

The future of stem cell therapies for Parkinson disease

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Abstract | Cell-replacement therapies have long been an attractive prospect for treating Parkinson disease. However, the outcomes of fetal tissue-derived cell transplants in individuals with Parkinson disease have been variable, in part owing to the limitations of fetal tissue as a cell source, relating to its availability and the lack of possibility for standardization and to variation in methods. Advances in developmental and stem cell biology have allowed the development of cell-replacement therapies that comprise dopamine neurons derived from human pluripotent stem cells, which have several advantages over fetal cell-derived therapies. In this Review, we critically assess the potential trajectory of this line of translational and clinical research and address its possibilities and current limitations and the broader range of Parkinson disease features that dopamine cell replacement based on generating neurons from human pluripotent stem cells could effectively treat in the future.

Parkinson disease (PD) is a progressive neurodegenerative disorder that is characterized by relatively focal degeneration of mesencephalic dopamine (mesDA) neurons, the cell bodies of which are located within the substantia nigra pars compacta in the ventral mesencephalon (VM)¹. The associated loss of axonal projections and subsequent deficit in DA release onto the medium spiny neurons of the striatum results in disability, particularly bradykinesia, resting tremor and muscle rigidity. The fact that many of the key motor symptoms and signs of PD result from the loss of mesDA neurons means that, unlike in many neurodegenerative disorders, replacing just one cell type in a single localized brain region holds promise for relieving some of the significant deficits that affect individuals with PD. As such, PD has long been a trailblazer for cell-replacement therapy.

Initial efforts in this area started more than three decades ago (FIG. 1a) and provided proof-of-principle evidence that DAergic neuron precursor cells isolated from the developing human VM could survive and function in graft recipients with PD when surgically delivered to the putamen, a main target area of mesDA neurons². However, the use of human fetal VM tissue posed serious limitations (discussed later) that precluded clinical application of a cell-based therapy until recently¹. Several notable advances in culturing and differentiating human pluripotent stem cells (hPSCs)^{3–6} have now allowed the formation of transplantable VM progenitors that mature into DA neurons that are virtually indistinguishable from human fetal mesDA neurons with respect to molecular identity, in vivo functionality, potency and target-specific axonal outgrowth⁷. On this basis, we refer to the mesDA-like neurons sourced from hPSCs as ‘hPSC-derived

mesDA neurons’. The field is now on the verge of using hPSC-derived mesDA neurons instead of fetal cells to provide unlimited numbers of quality-controlled cells for clinical trials as a more robust and available future therapy. Indeed, the international collaborative network GForce-PD (<http://www.gforce-pd.com>) has enabled several consortia working on stem cell-based DAergic replacement therapy for PD to share their experiences in the design and execution of the first-in-human clinical trial of hPSC-derived mesDA neurons for PD⁸. The GForce-PD network is continuously growing, and its last meeting was attended by two USA-based, one Europe-based and two Asia-based teams, all at the verge of entering clinical trials. Together, the efforts of these teams and the broader community have catapulted hPSCs for cell-replacement therapy for PD to the forefront of regenerative medicine (FIG. 1b).

In this Review, we describe the key developments in research into DA cell-replacement therapy for PD so far, as well as current and future research aimed at improving graft function and reproducibility by increasing the survival and purity of mesDA neurons in the graft, accelerating their fate acquisition and/or functional maturation and making them less susceptible to attack by the immune system. Furthermore, we speculate on the trajectory of this line of translational and clinical research and address the broader range of PD features that hPSCs might effectively treat.

Parkinson disease

PD is a common, age-related, progressive neurological disease that is traditionally characterized as a neurodegenerative movement disorder⁹ and that, with the exception of a small number of specific genetically linked

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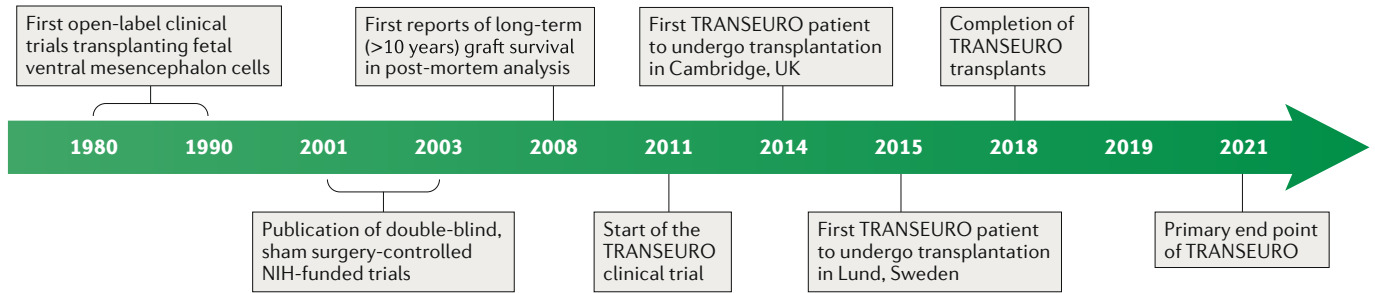
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a Fetal tissue transplantation



b hESC and hiPSC transplantation

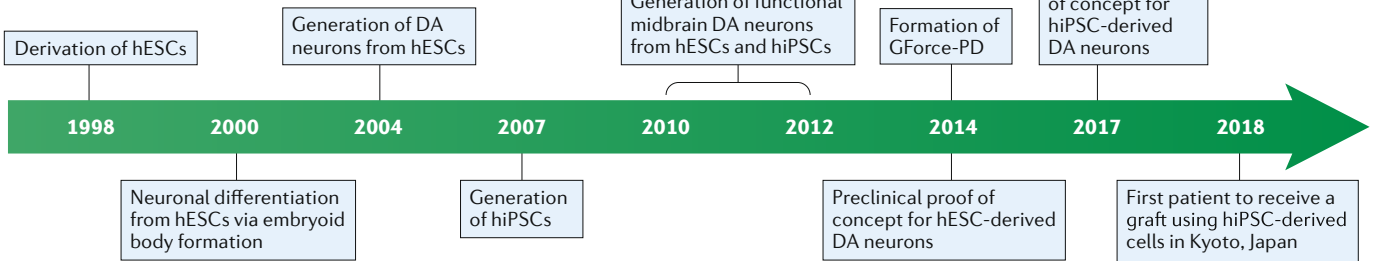


Fig. 1 | Timeline of developing cell therapies for Parkinson disease. a | Timeline for the development of fetal tissue transplantation in Parkinson disease. **b** | Timeline for the development of human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) transplantation in Parkinson disease. DA, dopamine; NIH, US National Institutes of Health.

cases, is linked to cytoplasmic aggregation of α -synuclein and the formation of Lewy bodies in the DA neurons of the substantia nigra pars compacta. Our understanding of its complexity has evolved enormously over the past few decades. PD is now widely appreciated to lead to diverse motor and non-motor symptoms and signs through dysfunction of multiple CNS and peripheral nervous system pathways, affecting the release of various neurotransmitters^{10,11}. It seems to arise from a complex interplay of genetic factors and environmental exposures that differ between individuals with the disease^{12–15}. Unsurprisingly, therefore, endophenotypes of PD differ extensively between individuals, and even the types of protein aggregates in the brain can differ between people with PD^{16,17}. These differences account, at least in part, for the heterogeneity in clinical presentations¹⁸ and have contributed not only to the challenges in the clinical care of individuals with PD but also to the failures in developing novel therapeutics, such as neuroprotective and neurorestorative interventions.

Despite this heterogeneity, the traditional view of the core PD pathology — namely, the degeneration of mesDA neurons resulting in a loss of DA in the striatum — is the unifying feature for the vast majority of individuals with this diagnosis. As such, the most successful current pharmacotherapies, including the gold standard treatment levodopa, mostly act by augmenting nigrostriatal DA inputs to the medium spiny neurons in the striatum (BOX 1). Unfortunately, the systemically administered medications have major drawbacks: the increases in intracerebral DA concentrations that they induce cannot be optimally temporally regulated and the delivery cannot be spatially regulated at all. For example,

the pulsatility of levodopa therapy and its continued use result in complications that include end-of-dose wearing off and levodopa-induced dyskinesia¹⁹. Other common adverse effects include hallucinations, impulse control disorders and other psychiatric problems^{20,21}.

In individuals with PD for whom medications eventually provide insufficient relief, surgical approaches have the potential to pseudonormalize the basal ganglia outputs that are disrupted in this disease, to reduce key motor symptoms (BOX 1). These approaches include deep brain stimulation (DBS), which is commonly used when medical therapy is exhausted, to modulate the dysregulated output nuclei of the basal ganglia in an attempt to normalize motor function. Newer methods for non-invasive and more precise targeting of lesions in similar tracts and structures using magnetic resonance-guided focused ultrasound ablation are approved in the USA for clinical use for tremor-predominant PD and are also currently in randomized controlled clinical trials²².

A gene therapy approach has been investigated in a phase II clinical trial, in which viral vectors are surgically delivered by precise local injection into the subthalamic nucleus to induce expression of genes encoding the synthesis of the inhibitory neurotransmitter GABA in an attempt to inhibit aberrant neuronal function²³. Other potential gene therapies that have reached clinical trials focus on either neuroprotection by growth factor delivery²⁴ or the delivery of genes encoding key enzymes required for DA synthesis to enable other neural cell types to compensate for the loss of mesDA neurons and to synthesize DA locally in the putamen^{25–27}, or will aim to correct underlying genetic deficits²⁷.

Magnetic resonance-guided focused ultrasound ablation
An intracranial thermal ablation procedure with multiple ultrasound beams that are precisely targeted to facilitate non-incisional pallidotomy or subthalamotomy that may potentially alleviate symptoms of Parkinson disease.

Another potentially powerful strategy to restore the ‘normal’ physiological pattern of striatal DA transmission and thus avoid complications associated with systemic DA delivery is to replace the DA neurons that are lost in the disease. This was the focus of historical efforts and continues to be the focus of current efforts to develop experimental cell-based DA replacement therapies for PD^{2,8,28}.

Cell replacement therapy in PD

In pioneering clinical studies performed more than 30 years ago, mesDA neuron precursors from allogeneic fetal tissue were transplanted to the striata of individuals with PD^{29–37}. The first-in-human clinical studies provided proof of concept that tissue could be implanted into the brains of patients with no overt negative effects at the target site of transplantation, but variable clinical benefits were observed. Since then, other sources of cells for transplantation have been tested. These alternative cell sources have included autologous adrenal medullary tissue as a source of DA derived from neuroendocrine chromaffin cells³⁸, autologous carotid body tissue as a source of DAergic glomus cells³⁹ and retinal pigmented epithelial cells as a source of levodopa^{40,41}. However, transplants of cells other than authentic fetal

VM DAergic neurons have failed to demonstrate key requisite properties, including robust survival, DA release and clinical benefit^{38,40,41}. In particular, the rationale for transplanting adrenal medullary tissue, which is based on the fact that neuroendocrine chromaffin cells can produce DA, has been questioned⁴². Importantly, the open-label studies demonstrated that fetal VM was the only cell source that conferred the potential for clinical improvement and, notably, for graft survival and function as demonstrated by clinical measures, neuroimaging^{33,36,43–45} and post-mortem histological analysis^{46–50}. In addition to open-label studies, two randomized, double-blinded, sham-surgery-controlled clinical trials using fetal VM tissue as the donor cell source also demonstrated some specific benefits in function, despite neither trial reaching its primary end points^{51,52}. Although some individuals with PD showed normalized DA signalling coupled with clinical benefits, other participants did not benefit from the graft, and some individuals even developed adverse effects, such as graft-induced dyskinesias (GIDs)^{2,51,52}.

Despite this variation in clinical outcome, fetal VM tissue has gathered the greatest momentum and evidence base that the cells survive and function over the long term^{2,49,53–55}. To understand the divergent

Box 1 | Current therapeutic landscape for Parkinson disease

Currently, the mainstay of treatment for Parkinson disease (PD) remains pharmacotherapy and, in some cases, surgery, with attention to patient education, lifestyle adjustments, occupational therapy, diet and exercise.

Pharmacotherapy

Pharmacotherapy to alleviate motor symptoms mostly aims to restore striatal dopaminergic (DAergic) tone, using DA agonists, monoamine oxidase B inhibitors and/or levodopa plus carbidopa^{20,21}. Anticholinergic medications and amantadine (which has a mixed mechanism of action) are also useful in some patients²⁰. Moreover, pharmacotherapy is efficacious in treating some non-motor symptoms via a broad array of possible drug targets; for example, antidepressant medications that manipulate the serotonergic system¹⁴⁶. However, off-target effects inherent to systemic administration (such as nausea, drowsiness and orthostasis) and adverse on-target effects (for example, impulse control disorders) can be limiting, and complications (such as end-of-dose wearing off and dyskinesia) can emerge in the intermediate and long term with levodopa therapy^{19,147}. In particular, there is a progressive shortening of the duration of action of levodopa, and ‘wearing off’ of the therapeutic effect occurs with emergence of poorly controlled PD signs and symptoms before the next scheduled levodopa dose.

The pharmacological competitive landscape to reduce wearing off¹⁴⁷ includes adjunctive oral medications, fast-onset injectable or inhalable ‘rescue’ therapies or continuous subcutaneous infusion of apomorphine, a DA receptor agonist. Levodopa–carbidopa gel can be delivered to the intestines via a percutaneous jejunal tube to reduce motor fluctuations; however, this requires a gastrostomy, and so the benefits of this approach are limited by the need for care and maintenance of the stoma and/or production of hardware-related adverse effects. Cell-based therapies that aim to restore DAergic inputs to the striatum may overcome many limitations of current oral medications, particularly levodopa wearing off, unpredictability, burden of frequent dosing and off-target effects.

The advantages of current pharmacotherapies are that they are easily administered, thus facilitating flexible timing to tailor to an individual’s wearing-off profile; there are decades of data demonstrating long-term safety, tolerability and efficacy; in general, they are widely available and affordable; and they mostly target the DA system, although other drugs

targeting different neurotransmitter systems may be useful in alleviating motor and non-motor symptoms. The main drawbacks include the fact that many of the clinically important symptoms respond poorly to current treatments (for example, imbalance, falls and freezing of gait) and that as the disease progresses and symptoms worsen, the dosing and dose frequency increase, resulting in complex regimens with increasing adverse events. The latter also leads to increased wearing off and dyskinesias, often occurring at levodopa peak dose (which will increase with increased frequency of administrations throughout the day).

Surgical treatments

When pharmacotherapy provides insufficient control of motor symptoms, surgical therapies may benefit certain patients. Deep brain stimulation (DBS) is the gold standard against which new experimental surgical interventions are measured¹⁴⁸. It is somewhat reversible, in that misplaced or infected DBS leads may be removed. However, implanted hardware may be damaged, erode or migrate; non-rechargeable batteries must be surgically replaced; and there are lingering concerns about the possible effects of DBS on cognition, mood, speech, gait and balance^{149,150}. Ongoing advances include the use of directional leads for superior field shaping to increase the benefit–risk ratio, improved programming paradigms and new approaches such as use of adaptive systems^{151,152}.

The pipeline

Magnetic resonance-guided focused ultrasound ablation²⁷ is a non-invasive procedure that depends on the precise focusing of multiple ultrasound beams. It is currently in clinical use for tremor-predominant PD and is in further clinical trials. Gene therapy could potentially provide symptomatic relief by increasing DA synthesis¹⁵³ or modulating glutamate production²³; deliver neuroprotective agents such as glial cell-derived neurotrophic factor or neurturin; or correct underlying genetic deficits²⁷. These pipeline approaches hold promise but are early in development; none has undergone a successful phase III clinical trial.

An optimal cell-based therapy would avoid the hardware-associated problems of DBS and the adverse effects that limit dose delivery. It would also be advantageous over magnetic resonance-guided focused ultrasound ablation by providing a restorative rather than an ablative intervention.

patient responses, a working group was created in 2006, to which key researchers involved with human fetal VM-derived transplantation trials internationally were invited to discuss and decipher the factors that could influence the variability in outcome, including several main parameters that were deemed to have a significant impact related to the cells, the method of implantation, immunosuppression and patient selection (extensively reviewed in⁵³). A systematic review focused on patient selection in all PD transplantation trials using human fetal VM tissue where sufficient data were available identified that age at transplantation was associated with poorer outcomes². Other analyses have identified that younger patients⁵¹ and those with greater levodopa response before surgery⁵² have better outcomes after transplantation. Notably, patients in whom the loss of DAergic innervation was restricted to the putamen benefited more than those with more extensive denervation of the ventral striatum^{56,57}. Moreover, some studies showed that good clinical effects required sufficient amounts of tissue (minimum of three fetal VMs per hemisphere), sufficient fibre outgrowth and prevention of graft rejection^{50,52,58,59}.

Rapid advances in understanding the genetic basis of PD have opened another avenue to potentially define the optimal cohort for cell transplantation. DA neuron replacement is expected to primarily alleviate motor symptoms. In cases in which the predominant features are cognitive impairment and dementia, DA replacement would therefore be of limited benefit. For example, cases of PD linked to certain *GBA* mutations are associated with more rapid cognitive decline^{60,61}, including after DBS⁶². Moreover, novel approaches targeting *GBA*⁶³ and *LRRK2* (REF.⁶⁴) have the potential to provide a therapeutic effect more broadly throughout the nervous system and therefore, if they are successfully developed, could be preferential. By contrast, individuals with *PARK2* mutations primarily have a motor phenotype and are highly susceptible to developing levodopa-induced dyskinesias⁶⁵, suggesting that early intervention with cell therapy in these patients might be more beneficial.

The cumulative reanalysis of the previous open-label and double-blind, placebo-controlled trials led to the design of an optimized clinical trial design for cell transplantation, called 'TRANSEURO'⁶⁶. This is a European open-label, assessor-blinded multicentre trial of transplants of fetal VM tissue into individuals with sporadic PD. The transplant patients and their age-matched and disease severity-matched non-transplant controls will be compared with a similar population of patients in the observational cohort who were not randomized to the active arm of the trial and will instead be followed up as a natural history cohort. The primary end point of this study is the change in the Unified Parkinson's Disease Rating Scale score, in the 'off'-medication state, at 3 years after the second transplant. Multiple secondary end points will also provide valuable data comprising other motor scores, non-motor assessments and non-invasive imaging of transplant function using positron emission tomography (PET) and MRI⁵³. In total, 11 patients have received human

fetal VM tissue transplants in 21 grafting sessions performed between 2015 and 2018. A recent update from the trial has been published⁶⁶, and the first report on outcomes in patients who received transplants is expected 3 years after the last transplantation surgery, which occurred at Skane University Hospital in Lund, Sweden, in early 2018 (FIG. 1a).

One major remaining hurdle for clinical application of transplantation-based cell replacement is the use of fetal tissue as a cell source. In the TRANSEURO study, many surgical procedures were cancelled owing to lack of sufficient or suitable amounts of fetal tissue, resulting in substantial delays, logistic complications and burdensome experiences for the patients⁶⁶. In addition to its low availability, there are multiple other barriers to the use of fetal tissue as a cell source (BOX 2), thus highlighting the critical need for new sources of authentic and functional human mesDA neurons of high purity and consistent quality (FIG. 2).

Pluripotent stem cells

PSCs — such as human embryonic stem cells (hESCs), first reported in 1998 (REF.⁶⁷), and induced human PSCs (hiPSCs), first reported in 2007 (REF.⁶⁸) — offer a renewable source of human cells of a very early developmental stage with the potential to form any cell type in the adult body. As such, hPSCs offer a scalable cell source from which standardized and quality-controlled cell derivatives can be obtained for therapeutic use (FIG. 2). When hPSCs are cultured under serum-free conditions, they readily differentiate into neuroectoderm^{69,70}. This makes hPSCs relatively easy to use for the generation of regionally specified neural cell types, and the protocols for such purposes have gradually evolved⁷¹. In early studies, different subtypes of neural cells were typically induced via stromal feeder cells, aggregation into embryoid bodies or stepwise addition of small molecules. These studies were instrumental in showing that DA neurons can be formed from either hESCs or hiPSCs^{72–75}, but the purity and yield of DA neurons was highly variable owing to the unsynchronized and incomplete differentiation achieved with these methods.

In 2009, a protocol was developed for very efficient and synchronized neuralization of hPSCs using inhibitors of the SMAD-dependent transforming growth factor- β and bone morphogenetic protein signalling pathways⁷⁶. This method for neuralization is commonly referred to as 'dual-SMAD inhibition'. When combined with extrinsic patterning factors that normally control regional identity during neural development, several therapeutically relevant neuronal subtypes can be obtained under defined conditions^{77,78}. Dual-SMAD inhibition was successfully used to generate mesDA neurons via a floorplate intermediate in 2011 (REF.³). Since then, several protocols for generating mesDA neurons have been developed in which the timing and concentration of the patterning factors sonic hedgehog protein (SHH) for ventralization and glycogen synthase kinase 3 inhibitor (which results in activation of the canonical WNT pathway) and/or fibroblast growth factor 8 (FGF8) for sufficient caudalization have been optimized^{4,5,79–81}. These differentiation strategies, based

Unified Parkinson's Disease Rating Scale

A widely used, validated clinical rating scale that evaluates non-motor symptoms, activities of daily living, motor signs and complications of levodopa therapy.

Neuroectoderm

Derived from the ectoderm, the outermost of the three primary germ layers in the early embryo; its formation is the first step in development of the nervous system.

Stromal feeder cells

Feeder cells that provide extracellular secretions to help another cell to proliferate or differentiate. Often the cells of the feeder layer are irradiated or otherwise treated to arrest growth.

Box 2 | Advantages of stem cell-derived cells over fetal cells

Stem cell-derived cells have several advantages over fetal cells, including near-unlimited availability, standardized manufacture, the ability to be cryopreserved, and increased purity, allowing more facile surgery, dosing and distribution.

Availability

Human fetal brain tissue to be used for transplantation is scarce. To obtain sufficient surviving dopamine (DA) neurons in the transplants, the ventral mesencephalon (VM) of at least three fetuses must be collected and transplanted into each hemisphere¹⁵⁴. Moreover, the cells remain viable for only a short time in hibernation medium¹⁵⁵, meaning that all the material used for grafting in one patient must be collected over a short period. The earlier fetal VM transplantation trials used tissue from surgical terminations of pregnancy⁷, but now medical (non-surgical) terminations are often used in clinics¹⁵⁶. Although this change does not preclude tissue use, as fetal tissue is subject to carefully defined criteria, the collection is more challenging, the embryos are often too young, and supply determines surgical transplantation date⁶⁶. By contrast, human pluripotent stem cell (hPSC)-derived DAergic neural progenitors can be produced in near-unlimited numbers, cryopreserved and used on demand.

Standardized manufacture

Although all possible measures are taken to collect and process fetal tissue using standard operating procedures to ensure the highest quality achievable, the cells used for transplantation inevitably differ between each patient. The ages of the three fetal VMs are different (in the TRANSEURO trial, the crown–rump lengths of the fetuses used were 15–35 mm) and thus the dissections and, as a result, cell composition vary each time, as does the cell viability after processing⁶⁶. By contrast, stem cell production and differentiation can be performed under fully defined conditions that meet rigorous and standardized good manufacturing practice standards, thus reducing the cell variability between batches and within a single batch⁶.

Cryopreservation

If sufficiently scaled, large-scale production of hPSC-derived cell derivatives for transplantation is possible. An hPSC-derived product can be cryopreserved^{6,83}, providing enormous advantages over previous

approaches. Importantly, cryopreservation would allow rigorous preclinical studies of the efficacy, safety and adverse effects of exactly the same cell batches that are to be transplanted into humans (FIG. 3). These cells are anticipated to survive, mature and function as authentic adult A9 mesencephalic DA neurons, as demonstrated preclinically (see the main text). Critical issues including lack of tumorigenicity, lack of off-target effects and compatibility with surgical delivery devices and immunosuppressive regimens (if used) may also be addressed before clinical trials in cells identical to those to be transplanted into humans.

Purity

The major concern with any stem cell-based therapy for PD is the risk of remaining PSCs or other proliferative cell populations that can lead to tumours, teratoma formation or neural overgrowth after transplantation. Use of stem cell-derived preparations allows the use of cell purification strategies based on cell surface markers^{5,157} and/or a thorough preclinical characterization to rule out the presence of such cell types before grafting. A second concern is that a subset of patients who previously received fetal VM cell transplants developed graft-induced dyskinesia that persisted in the absence of levodopa^{51,52}. This unwanted side effect has, at least in part, been linked to the activity of serotonin neurons in the graft^{97,98}. Use of well-characterized cells for grafting, as is possible with hPSC-derived cell products, will allow exclusion of this type of cell in the graft, thus minimizing one of the factors associated with the risk of graft-induced dyskinesia. The homogeneity of the cell products for transplantation is therefore thought to be desirable, at least in early studies for this reason, in addition to facilitating reproducibility between grafts, ensuring that no unwanted cell types are present, and allowing greater control of the precise cell doses delivered.

Surgery, dosing and distribution

Transplantable DAergic neuron progenitors can now be manufactured in near-unlimited numbers from stem cells^{77,91,92}. Thus, the optimal number of cells to be transplanted and the scheduling of bilateral transplants can be based on scientific and clinical considerations rather than on (limited) cell availability. Factors such as the concentration of cells and volume transplanted can easily be standardized across transplantation tracts, hemispheres and patients.

on deriving mesDA neurons via a developmentally correct mesencephalic floorplate intermediate, resulted in the first grafts with robust *in vivo* performance, and this positive outcome spurred intensive preclinical evaluation, good manufacturing practice manufacture of cells and pioneering clinical trials (BOX 3; FIG. 1b).

The initial preclinical studies in which DA neurons generated via a floorplate intermediate were used demonstrated good *in vivo* survival of the transplanted cells and functional recovery of motor deficits in animal models of PD, as assessed with amphetamine-induced rotation^{3,4}. Moreover, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned non-human primates, transplantation of autologous iPSC-derived DA neurons led to a marked rescue of motor deficits⁸². Several more studies demonstrated the long-term functionality of xenografted human and non-human primate mesDA neurons in rodent models of PD^{5,49,75,81,83–86}. One study⁷ reported that hESC-derived mesDA neurons became functional by 6 months after transplantation in the 6-hydroxydopamine rat model of PD, as visualized by PET and single photon emission computed tomography, and demonstrated that the grafts matured into DA neurons that could release DA *in vivo*, without any overgrowth or contamination by unwanted cell types.

Importantly, functional recovery in the amphetamine-induced rotation test was achieved with a comparable number of surviving DA neurons (measured by immunostaining of tyrosine hydroxylase) from the hESCs to that achieved with human fetal VM in the same rodent model, implying that these cell types are equally potent⁷. Further studies using optogenetic and chemogenetic manipulations demonstrated that the functional recovery achieved using hPSC-derived mesDA neurons is mediated by spontaneous *in vivo* DA release in both spontaneous and drug-induced behaviours in mouse models of PD^{80,85}. Moreover, homotopic intranigally grafted neurons showed target-specific innervation of appropriate neuroanatomical structures across distances of at least 10 mm in a rat model of PD^{7,87}. Importantly, the ability of engrafted neurons to form long-distance projections that release DA and to support functional recovery has also been reported in non-human primate models of PD^{81,82}.

Cumulatively, a large number of transplants of DAergic neurons derived from PSCs have now been reported in animal models of PD, and neither tumours nor uncontrolled proliferation has been reported from these newer protocols. However, there remains a need for caution as these approaches are translated to the clinic.

Amphetamine-induced rotation

Drug-induced turning behaviour used to assess unilateral dopaminergic lesions and effects of transplants in rodent models of Parkinson disease.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

A prodrug of 1-methyl-4-phenylpyridinium, a neurotoxin leading to loss of dopamine neurons in the substantia nigra, and used to create animal models of Parkinson disease.

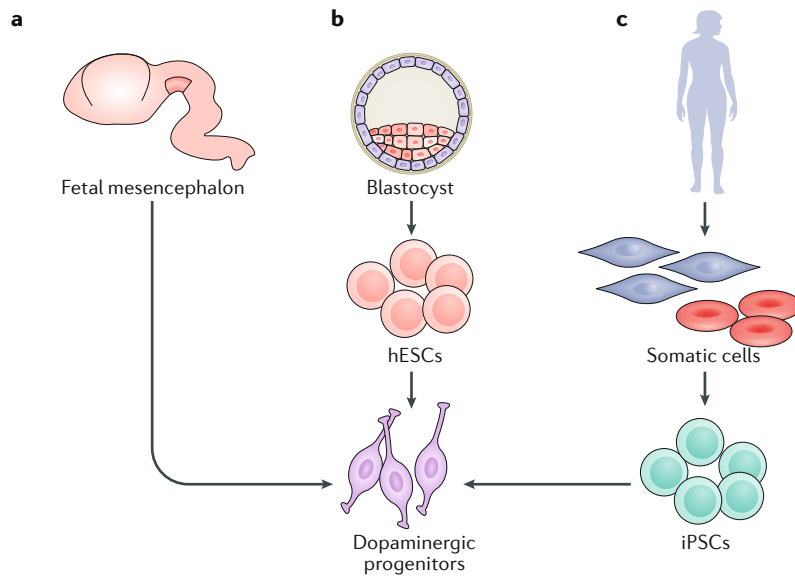


Fig. 2 | Cell sources being trialled for clinical cell-replacement therapy in Parkinson disease. **a** | Transplantable dopaminergic (DAergic) progenitors isolated from fetal ventral mesencephalon were first tested in the 1980s and are also used in the ongoing TRANSEURO study. **b** | DAergic progenitors with equal functionality and potency can be isolated from human embryonic stem cells (hESCs) that are obtained from the inner cell mass of the preimplantation blastocyst. **c** | Somatic cells, commonly skin biopsy or blood cells, isolated from adult donors can be reprogrammed into induced pluripotent stem cells (iPSCs), which have a capacity similar to that of hESCs to form transplantable DAergic progenitors.

Detailed characterization of source cells is critical, in particular since accumulated genetic mutations have been demonstrated in large cell banks that may, in many cases, be associated with growth advantage: for example, dominant negative *TP53* mutations^{88–90}. Furthermore, despite extensive preclinical testing, there are inherent limitations of this approach, with the relatively short duration of testing when compared with that in humans and the smaller cell doses tested. In addition, multiple other factors differ between animals and humans, such as the immune environment and the precise effects of immunosuppression. Therefore, potential lack of safety and the possibility of tumour formation after grafting are the main concerns for these first pioneering clinical trials, and strategies to ensure that such risks are sufficiently mitigated have been described by several research groups^{77,91,92}.

Early clinical trial expectations

In 2018, a groundbreaking clinical trial of surgical transplantation of allogeneic human iPSC (hiPSC)-derived DAergic neuron precursors into the putamen of individuals with PD launched in Japan^{92,93}. Further clinical trials of cell transplants in PD are expected to start soon^{8,77,79}. The first trials of hPSC-derived transplants in PD will have a primary focus on evaluating feasibility, safety and tolerability. If successful, these trials will be followed by efficacy trials. The current trials conducted by academic groups are summarized and compared with the TRANSEURO clinical trial in TABLE 1. In addition to these trials, several companies have announced that they are developing commercial cell preparations which,

if the trials are successful, will ensure widely available therapies in the future.

Stem cell-based approaches have major advantages over previous efforts using fetal cells (BOX 2). Given the expectations of cell survival, maturation and integration into a host’s circuitry that are supported by the extensive preclinical safety and efficacy data^{3,4,7,81,82,87,94}, stem cell-based approaches have the potential to deliver DA to a host’s medium spiny neurons in a near-physiological manner, maintaining steady intrasynaptic DA levels. Stimulation of postsynaptic DA D1 and D2 receptors would, in turn, modulate key downstream pathways⁹⁵ necessary for properly regulated and implemented motor activity, thus ameliorating the key clinical features of PD that result from DA loss.

What can we expect from these pioneering hPSC clinical trials? It is likely that the pattern of signs and symptoms to benefit will reflect those that are DA responsive in patients; for example, those that respond to levodopa (BOX 3). This includes bradykinesia and rigidity, as well as ‘off’ dystonia, resulting in improved fine coordination skills, reduced tremor, improved facial expression and alleviation of pain due to ‘off’ dystonia. Moreover, since these trials are based on the same concept as the fetal transplantation trials — that is, mesDA neuron replacement — one might expect an outcome similar to that of the best patient responses in the fetal cell trials but in a more robust and reproducible manner as the PSC-derived cells can be standardized and precisely dosed for transplantation. Thus, there may be not only improvements in motor scores but also reductions in motor fluctuations, and patients may be able to reduce or stop taking DAergic drugs, consistent with the postulated mechanism of action of the engrafted cells.

Certain symptoms of PD respond less well and variably to DAergic medications, including speech difficulties, imbalance and freezing of gait. In such cases, it is anticipated that response to mesDA neuron replacement will reflect, at least partially, response to levodopa. One problem is that some symptoms, such as imbalance, may arise owing to multiple areas of pathology involving multiple neurotransmitters, and therefore might not respond fully to a DA neuron replacement strategy. Although case reports of long-term follow-up of patients who have received fetal cell grafts have indicated an absence of freezing and falls often seen in individuals with advanced PD⁵⁵, data from systematic long-term follow-up from sham-surgery-controlled trials is lacking.

The expected effects on motor symptoms that are generally worsened by additional DAergic stimulation, such as levodopa-induced dyskinesias, are also less clear. It is possible that by eliminating fluctuating DA stimulation in the striatum, some individuals may experience alleviation of dyskinesia related to the DAergic drugs. However, a potential complicating factor is that GIDs were reported in a differing proportion of graft recipients in the open-label trials and in 15% and 56% of fetal tissue transplant recipients, respectively, in the two sham-surgery-controlled trials^{51,52,55}. Although the mechanisms underlying GIDs are not fully understood, one proposed mechanism is that contaminating serotonin neurons present in the graft may have a role⁹⁶.

Indirect pathway

A neuronal network pathway from the striatal medium spiny neurons primarily expressing dopamine D2 receptors via the globus pallidus pars externa to the subthalamic nucleus.

Activation of this pathway inhibits movement and action selection. This pathway is abnormally active in Parkinson disease, as the loss of dopaminergic tone leads to disinhibition of the pathway.

Evidence in support of this proposed mechanism has been obtained from studies using PET in patients with PD who have received a graft who express GIDs. The GIDs could also be suppressed with drugs targeting the serotonergic system^{97,98}. The serotonin neurons develop caudal to but in close proximity to and sometimes intermingled with the DA neurons⁹⁹ and because of this they cannot be completely excluded when fetal tissue is dissected. Unlike for cells from fetal VM, the serotonergic contamination can be minimized in, or completely excluded from, stem cell-derived preparations (BOX 2), and thus the risk of GIDs mediated via serotonin neurons is reduced. However, it cannot be ruled out that other mechanisms may also contribute to the development of GIDs.

One open question is whether the trialled hPSC-derived transplants will affect non-motor symptoms or

the progression of the disease. Most of the non-motor symptoms in PD do not respond to levodopa treatment, and therefore might not be expected to experience meaningful relief with a DA neuron replacement strategy. However, certain non-motor symptoms that are exacerbated by off-target effects of anti-PD medication, such as psychosis or orthostasis, might be relieved indirectly as a result of it being possible to reduce oral medications. The current surgical gold standard for moderate-to-advanced PD, DBS, may also offer clues as to the potential effects of PSC transplants, given the known effects of DBS on PD-related circuits. This surgical intervention commonly targets the subthalamic nucleus and the globus pallidus pars interna — integral parts of the indirect pathway, which is inhibited by DAergic inputs that are lost in PD. DBS provides relief not only from the motor symptoms but also from

Box 3 | The first wave of trials of hPSC-based DA neuron transplants in PD

Several distinct human pluripotent stem cell (hPSC)-based cell sources are currently being or will soon be tested in early clinical trials, including fetal ventral mesencephalon (VM) tissue and dopaminergic (DAergic) neuron progenitors derived from stem cells^{66,77,91,92} (TABLE 1). There is a clear rationale with insight into the mechanism of action of the therapeutic hPSC-derived cells, as is recommended by International Society for Stem Cell Research guidelines for clinical translation¹⁵⁸. Various aspects of clinical trial design, including the optimal cohort, dosing, outcome measures and follow-up periods, should be accordingly defined. A list of design considerations for clinical trials of mesencephalic DAergic neuron progenitors derived from human embryonic stem cells (hESCs) and induced PSCs is given below. Other cell sources with less extensive preclinical evidence and less well-defined effects, such as non-DAergic neural progenitor cells, present greater challenges in terms of clinical trial design.

Clinical trial cohort considerations

Age

- Younger patients are at higher risk of Parkinson disease (PD)-related gene mutations (and so results from these individuals may be less generalizable), although one fetal VM transplantation trial demonstrated greater benefit in patients younger than 60 years⁵¹.
- In one study, graft-induced dyskinesias (GIDs) developed only in ‘younger’ patients (up to 60 years old), and so until the mechanism of GIDs is better understood, any advantage conferred by younger age may be mitigated by the risk of GIDs⁵¹.
- Older patients are more likely to have comorbid and confounding disorders and therefore may be at higher risk of adverse events from surgical intervention in addition to immunosuppressive drugs if they are used after transplantation¹⁵⁹.

PD stage

Individuals with early PD with localized pathology are predicted to be more likely to benefit⁶⁶, but the first-in-human clinical trials must balance the unknown benefit with important ‘known’ risks, such as the potential for graft overgrowth or tumorigenesis, as well as ‘unknown’ risks, thus favouring inclusion of patients with more advanced PD.

Predicted responses of PD characteristics

DA-responsive motor signs and symptoms likely to benefit

- Bradykinesia
- Rigidity
- End of levodopa dose ‘wearing off’
- Off-phase dystonia
- Tremor^a
- Gait^a

- Freezing of gait^a
- Balance^a
- Adverse effects of medications such as dyskinesia, psychosis and orthostasis (indirect effects due to lower dose requirement)

Predicted indirect benefits as a result of improved motor function

- Activities of daily living
- Quality of life
- Some non-motor symptoms (for example, sleep)

Features unlikely to benefit

- Mild cognitive impairment and dementia
- Dysautonomia (unless due to medications)
- Weight loss
- Dysosmia
- Sialorrhoea
- Dysphagia
- Pain syndromes

Primary and secondary outcomes

Primary outcomes: safety and tolerability

- Serious and non-serious adverse events to be measured by clinical, imaging and laboratory tests
- Safety considerations include risks related to surgery (haemorrhage, stroke, infection or seizure); risks related to transplanted cells (tumorigenesis, overgrowth or growth of unwanted cells, spread to off-target sites, dysregulation resulting in GIDs, inflammatory reaction); and risks related to immunosuppression (increased risk of infection, increased risk of certain cancers, renal dysfunction and other effects — probably short term, owing to the duration of immunosuppression)

Secondary and exploratory outcomes

- Graft survival, growth and neurochemical function as reflected by neuroimaging, such as MRI, positron emission tomography using fluorodopa or DA transporter ligands, and single photon emission computed tomography^{56,160}
- Clinical effects on motor symptoms as reflected by standardized rating scales, applications or wearable devices
- Patient-reported outcomes, including activities of daily living, quality of life and global impressions
- Exploratory blood and/or cerebrospinal fluid biomarker measures

^aMay differ between individuals, as it differs for levodopa response

Table 1 | Academic clinical cell transplantation trials in Parkinson disease

Trial (NCT number)	Transplantations initiated	Donor cells (cryopreserved product)	Number of transplant recipients (age in years)	Disease duration (years)	Disease severity	Primary end point
TRANSEURO ^a (0189839) ⁶⁶	Completed	Human fetal VM tissue (no)	11 (30–68)	2–13	Early to moderate	Efficacy
STEM-PD (NA) ⁹²	No	hESC-derived mesDA progenitors	8 (<70)	5–15	Moderate	Tolerability and feasibility
NYSTEM-PD (NA) ⁷⁸	No	hESC-derived mesDA progenitors	10 (45–72)	5–15	Severe	Safety, tolerability and feasibility
CiRA (NA) ⁹³	Yes	hiPSC-derived mesDA progenitors	5–10 (50–69)	>5	Severe	Safety and tolerability
Chinese Academy of Sciences (03119636) ¹⁶¹	Yes	Stem cell-derived neural precursors	50 (50–80)	>5	Severe	Safety
Bundang CHA Hospital, Korea (01860794)	No	Human fetal VM neural precursors	15 (18–70)	NA	Severe	Safety and tolerability

Information on current and upcoming clinical transplantation trials is based on published data and clinical trial registration information at <https://clinicaltrials.gov>. The trials listed are not blinded. hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; mesDA, mesencephalic dopamine; NA, not available; VM, ventral mesencephalon. ^aTrial includes randomization of participants to treatment groups.

some of the non-motor symptoms and also improves quality of life^{100,101}. For example, the beneficial effects of subthalamic nucleus-targeted DBS on sleep¹⁰² include improvements in overall sleep quality and maintenance, possibly reflecting indirect effects of the motor benefits of the stimulation and/or enabling patients to take lower doses of other medications, in addition to possible direct effects on sleep architecture. Of course, certain symptoms might not be expected to respond to current transplant strategies, such as mild cognitive impairment and dementia, owing to the underlying nature of their pathology being widespread and involving neurotransmitters other than DA, such as acetylcholine.

Immune rejection

The brain has traditionally been considered to be immunologically privileged, thus obviating concerns for graft rejection following allogeneic cell and tissue transplants. However, the recent description of the glymphatic system¹⁰³ and the identification of CNS lymphatic vessels connected to the deep cervical lymph nodes¹⁰⁴ have challenged this traditional view¹⁰⁵. Immune reactions to grafts of fetal neural cells that are not major histocompatibility complex (MHC) matched to the recipient have been demonstrated in animal models^{84,106}. Moreover, the implantation surgery itself breaches the blood–brain barrier, compromising the immune-privileged status of the brain and potentially triggering the entry of immune cells. Therefore, there is a rationale for at least short-term immunosuppression to prevent graft rejection and promote cell survival and innervation¹⁰⁷. In addition, PD compromises the blood–CNS barrier¹⁰⁸ and may require more aggressive immunosuppression, possibly using multiple drugs.

Previous transplantation trials targeting the CNS have ranged from using no immunosuppression to various protocols that are largely based on those used for solid-organ transplants; others have taken an intermediate approach^{31,51,52,109}. When immunosuppressants have been used, the duration of administration has also been variable. Therefore, it is near impossible to draw

firm conclusions from previous clinical trials on the optimal type, dose and duration of post-transplant immunosuppressive regimens. Although the predicted benefits of immunosuppression need to be formally confirmed, there is extensive experience in solid-organ transplants that will help guide investigators in the choice of drug regimen and its potential impact on safety and tolerability of clinical trial interventions. Unfortunately, immunosuppression is burdensome and can limit eligibility to partake in trials¹¹⁰. Drug toxic effects, infections, such as urinary tract infections, sepsis and pneumonia, and specific malignancies can be linked to immunosuppressive drugs¹¹¹. Some of the complications of immunosuppressive therapy described after solid-organ transplants are associated with the general health of the recipient and the potential for reactivating pre-existing infections in both the donor and the recipient¹¹², and thus may not be as prevalent in most cases of stem cell-based transplants in PD. Nonetheless, the potential burden of immunosuppression on graft recipients has led to the desire to limit its administration, and this needs to be balanced with predicted benefits for engrafted cell survival and function. In the first clinical studies using PSCs, there will be opportunities to learn and thus refine the optimal role of immunosuppression regimens.

Similarly to fetal VM cells, hPSC-derived cells are at risk of rejection because although their expression of human leukocyte antigen (HLA) antigen is initially low, it increases after differentiation both in vitro and in vivo. The first clinical trials of allogeneic hPSC transplants will therefore incorporate a transient immunosuppressive regimen⁸. However, removing the need for burdensome and expensive immunosuppressive treatment would be highly desirable in the future and may be achieved by autologous grafts — that is, using cells derived from the recipient — thus ensuring immune compatibility and minimizing the risk of transplant rejection. Although patient-specific hESCs can be generated through nuclear transfer¹¹³, it was the discovery of hiPSCs in 2007 (REF.⁶⁸) that made the potential generation of HLA-matched and patient-specific hiPSCs for therapy

Lewy body

Abnormal intracytoplasmic protein aggregates occurring in neurons in certain neurodegenerative disorders, including Parkinson disease.

a tangible target. This approach has proved successful in animal models of PD and is of intense interest for further clinical development⁸².

Within 10 years of their discovery, the first hiPSC lines have already entered clinical trials for age-related macular degeneration¹¹⁴ and PD^{92,93}. These trials are investigating the use of allogeneic grafts from donor iPSC lines that were carefully characterized before transplantation and then delivered to all patients in the trial. An alternative being actively explored is to make ‘banks’ of hiPSCs from individuals whose genetic HLA profiles make their cells more compatible for use in non-related recipients; these donors are so-called super donors. Most super donors are individuals who have blood group O and are homozygous at HLA loci, meaning that their cells can be tolerated with matching of just one HLA of the recipient. Such banks are currently being established in Japan, Europe, China and

the USA¹¹⁵ and could become a supply of ‘off-the-shelf’ cells for a wide range of individuals who could benefit from cell therapies. However, it is thought that despite HLA matching based on the major HLA proteins, immunosuppression may still be beneficial⁸⁴. It is as yet unknown whether and how much outcomes could be improved by matching specific minor HLA antigens, but more extended matching would render the super donor approach more challenging logistically. Moreover, super donors are extremely rare, and calculations show that the 50 highest-ranked homozygous HLA-A, HLA-B and HLA-DR types cumulatively provide an HLA match for only 79% of the 10,000 potential recipients of these iPSC-derived cells in the UK¹¹⁶.

An alternative strategy that is being actively pursued as an alternative to donor–recipient matching is to generate hPSCs that evade recognition by the immune system. With modern genetic engineering and gene-editing techniques^{117,118}, strategies to target β_2 -microglobulin (which is essential for surface expression of HLA class I proteins), to overexpress non-canonical HLA-G (which mediates fetomaternal tolerance) or to co-express immunosuppressive molecules are being actively considered, among others. In principle, such approaches could potentially be used to make generic, ‘one-type-fits-all’ donor cells with global compliance. The cell derivatives would be cryopreserved, allowing extensive safety and efficacy testing of exactly the same cells in preclinical models before clinical use in an economically and practically feasible manner (FIG. 3). However, the use of such cells raises additional safety concerns, as they would be able to evade immune surveillance. Their use would require development of tight safety checkpoints so that grafted cells can be effectively eliminated if any cellular transformation or unwanted proliferation is detected. The most common systems for this today are based on the expression of suicide genes such as the herpes simplex virus thymidine kinase gene, which can be activated by the FDA-approved thymidine kinase-targeting drug ganciclovir^{119–121}.

Pathology in grafted cells

Post-mortem analysis of patients who have received fetal VM grafts has shown evidence of Lewy body pathology in the transplanted cells in some patients^{48,122}, leading to the hypothesis that pathology may spread from host to graft¹²³. However, such pathology has not been observed in all studies^{49,54}, and in the patients in whom pathology was observed, it affected only a small percentage of the DA cells^{47,124} and has not been directly linked to diminished graft function. Nevertheless, it is possible that the appearance of pathology in the transplant may compromise the function of the graft over time. As the field develops, the demands for transplants with longer-lasting effects will be greater, especially in those patients who are younger and/or whose disease is at an earlier phase^{51,52}. A more detailed understanding of the processes involved will help to predict whether autologous grafts (which are envisioned to combat graft rejection; see earlier) may in theory risk accelerated pathology in some cases. It cannot be ruled out that cells sourced from individuals with PD may be more vulnerable to

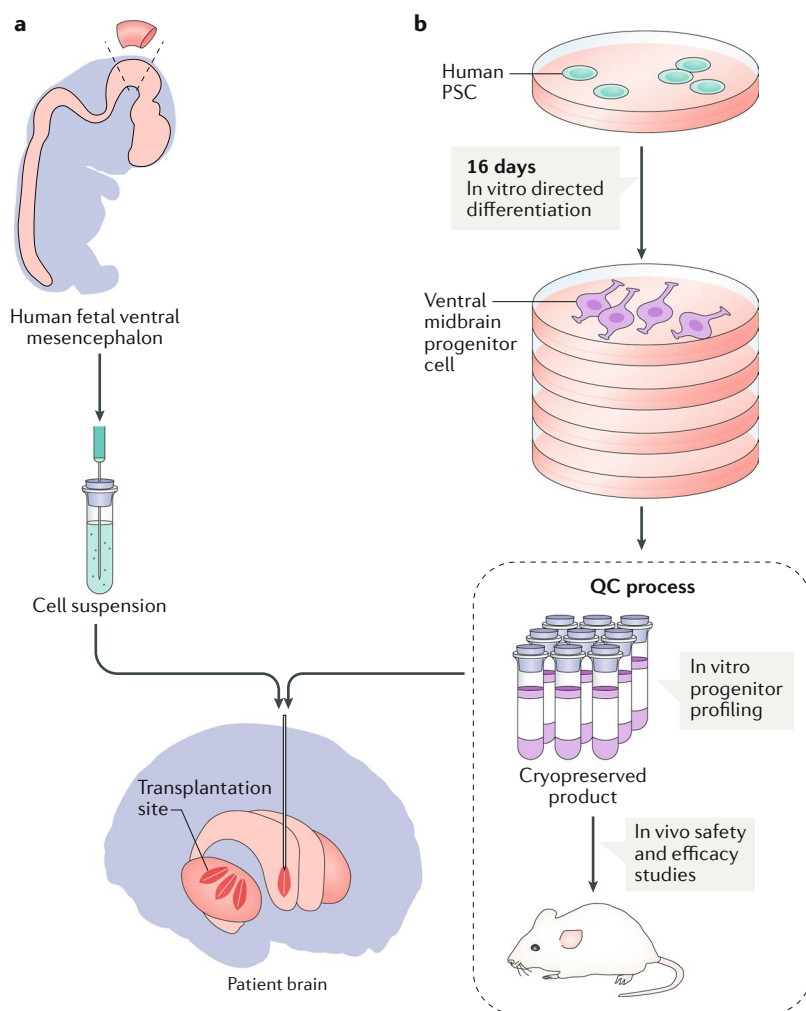


Fig. 3 | Advantages of PSCs versus fetal ventral mesencephalon tissue as a cell source for transplantation. a | Fetal cells can be isolated from the developing ventral mesencephalon and directly transplanted into the brain of patients with Parkinson disease. **b** | The use of pluripotent stem cells (PSCs) is currently being developed as a therapeutic approach that allows large-scale manufacturing and cryopreservation, with robust quality control (QC). The process takes as little as 16 days, and results in hundreds of patient doses that can be cryopreserved, which allows extensive in vitro and in vivo testing for safety and efficacy before clinical transplantation.

Autophagy

A complex intracellular process that uses hydrolytic enzymes in the lysosomes to degrade modified or damaged macromolecules and organelles.

Corridor test

A drug-free behavioural test of lateralized neglect in animals, which is sensitive to unilateral dopamine-denervating lesions and subsequent graft-derived striatal dopaminergic replacement.

Dysosmia

Distortion in sense of smell.

the pathological environment than cells from healthy donors, especially over long time frames. Supporting this concept are multiple studies in which cells derived from patients' iPSCs have disturbances in cellular processes relevant to PD pathophysiology^{125–131}. This concern extends beyond monogenetic forms of the disease, as disease-related pathology such as defects in mitophagy and autophagy as well as epigenomic and transcriptomic alterations have been detected also in DA neurons derived from people with sporadic PD^{132–134}.

From cell replacement to circuits

A key requirement for cell-replacement therapy to work is that engrafted neurons connect to resident neuronal networks, thereby reconstructing damaged circuits or establishing alternative circuitries that can compensate for the functional deficits elicited by neurodegeneration.

Studies in rodents using allografted fetal VM demonstrate synapse formation from host to graft and from graft to host^{135,136}. Novel technologies have made the assessment of synaptic integration of hPSC-derived neurons more accessible. For example, retrograde tracing based on modified rabies virus has allowed monosynaptic connections of afferent neurons to and from the graft to be mapped in the 6-hydroxydopamine rat model of PD, and revealed that the host circuitry, specifically nuclei of the intact mesDA system, formed appropriate synaptic contacts with grafted hPSC-derived neurons^{97,137}. Using the same experimental design, another study demonstrated that grafted neurons also formed synaptic contacts with host circuitry, namely with surrounding striatal medium spiny neurons (the main synaptic targets of A9 mesDA neurons that are associated with motor function) and neurons of the medial prefrontal cortex that are normally targeted by A10 DA neurons¹³⁸. These studies demonstrate that synaptic integration between host and transplanted neurons is a dynamic process, starting as early as 6 weeks after transplantation and maintained for at least 6 months, that occurs between reconstructed physiologically relevant circuits.

Functional connectivity in stem cell-derived grafts has been investigated with use of optogenetic and chemogenetic techniques that allow transplanted neurons to be switched 'on' or 'off' in vivo. In the 6-hydroxydopamine mouse model of PD, optogenetic inhibition of transplanted hESC-derived cells recreated pretransplantation deficits in a spontaneous behavioural task, the corridor test, thus demonstrating that the behavioural recovery is indeed mediated via the transplanted cells⁸⁵. In another study using the same PD mouse model, chemogenetic excitation and inhibition of implanted hPSC-derived cells controlled graft function and in turn modulated drug-induced and spontaneous behaviours³⁰. Thus, the activity of hPSC-derived mesDA neurons can be manipulated in vivo to test their interactions with host motor-behaviour circuits and their effects on functional recovery.

Extranigral pathology in PD

Over time, most individuals with PD develop pathology at various CNS and non-CNS sites, leading to levodopa-unresponsive motor symptoms as well as non-motor symptoms. Frequent falls and freezing of gait are motor

symptoms that often do not respond to levodopa, and thus are not likely to be ameliorated by DA neuron replacement. Non-motor symptoms such as dementia, psychosis and some sleep disturbances are also not likely to be substantially ameliorated by replacing DA neurons, as these phenomena are thought to relate, at least in part, to damage to neuronal networks besides those traditionally studied in PD, such as the cholinergic and noradrenergic systems^{11,139}.

Although the precise mechanisms and patterns of the spread of PD pathology are debated, the Braak staging model has been useful in emphasizing the broad reach of α -synuclein pathology and provides some basis for understanding anatomical correlates of the myriad PD signs and symptoms that affect patients^{12,140,141}. Lewy body pathology and loss of neurons in various specific locations have been found to correlate with particular non-motor symptoms¹⁴². This concordance raises the intriguing possibility that specific cell types could be replaced at particular locations to treat specific symptoms in a patient-tailored precision approach. For example, cholinergic pathways are disrupted in many individuals with PD, and this can be associated with slowed gait, falls, cognitive decline, rapid eye movement sleep behaviour disorder and dysosmia. Cholinergic dysfunction and, in particular, loss of cholinergic neurons in the nucleus basalis of Meynert have been implicated in PD dementia¹⁴³, and has therefore been recently targeted, albeit with limited effects, in initial attempts to treat PD dementia using DBS¹⁴⁴. Combination cell-replacement therapy including stem cell-derived cholinergic neurons could therefore be tested in the future to potentially help manage these aspects of PD. Similarly, multiple types of neurons in the pedunculopontine nucleus are affected in PD, including GABAergic neurons. This structure is under investigation as a potential target for DBS as it seems to be important for gait control¹⁴⁵.

Recent advances in our understanding of the networks affected in PD have highlighted their complexity, but compared with the DAergic pathways, the networks involved in non-levodopa-responsive systems are poorly described. It is therefore likely that an optimal effect requires greater characterization of the precise networks affected in each patient and a tailored combination of cells delivered to the relevant locations.

Conclusions

Over the past few decades, rapid advances in stem cell technology, including development of robust differentiation protocols and manufacturing processes, have facilitated the development of a first generation of hPSC-derived DA neuron technologies that are now in the pipeline for first-in-human clinical trials (FIG. 1b). Transplantation of hPSC-derived DAergic neuron precursors to the striatum — the site of DA loss in PD — is predicted to generate more robust and consistent outcomes than previously tested regenerative therapies using fetal VM tissue. If such transplants do alleviate motor deficits, cell-replacement therapies could conceivably be highly competitive in the current and pipeline therapeutic landscape, alongside continuous infusion therapies, surgical interventions such as DBS

and magnetic resonance-guided focused ultrasound ablation, and gene therapy.

However, this approach is not likely to have an effect on symptoms related to extranigral pathology. Extranigral networks could be ‘pseudonormalized’ by delivering cells from other lineages, but such combination cell therapies seem farther away than DA neuron-based approaches. For cell therapy to be optimized, effective and clinically relevant for a wider range of symptoms, key limitations must be addressed in the future using emerging technologies and new disease insights: as trials progress, optimal and probably individualized dosing and spatial delivery schemes, possibly based on PET biomarkers that quantify and map out existing DA inputs, will improve. In addition, adjunct interventions to increase cell survival, enhance physiological synaptogenesis and promote development of ‘normal’ neuronal controls on the engrafted cells are likely to be put in place. This could be attempted, for

example, using gene modification to express neurotrophic or other factors, or by simultaneous delivery of adjunctive therapeutics. The effects of host tissue on the grafts, including potential spread of pathology and the role of inflammation, will need to be defined in these new cell-based interventions. When patient-derived cells are used, the risk of inherent pathology in the cells also needs to be taken into consideration. However, the true potential of stem cell-based therapeutics in PD may lie in the ability to manipulate the donor cells; for example, in enhancing resistance to pathology, or engineering the cells to deliver disease-modifying or neuroprotective products besides DA. In summary, although we are not yet looking at a disease-modifying treatment, nor a cure, stem cell technologies have the potential to be at the forefront of such PD treatments in the future.

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1. Henchcliffe, C. & Parmar, M. Repairing the brain: cell replacement using stem cell-based technologies. *J. Parkinsons Dis.* **8**, S131–S137 (2018).
2. Barker, R. A., Barrett, J., Mason, S. L. & Bjorklund, A. Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. *Lancet Neurol.* **12**, 84–91 (2013).
3. **A systematic review of transplantation trials using human fetal tissue and that includes critical reappraisal of data from the clinical trials.**
4. Kirkeby, A. et al. Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. *Cell Rep.* **1**, 703–714 (2012).
5. Doi, D. et al. Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. *Stem Cell Rep.* **2**, 337–350 (2014).
6. Nolbrant, S., Heuer, A., Parmar, M. & Kirkeby, A. Generation of high-purity human ventral midbrain dopaminergic progenitors for in vitro maturation and intracerebral transplantation. *Nat. Protoc.* **12**, 1962–1979 (2017).
7. Grealish, S. et al. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. *Cell Stem Cell* **15**, 653–665 (2014).
8. **Side-by-side comparison of hESC-derived and fetal VM grafts in a preclinical model of PD demonstrated that stem cell-derived DA neurons show similar subtype-specific maturation, targeted innervation and functional potency to fetal cells.**
9. Barker, R. A., Parmar, M., Studer, L. & Takahashi, J. Human trials of stem cell-derived dopamine neurons for Parkinson's disease: dawn of a new era. *Cell Stem Cell* **21**, 569–573 (2017).
10. Kalia, L. V. & Lang, A. E. Parkinson's disease. *Lancet* **386**, 896–912 (2015).
11. Pagano, G. & Politis, M. Molecular imaging of the serotonergic system in Parkinson's disease. *Int. Rev. Neurobiol.* **141**, 173–210 (2018).
12. Bohnen, N. I., Kanel, P. & Muller, M. Molecular imaging of the cholinergic system in Parkinson's disease. *Int. Rev. Neurobiol.* **141**, 211–250 (2018).
13. Johnson, M. E., Stecher, B., Labrie, V., Brundin, L. & Brundin, P. Triggers, facilitators, and aggravators: redefining Parkinson's disease pathogenesis. *Trends Neurosci.* **42**, 4–13 (2019).
14. Bronstein, J. et al. Meeting report: consensus statement-Parkinson's disease and the environment: collaborative on health and the environment and Parkinson's action network (CHE PAN) conference 26–28 June 2007. *Env. Health Perspect.* **117**, 117–121 (2009).
15. Sampson, T. The impact of indigenous microbes on Parkinson's disease. *Neurobiol. Dis.* <https://doi.org/10.1016/j.nbd.2019.03.014> (2019).
16. Billingsley, K. J., Bandres-Ciga, S., Saez-Atienzar, S. & Singleton, A. B. Genetic risk factors in Parkinson's disease. *Cell Tissue Res.* **373**, 9–20 (2018).
17. Burack, M. A. et al. In vivo amyloid imaging in autopsy-confirmed Parkinson disease with dementia. *Neurology* **74**, 77–84 (2010).
18. Coughlin, D. et al. Cognitive and pathological influences of tau pathology in Lewy body disorders. *Ann. Neurol.* **85**, 259–271 (2019).
19. Trinh, J. et al. Genotype-phenotype relations for the Parkinson's disease genes SNCA, LRRK2, VPS35: MDSGene systematic review. *Mov. Disord.* **33**, 1857–1870 (2018).
20. Picconi, B., Hernandez, L. F., Obeso, J. A. & Calabresi, P. Motor complications in Parkinson's disease: striatal molecular and electrophysiological mechanisms of dyskinesias. *Mov. Disord.* **33**, 867–876 (2018).
21. Connolly, B. S. & Lang, A. E. Pharmacological treatment of Parkinson disease: a review. *JAMA* **311**, 1670–1683 (2014).
22. Zeuner, K. E., Schaffer, E., Hopfner, F., Bruggemann, N. & Berg, D. Progress of pharmacological approaches in Parkinson's disease. *Clin. Pharmacol. Ther.* **105**, 1106–1120 (2019).
23. Martinez-Fernandez, R. et al. Focused ultrasound subthalamotomy in patients with asymmetric Parkinson's disease: a pilot study. *Lancet Neurol.* **17**, 54–63 (2018).
24. LeWitt, P. A. et al. AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. *Lancet Neurol.* **10**, 309–319 (2011).
25. Kirik, D., Cederfjall, E., Halliday, G. & Petersen, A. Gene therapy for Parkinson's disease: disease modification by GDNF family of ligands. *Neurobiol. Dis.* **97**, 179–188 (2017).
26. Palfi, S. et al. Long-term follow-up of a phase I/II study of ProSavin, a lentiviral vector gene therapy for Parkinson's disease. *Hum. Gene Ther. Clin. Dev.* **29**, 148–155 (2018).
27. Palfi, S. et al. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. *Lancet* **385**, 1138–1146 (2014).
28. Axelsen, T. M. & Woldbye, D. P. D. Gene therapy for Parkinson's disease, an update. *J. Parkinsons Dis.* **8**, 195–215 (2018).
29. Parmar, M. Towards stem cell based therapies for Parkinson's disease. *Development* **145**, dev156117 (2018).
30. Lindvall, O. et al. Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch. Neurol.* **46**, 615–631 (1989).
31. **The first description of stereotaxic implantation of human embryonic ventral mesencephalic cells in two individuals with PD. This open-label study supported further clinical transplantation trials in PD.**
32. Lindvall, O. et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* **247**, 574–577 (1990).
33. Lindvall, O. et al. Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putamen implants. *Ann. Neurol.* **31**, 155–165 (1992).
34. Freed, C. R. et al. Transplantation of human fetal dopamine cells for Parkinson's disease. Results at 1 year. *Arch. Neurol.* **47**, 505–512 (1990).
35. Freed, C. R. et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N. Engl. J. Med.* **327**, 1549–1555 (1992).
36. Molina, H. et al. Computer assisted CT-guided stereotactic transplantation of foetal ventral mesencephalon to the caudate nucleus and putamen in Parkinson's disease. *Acta Neurochir. Suppl.* **58**, 17–19 (1993).
37. Spencer, D. D. et al. Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease. *N. Engl. J. Med.* **327**, 1541–1548 (1992).
38. Peschanski, M. et al. Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalon. *Brain* **117**, 487–499 (1994).
39. Mendez, I. et al. Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study. Report of three cases. *J. Neurosurg.* **96**, 589–596 (2002).
40. Goetz, C. G. et al. United Parkinson foundation neurotransplantation registry on adrenal medullary transplants: presurgical, and 1- and 2-year follow-up. *Neurology* **41**, 1719–1722 (1991).
41. Minguez-Castellanos, A. et al. Carotid body autotransplantation in Parkinson disease: a clinical and positron emission tomography study. *J. Neurol. Neurosurg. Psychiatry* **78**, 825–831 (2007).
42. Gross, R. E. et al. Intrastriatal transplantation of microcarrier-bound human retinal pigment epithelial cells versus sham surgery in patients with advanced Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol.* **10**, 509–519 (2011).
43. Farag, E. S., Vinters, H. V. & Bronstein, J. Pathologic findings in retinal pigment epithelial cell implantation for Parkinson disease. *Neurology* **73**, 1095–1102 (2009).
44. Stoddard, S. L. et al. Decreased adrenal medullary catecholamines in adrenal transplanted parkinsonian patients compared to nephrectomy patients. *Exp. Neurol.* **104**, 218–222 (1989).
45. Wenning, G. K. et al. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann. Neurol.* **42**, 95–107 (1997).

44. Hagell, P. et al. Sequential bilateral transplantation in Parkinson's disease: effects of the second graft. *Brain* **122**, 1121–1132 (1999).
45. Brundin, P. et al. Bilateral caudate and putamen grafts of embryonic mesencephalic tissue treated with lazarets in Parkinson's disease. *Brain* **123**, 1380–1390 (2000).
46. Mendez, I. et al. Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. *Brain* **128**, 1498–1510 (2005).
47. Li, W. et al. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. *Proc. Natl Acad. Sci. USA* **113**, 6544–6549 (2016).
Post-mortem analysis of a transplant patient 24 years after grafting showing a dense graft-derived DAergic reinnervation of the putamen and providing important proof that graft integrity can be maintained long term in a pathological environment.
48. Li, J. Y. et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat. Med.* **14**, 501–503 (2008).
The first report of α -synuclein-positive Lewy bodies in a subset of grafted neurons in two patients assessed after death, which, together with Kordower et al. (2008), provides the first evidence that the PD-like pathology can develop in the grafted cells. These observations also led to the still controversial theory that misfolded proteins may spread via a prion-like mechanism.
49. Hallett, P. J. et al. Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. *Cell Rep.* **7**, 1755–1761 (2014).
50. Kordower, J. H. et al. Fetal nigral grafts survive and mediate clinical benefit in a patient with Parkinson's disease. *Mov. Disord.* **13**, 385–393 (1998).
51. Freed, C. R. et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **344**, 710–719 (2001).
52. Olanow, C. W. et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann. Neurol.* **54**, 403–414 (2003).
53. Barker, R. A., Drouin-Ouellet, J. & Parmar, M. Cell-based therapies for Parkinson disease—past insights and future potential. *Nat. Rev. Neurol.* **11**, 492–503 (2015).
54. Mendez, I. et al. Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years. *Nat. Med.* **14**, 507–509 (2008).
55. Kefalopoulou, Z. et al. Long-term clinical outcome of fetal cell transplantation for Parkinson disease: two case reports. *JAMA Neurol.* **71**, 83–87 (2014).
Long-term (15 and 18 years) follow-up of patients who received human fetal tissue transplants demonstrating sustained DA release and long-term motor improvements.
56. Ma, Y. et al. Dopamine cell implantation in Parkinson's disease: long-term clinical and [¹⁸F]FDOPA PET outcomes. *J. Nucl. Med.* **51**, 7–15 (2010).
57. Piccini, P. et al. Factors affecting the clinical outcome after neural transplantation in Parkinson's disease. *Brain* **128**, 2977–2986 (2005).
58. Freeman, T. B. et al. Bilateral fetal nigral transplantation into the postcommisural putamen in Parkinson's disease. *Ann. Neurol.* **38**, 379–388 (1995).
59. Kordower, J. H. et al. Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural, and metabolic studies. *J. Comp. Neurol.* **370**, 203–230 (1996).
60. Liu, G. et al. Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann. Neurol.* **80**, 674–685 (2016).
61. Alcalay, R. N. et al. Cognitive performance of GBA mutation carriers with early-onset PD: the CORE-PD study. *Neurology* **78**, 1434–1440 (2012).
62. Angeli, A. et al. Genotype and phenotype in Parkinson's disease: lessons in heterogeneity from deep brain stimulation. *Mov. Disord.* **28**, 1370–1375 (2013).
63. Riboldi, G. M. & Di Fonzo, A. B. GBA, Gaucher disease, and Parkinson's disease: from genetic to clinic to new therapeutic approaches. *Cells* **8**, E364 (2019).
64. West, A. B. Achieving neuroprotection with LRRK2 kinase inhibitors in Parkinson disease. *Exp. Neurol.* **298**, 236–245 (2017).
65. Sassone, J., Valtorta, F. & Ciammola, A. Early dyskinesias in Parkinson's disease patients with parkin mutation: a primary corticostriatal synaptopathy? *Front. Neurosci.* **13**, 273 (2019).
66. Barker, R. A., TRANSEURO consortium. Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nat. Med.* **25**, 1045–1053 (2019).
Description of the rationale for the design of the currently ongoing TRANSEURO trial with critical perspectives on what is to be considered when designing future stem cell-based trials.
67. Thomson, J. A. et al. Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147 (1998).
68. Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872 (2007).
69. Reubinoff, B. E. et al. Neural progenitors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1134–1140 (2001).
70. Zhang, S. C., Wernig, M., Duncan, I. D., Brustle, O. & Thomson, J. A. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1129–1133 (2001).
71. Tao, Y. & Zhang, S. C. Neural subtype specification from human pluripotent stem cells. *Cell Stem Cell* **19**, 573–586 (2016).
72. Roy, N. S. et al. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat. Med.* **12**, 1259–1268 (2006).
73. Vazin, T., Chen, J., Lee, C. T., Amable, R. & Freed, W. J. Assessment of stromal-derived inducing activity in the generation of dopaminergic neurons from human embryonic stem cells. *Stem Cell* **26**, 1517–1525 (2008).
74. Smith, J. R. et al. Inhibition of activin/nodal signaling promotes specification of human embryonic stem cells into neuroectoderm. *Dev. Biol.* **313**, 107–117 (2008).
75. Hargus, G. et al. Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc. Natl Acad. Sci. USA* **107**, 15921–15926 (2010).
76. Chambers, S. M. et al. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* **27**, 275–280 (2009).
Demonstration of rapid, synchronized and efficient neuralization of iPSCs with dual-SMAD inhibition.
77. Studer, L. Strategies for bringing stem cell-derived dopamine neurons to the clinic—the NYSTEM trial. *Prog. Brain Res.* **230**, 191–212 (2017).
78. Steinbeck, J. A. & Studer, L. Moving stem cells to the clinic: potential and limitations for brain repair. *Neuron* **86**, 187–206 (2015).
79. Kirkeby, A. et al. Predictive markers guide differentiation to improve graft outcome in clinical translation of hESC-based therapy for Parkinson's disease. *Cell Stem Cell* **20**, 135–148 (2017).
80. Chen, Y. et al. Chemical control of grafted human PSC-derived neurons in a mouse model of Parkinson's disease. *Cell Stem Cell* **18**, 817–826 (2016).
81. Kikuchi, T. et al. Human iPSC cell-derived dopaminergic neurons function in a primate Parkinson's disease model. *Nature* **548**, 592–596 (2017).
82. Hallett, P. J. et al. Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. *Cell Stem Cell* **16**, 269–274 (2015).
The first demonstration of long-term survival and functional efficacy of autologous iPSC-derived DA neurons in a non-human primate model of PD.
83. Wakeman, D. R. et al. Cryopreservation maintains functionality of human iPSC dopamine neurons and rescues Parkinsonian phenotypes in vivo. *Stem Cell Rep.* **9**, 149–161 (2017).
84. Morizane, A. et al. MHC matching improves engraftment of iPSC-derived neurons in non-human primates. *Nat. Commun.* **8**, 385 (2017).
This investigation of the effects of MHC-matched allograft and immunosuppression in non-human primates finds that MHC-matched iPSC-derived neurons provide better engraftment in the brain, with a lower immune response and higher survival of the transplanted neurons.
85. Steinbeck, J. A. et al. Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. *Nat. Biotechnol.* **33**, 204–209 (2015).
86. Sundberg, M. et al. Improved cell therapy protocols for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC-, and non-human primate iPSC-derived dopaminergic neurons. *Stem Cell* **31**, 1548–1562 (2013).
87. Cardoso, T. et al. Target-specific forebrain projections and appropriate synaptic inputs of hESC-derived dopamine neurons grafted to the midbrain of parkinsonian rats. *J. Comp. Neurol.* **526**, 2133–2146 (2018).
88. Merkle, F. T. et al. Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations. *Nature* **545**, 229–233 (2017).
89. Garitaonandia, I. et al. Increased risk of genetic and epigenetic instability in human embryonic stem cells associated with specific culture conditions. *PLoS One* **10**, e0118307 (2015).
90. Amir, H. et al. Spontaneous single-copy loss of TP53 in human embryonic stem cells markedly increases cell proliferation and survival. *Stem Cell* **35**, 872–885 (2017).
91. Kirkeby, A., Parmar, M. & Barker, R. A. Strategies for bringing stem cell-derived dopamine neurons to the clinic: a European approach (STEM-PD). *Prog. Brain Res.* **230**, 165–190 (2017).
92. Takahashi, J. Strategies for bringing stem cell-derived dopamine neurons to the clinic: the Kyoto trial. *Prog. Brain Res.* **230**, 213–226 (2017).
93. Cyranoski, D. 'Reprogrammed' stem cells implanted into patient with Parkinson's disease. *Nature* <https://doi.org/10.1038/d41586-018-07407-9> (2018).
94. Steinbeck, J. A. et al. Functional connectivity under optogenetic control allows modeling of human neuromuscular disease. *Cell Stem Cell* **18**, 134–143 (2016).
95. Calabresi, P., Picconi, B., Tozzi, A., Ghiglieri, V. & Di Filippo, M. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat. Neurosci.* **17**, 1022–1030 (2014).
96. Lane, E. L., Bjorklund, A., Dunnett, S. B. & Winkler, C. Neural grafting in Parkinson's disease unraveling the mechanisms underlying graft-induced dyskinesia. *Prog. Brain Res.* **184**, 295–309 (2010).
97. Politis, M. et al. Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. *Sci. Transl. Med.* **2**, 38ra46 (2010).
98. Politis, M. et al. Graft-induced dyskinesias in Parkinson's disease: high striatal serotonin/dopamine transporter ratio. *Mov. Disord.* **26**, 1997–2003 (2011).
99. Marklund, U. et al. Detailed expression analysis of regulatory genes in the early developing human neural tube. *Stem Cell Dev.* **23**, 5–15 (2014).
100. Erola, T. et al. Bilateral subthalamic nucleus stimulation improves health-related quality of life in Parkinsonian patients. *Parkinsonism Relat. Disord.* **11**, 89–94 (2005).
101. Rodrigues, J. P., Walters, S. E., Watson, P., Stell, R. & Mastaglia, F. L. Globus pallidus stimulation improves both motor and nonmotor aspects of quality of life in advanced Parkinson's disease. *Mov. Disord.* **22**, 1866–1870 (2007).
102. Kharkar, S. et al. Changes in Parkinson's disease sleep symptoms and daytime somnolence after bilateral subthalamic deep brain stimulation in Parkinson's disease. *NPJ Parkinsons Dis.* **4**, 16 (2018).
103. Yang, L. et al. Evaluating glymphatic pathway function utilizing clinically relevant intrathecal infusion of CSF tracer. *J. Transl. Med.* **11**, 107 (2013).
104. Louveau, A. et al. Structural and functional features of central nervous system lymphatic vessels. *Nature* **523**, 337–341 (2015).
105. Daneman, R. The blood-brain barrier in health and disease. *Ann. Neurol.* **72**, 648–672 (2012).
106. Sayles, M., Jain, M. & Barker, R. A. The cellular repair of the brain in Parkinson's disease—past, present and future. *Transp. Immunol.* **12**, 321–342 (2004).
107. Kim, J. W. et al. Acute brain reaction to DBS electrodes after deep brain stimulation: chronological observation. *Acta Neurochir.* **155**, 2365–2371; discussion 2371 (2013).
108. Cabezas, R. et al. Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. *Front. Cell Neurosci.* **8**, 211 (2014).
109. Lindvall, O. et al. In reply: fetal brain grafts and Parkinson's disease. *Science* **250**, 1435 (1990).
110. Ponticelli, C. & Glasscock, R. J. Prevention of complications from use of conventional immunosuppressants: a critical review. *J. Nephrol.* **32**, 851–870 (2019).
111. Yadav, D. K., Bai, X. L. & Liang, T. Dermatological disorders following liver transplantation: an update. *Can. J. Gastroenterol. Hepatol.* **2019**, 9780952 (2019).

112. Green, M. Introduction: infections in solid organ transplantation. *Am. J. Transpl.* **13**(Suppl 4), 3–8 (2013).
113. Matoba, S. & Zhang, Y. Somatic cell nuclear transfer reprogramming: mechanisms and applications. *Cell Stem Cell* **23**, 471–485 (2018).
114. Mandai, M. et al. Autologous induced stem-cell-derived retinal cells for macular degeneration. *N. Engl. J. Med.* **376**, 1038–1046 (2017).
115. Wilmut, I. et al. Development of a global network of induced pluripotent stem cell haplobanks. *Regen. Med.* **10**, 235–238 (2015).
116. Taylor, C. J., Bolton, E. M. & Bradley, J. A. Immunological considerations for embryonic and induced pluripotent stem cell banking. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **366**, 2312–2322 (2011).
117. Brookhouser, N., Raman, S., Potts, C. & Brafman, D. A. May I cut in? Gene editing approaches in human induced pluripotent stem cells. *Cells* **6**, 5 (2017).
118. Ben Jehuda, R., Shemer, Y. & Binah, O. Genome editing in induced pluripotent stem cells using CRISPR/Cas9. *Stem Cell Rev.* **14**, 323–336 (2018).
119. Greco, R. et al. Improving the safety of cell therapy with the TK-suicide gene. *Front. Pharmacol.* **6**, 95 (2015).
120. He, J., Rong, Z., Fu, X. & Xu, Y. A safety checkpoint to eliminate cancer risk of the immune evasive cells derived from human embryonic stem cells. *Stem Cell* **35**, 1154–1161 (2017).
121. Zheng, D., Wang, X. & Xu, R. H. Concise review: one stone for multiple birds: generating universally compatible human embryonic stem cells. *Stem Cell* **34**, 2269–2275 (2016).
122. Kordower, J. H. et al. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat. Med.* **14**, 504–506 (2008).
123. Brundin, P. & Kordower, J. H. Neuropathology in transplants in Parkinson's disease: implications for disease pathogenesis and the future of cell therapy. *Prog. Brain Res.* **200**, 221–241 (2012).
124. Li, J. Y. et al. Characterization of Lewy body pathology in 12- and 16-year-old intrastriatal mesencephalic grafts surviving in a patient with Parkinson's disease. *Mov. Disord.* **25**, 1091–1096 (2010).
125. Bieri, G. et al. LRRK2 modifies alpha-syn pathology and spread in mouse models and human neurons. *Acta Neuropathol.* **137**, 961–980 (2019).
126. Beevers, J. E., Caffrey, T. M. & Wade-Martins, R. Induced pluripotent stem cell (iPSC)-derived dopaminergic models of Parkinson's disease. *Biochem. Soc. Trans.* **41**, 1503–1508 (2013).
127. Chung, S. Y. et al. Parkin and PINK1 patient iPSC-derived midbrain dopamine neurons exhibit mitochondrial dysfunction and alpha-synuclein accumulation. *Stem Cell Rep.* **7**, 664–677 (2016).
128. Devine, M. J. et al. Parkinson's disease induced pluripotent stem cells with triplication of the alpha-synuclein locus. *Nat. Commun.* **2**, 440 (2011).
129. Rakovic, A., Seibler, P. & Klein, C. iPSC models of parkin and PINK1. *Biochem. Soc. Trans.* **43**, 302–307 (2015).
130. Sanders, L. H. et al. LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. *Neurobiol. Dis.* **62**, 381–386 (2014).
131. Zambon, F. et al. Cellular alpha-synuclein pathology is associated with bioenergetic dysfunction in Parkinson's iPSC-derived dopamine neurons. *Hum. Mol. Genet.* **28**, 2001–2013 (2019).
132. Hsieh, C. H. et al. Functional impairment in miro degradation and mitophagy is a shared feature in familial and sporadic Parkinson's disease. *Cell Stem Cell* **19**, 709–724 (2016).
133. Fernandez-Santiago, R. et al. Aberrant epigenome in iPSC-derived dopaminergic neurons from Parkinson's disease patients. *EMBO Mol. Med.* **7**, 1529–1546 (2015).
134. Sanchez-Danes, A. et al. Disease-specific phenotypes in dopamine neurons from human iPSC-based models of genetic and sporadic Parkinson's disease. *EMBO Mol. Med.* **4**, 380–395 (2012).
135. Freund, T. F. et al. Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host neostriatum: a tyrosine hydroxylase immunocytochemical study. *J. Neurosci.* **5**, 603–616 (1985).
136. Bolam, J. P., Freund, T. F., Bjorklund, A., Dunnett, S. B. & Smith, A. D. Synaptic input and local output of dopaminergic neurons in grafts that functionally reinnervate the host neostriatum. *Exp. Brain Res.* **68**, 131–146 (1987).
137. Adler, A. F. et al. hESC-derived dopaminergic transplants integrate into basal ganglia circuitry in a preclinical model of Parkinson's disease. *Cell Rep.* **28**, 3462–3473.e5 (2019).
- This study uses rabies-based monosynaptic tracing to provide a refined understanding of how graft neurons integrate with host circuitry. It shows that DAergic grafts transplanted in a long-term preclinical rat model of PD receive synaptic input from subtypes of host cortical, striatal and pallidal neurons that are known to regulate the function of endogenous nigral DA neurons.**
138. Grealish, S. et al. Monosynaptic tracing using modified rabies virus reveals early and extensive circuit integration of human embryonic stem cell-derived neurons. *Stem Cell Rep.* **4**, 975–983 (2015).
139. Nahimi, A. et al. Noradrenergic deficits in Parkinson disease imaged with ¹¹C-MeNER. *J. Nucl. Med.* **59**, 659–664 (2018).
140. Braak, H. et al. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* **24**, 197–211 (2003).
141. Surmeier, D. J., Obeso, J. A. & Halliday, G. M. Parkinson's disease is not simply a prion disorder. *J. Neurosci.* **37**, 9799–9807 (2017).
142. Hall, H. et al. Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. *Brain* **137**, 2493–2508 (2014).
143. Gratwicke, J., Jahanshahi, M. & Foltynie, T. Parkinson's disease dementia: a neural networks perspective. *Brain* **138**, 1454–1476 (2015).
144. Gratwicke, J. et al. Bilateral deep brain stimulation of the nucleus basalis of meynert for Parkinson disease dementia: a randomized clinical trial. *JAMA Neurol.* **75**, 169–178 (2018).
145. Garcia-Rill, E. et al. Local and relayed effects of deep brain stimulation of the pedunculopontine nucleus. *Brain Sci.* **9**, E64 (2019).
146. Mills, K. A. et al. Efficacy and tolerability of antidepressants in Parkinson's disease: a systematic review and network meta-analysis. *Int. J. Geriatr. Psychiatry* **33**, 642–651 (2018).
147. Antonini, A., Moro, E., Godeiro, C. & Reichmann, H. Medical and surgical management of advanced Parkinson's disease. *Mov. Disord.* **33**, 900–908 (2018).
148. Rowland, N. C. et al. Combining cell transplants or gene therapy with deep brain stimulation for Parkinson's disease. *Mov. Disord.* **30**, 190–195 (2015).
149. Rossi, M., Bruno, V., Arena, J., Cammarota, A. & Merello, M. Challenges in PD patient management after DBS: a pragmatic review. *Mov. Disord. Clin. Pract.* **5**, 246–254 (2018).
150. Combs, H. L. et al. Cognition and depression following deep brain stimulation of the subthalamic nucleus and globus pallidus pars internus in Parkinson's disease: a meta-analysis. *Neuropsychol. Rev.* **25**, 439–454 (2015).
151. Bari, A. A., Thum, J., Babayan, D. & Lozano, A. M. Current and expected advances in deep brain stimulation for movement disorders. *Prog. Neuro. Surg.* **33**, 222–229 (2018).
152. Neumann, W. J. et al. Toward electrophysiology-based intelligent adaptive deep brain stimulation for movement disorders. *Neurotherapeutics* **16**, 105–118 (2019).
153. Bjorklund, T., Cederfjall, E. A. & Kirik, D. Gene therapy for dopamine replacement. *Prog. Brain Res.* **184**, 221–235 (2010).
154. Lindvall, O. & Bjorklund, A. Cell therapy in Parkinson's disease. *NeuroRx* **1**, 382–393 (2004).
155. Brundin, P. et al. Survival and function of dissociated rat dopamine neurons grafted at different developmental stages or after being cultured in vitro. *Brain Res.* **467**, 233–243 (1988).
156. Kelly, C. M. et al. Medical terminations of pregnancy: a viable source of tissue for cell replacement therapy for neurodegenerative disorders. *Cell Transpl.* **20**, 503–513 (2011).
157. Lehnen, D. et al. IAP-based cell sorting results in homogeneous transplantable dopaminergic precursor cells derived from human pluripotent stem cells. *Stem Cell Rep.* **9**, 1207–1220 (2017).
158. Daley, G. O. et al. Setting global standards for stem cell research and clinical translation: the 2016 ISSCR guidelines. *Stem Cell Rep.* **6**, 787–797 (2016).
159. Piccirilli, J. F. et al. The changing prevalence of comorbidity across the age spectrum. *Crit. Rev. Oncol. Hematol.* **67**, 124–132 (2008).
160. Cummings, J. L. et al. The role of dopaminergic imaging in patients with symptoms of dopaminergic system neurodegeneration. *Brain* **134**, 3146–3166 (2011).
161. Wang, Y. K. et al. Human clinical-grade parthenogenetic ESC-derived dopaminergic neurons recover locomotive defects of nonhuman primate models of Parkinson's disease. *Stem Cell Rep.* **11**, 171–182 (2018).

Author contributions

The authors all researched data for the article, contributed substantially to discussion of the article's content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests

M.P. is the owner of Parmar Cells AB and co-inventor on the US patent application 15/093,927 owned by Biolamina AB, EP17181588 owned by Miltenyi Biotec and PCT/EP2018/062261 owned by New York Stem Cell Foundation. C.H. has received consultancy fees from US WorldMeds, Adamas Pharmaceuticals, Prevail Therapeutics and Mitsubishi Tanabe Pharma America.

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