narrower confines. Thus it may be possible to create "augmented" iPSCs with designer genetic manipulations. These might endow the transplanted cells with a resistance to LB degeneration, or with other specific capabilities: for example, an improved ability to reinnervate the denervated striatum; or a sensitivity to modulation by exogenous pharmaceuticals (e.g., with DREADD technology); or simply the facility to have their survival, activity, or integration monitored remotely (Tønnesen et al., 2011). An exciting prospect might also be the use of cells engineered to produce GFs, which, when grafted, might then protect afferent (cortical) cells from degeneration.

Limits of technology

Neither CRT nor current gene therapies seem likely to solve the problems of PD in all their diversity. Even the early use of GF gene therapies to halt striatal disease progression (if their efficacy can ultimately be demonstrated) would not be expected to halt extrastriatal disease. Similarly, CRT would not reconstruct circuitry beyond the striatally placed transplant. Although this may well ameliorate nigrostriatal disease, with some consequent improvement in nonmotor symptoms (Lelos et al., 2012; Ostrosky-Solís et al., 1988; Sass et al., 1995), the most recent data suggest that a range of troublesome nonmotor features will still occur, related to more widespread degenerative changes in other neuromodulatory systems and in the cortex (Politis et al., 2012). Also, although transplants of relevant cells could be targeted to these other neuronal populations, the distributed nature of the projections in these cases, and the lack of good animal models for the nonmotor symptoms to which they relate, is likely to critically hinder the development of such technologies.

A mixed future

Ultimately, answers to the problems of disease modification will likely derive from our evolving understanding of the underlying disease mechanisms rather than from CRT. Indeed, iPSC technologies will probably be pivotal here, through their expanding role in disease modeling with patient-derived cells. Such work promises a steady trickle of novel disease targets to add to those currently under scrutiny—for example, molecular pathways governing mitochondrial biogenesis and function, lysosomal function, α-synuclein aggregation, and LRRK2 and Nurr1 activity (Aviles-Olmos et al., 2013; Kuan et al., 2012; Mazzulli et al., 2011; Obeso et al., 2010; Tofaris, 2012; Zhang et al., 2012).

In any emerging therapies, pharmaco-genetics may well play an increasing role, particularly through its implicit ability to avoid side effects of untargeted pharmaco-therapies. Thus, surgically delivered viral vectors could be used to target specific molecular pathways (for example, with micro-RNAs or with newer DREADDs) in selected neuronal populations. More exciting still is the prospect of improved nonsurgical delivery of pharmaco-genetic cargoes, using a combination of microsomal delivery and cell-type–specific promotors, which could allow genetic disease modification without surgical targeting. Genetically encoded growth factors, mitochondrial supporters, or α-synuclein aggregation inhibitors could thus be delivered to specific but dispersed neuronal (or astroglial) populations, while avoiding off-target effects in nonrelevant cells (El-Andaloussi et al., 2012; Lee et al., 2012).

These expanding prospects for pharmaco-genetics reflect the flexibility and adaptability of the technology. This does not preclude a niche for the use of CRT in treating PD, particularly if the remaining hurdles to robust and reliable generation of transplantable cells are overcome (and if upcoming trials show success). However, this niche may be limited to younger patients and, if useful disease-modifying treatments emerge, it may also be only temporary.

Powerful disease-modifying treatments for PD, if they are ever found, really would change the scene. And if they arrive, then no doubt stem cell technology will also move on, shifting focus to other conditions, and to

a continued and growing role in neurological disease modeling.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

ROLE OF AUTHORS

Both authors, who participated equally in the article preparation, had full access to all the information in the review and take responsibility for the integrity of the information and the accuracy of the analysis.

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