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 (PD). However, the outcomes of fetal-derived cell transplants in individuals with PD 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 methodologies. Advances in developmental and stem cell biology have enabled the development of cell replacement therapies that comprise dopamine neurons derived from human pluripotent stem cells (hPSCs), 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 PD features that dopamine cell replacement based on generating neurons from hPSCs 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, whose cell bodies are located within the substantia nigra pars compacta (SNpc) 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 DA neuron precursor cells isolated from the developing human VM could survive and function in graft recipients with PD when surgically delivered to the putamen, the target area of mesDA neurons[2]. However, the use of human fetal VM tissue posed serious limitations (discussed below) that have precluded clinical application of a cell-based therapy until recently1. Now, several notable advances in culturing and differentiating human pluripotent stem cells (hPSCs)[3-6] have enabled the formation of transplantable VM progenitors that mature into DAergic neurons that are virtually indistinguishable from human fetal mesDA neurons with respect to molecular identity, in vivo functionality, potency and target-specific axonal outgrowth[7]. Based on this, 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 DA 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 PD[8]. The GForce-PD network is continuously growing and its last meeting was attended by two US-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 in 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. Further, 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 disorder[9], and that, with the exception of a small number of specific genetically linked cases, is linked to cytoplasmic aggregation of α-synuclein and the formation of Lewy bodies in the DAergic neurons of the SNpc. 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 among individuals with the disease[12-15]. Unsurprisingly, therefore, endophenotypes of PD vary 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

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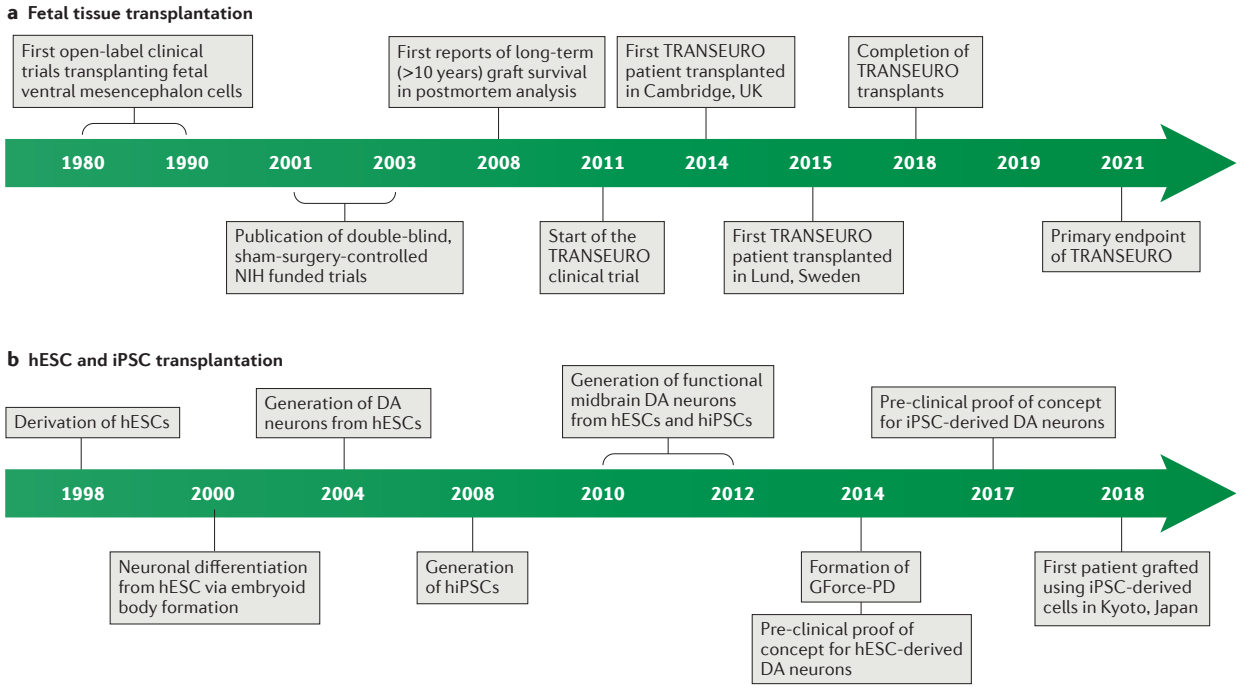

Fig. 1 | **Timeline of developing cell therapies for Parkinson disease. a** | Timeline for the development of fetal tissue transplantation in Parkinson disease (PD). **b** | Time for the development of human embryonic stem cell (hESC) and human induced-pluripotent stem cell (hiPSC)-derived cell transplantation in PD. DA, dopamine.

the heterogeneity in clinical presentations[18] 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 results in complications that include end-of-dose wearing off and levodopa-induced dyskinesia (LID)[19]. 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 pseudo-normalize 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 precisely targeted lesions in similar tracts and structures using magnetic resonance-guided focused ultrasound (MRgFUS) are approved in the US for clinical use for tremor-predominant PD and are also currently in randomized controlled clinical trials[22].

A gene therapy approach has been investigated in a Phase 2 clinical trial, in which viral vectors are surgically delivered by precise local injection to the subthalamic nucleus to induce expression of genes encoding for the synthesis of the inhibitory neurotransmitter GABA, in an attempt to inhibit aberrant neuronal function[23]. Other potential gene therapies that have reached clinical trials focus on either neuroprotection by growth factor delivery[24] 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 deficits[27].

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 has been, and continues to be, the focus of historical and 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 issue 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 souce of DA derived from neuroendocrine chromaffin cells[38], autologous carotid body tissue as a source of DAergic glomus cells[39] and retinal pigmented epithelial cells as a source of levodopa[40, 41]. However, transplants of cells other than authentic fetal VM DA

neurons have failed to demonstrate key requisite properties including robust survival, dopamine release or 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 questioned[42]. 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 their 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, 35, 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 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. Several main parameters that were deemed to have a significant impact related to the cells, the method of implantation, immune suppression and patient selection (extensively reviewed in[53]). 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 outcomes[2]. Other analyses have also identified that younger patients[51] and those with greater levodopa response prior to surgery[52] – have better outcomes post-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[57,58]. Moreover, some studies showed that good clinical effects required sufficient amounts of tissue (minimum 3 fetal VMs per hemisphere), sufficient fibre outgrowth and prevention of graft rejection[50, 52, 59, 60].

Rapid advances in understanding the genetic basis of PD have opened another avenue to potentially define the optimal cohort for cell transplantation. For example, the DA neuron replacement is expected to primarily alleviate motor symptoms. In cases in which predominant features are cognitive impairment and dementia, DA replacement would therefore be of limited benefit. For example, certain GBA mutations are associated with more rapid cognitive decline[61, 62] including after DBS[63]. Moreover, novel approaches targeting GBA[64] and LRRK2, have the potential to provide a widespread therapeutic effect more broadly throughout

Box 1 | **Current therapeutic landscape for Parkinson disease**

Currently, the mainstay of treatment for 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 patients[20]. 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 serotonin system[145]. 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, 146]. In particular, there is a progressive shortening duration of action of levodopa, and 'wearing off' of 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 off'[146], 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 delivery 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; 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: many of the clinically important symptoms respond poorly to current treatments (for example, imbalance and falls, and freezing of gait); as the disease progresses and symptoms worsen, the dosing and dose frequency increase, resulting in complex regimens with increasing adverse events; this 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 measured[147]. 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[148, 149]. Ongoing advances include the use of directional leads for superior field-shaping to improve the benefit:risk ratio, improved programming paradigms and new approaches such as use of adaptive systems[150, 151].

The pipeline

Magnetic resonance-guided focused ultrasound (MRgFUS)[22] 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 synthesis[152] or modulating glutamate production[23]; deliver neuroprotective agents such as glial cell-derived neurotrophic factor or neurturin; or correct underlying genetic deficits[27]. These pipeline approaches hold promise but are early in development; none has undergone a successful phase 3 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 MRgFUS by providing a restorative rather than an ablative intervention.

the nervous system and therefore, if successfully developed, could be preferential[65] On the other hand, individuals with PARK2 gene mutations primarily have a motor phenotype and are highly susceptible to developing LIDs[66], suggesting that early intervention with cell therapy in these patients might be more beneficial.

The cumulative re-analysis 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 TRANSEURO67. This is a European open-label, assessor-blinded multi-centre trial of transplants of fetal VM tissue into individuals with sporadic PD. The transplant patients and their age- 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 change in the Unified PD Rating Scale (UPDRS) 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 published67 and the first report on outcomes in transplanted patients is expected three years after the last transplantation surgery, which took place 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 surgeries were cancelled owing to lack of sufficient or suitable amounts of fetal tissue, resulting in substantial delays, logistic complications and burdensome experiences for the patients[67]. In addition to its low availability, there are multiple other barriers to using 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

Pluripotent stem cells (PSCs) — such as human embryonic stem cells (hESCs), first reported in 1998[68] and induced pluripotent stem cells (iPSCs), first reported in 2007[69] — 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[70, 71]. This makes hPSCs relatively easy to use for the generation of regionally specified neural cell types, and the protocols for such purposes have gradually evolved[72]. 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 hPSCs[73-76], but the purity and yield of DA neurons was highly variable, owing to the unsynchronized and incomplete differenation 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 (TGFβ) and bone morphogenic protein (BMP) signalling pathways[77]. 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[78, 79]. Dual-SMAD inhibition was successfully used to generate mesDA neurons via a floor plate intermediate in 2011[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 (GSKi; which results in activation of the canonical WNT pathway) and/or fibroblast growth factor 8 (FGF8) for sufficient caudalization have been optimized[4, 5, 80-82]. These differentiation strategies, based on deriving mesDA neurons via a developmentally correct mesencephalic floor plate intermediate, resulted in the first grafts with robust in vivo performance, and this positive outcome spurred intensive preclinical evaluation, good manufacturing process (GMP) manufacturing 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 using amphetamine-induced rotation[3, 4]. Moreover, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned non-human primates, transplantation of autologous iPSC-derived DA neurons led to a marked rescue of motor deficits[83]. Several more studies demonstrated the long-term functionality of xenografted human and non-human primate mesDA neurons in rodent models of PD[5, 49, 76, 82, 84-87]. One study7 reported that hESC-derived mesDA neurons became functional by 6 months post-transplantation in the 6-hydroxydopamine (6-OHDA) rat model of PD, as visualized by PET and single photon emission computerized tomography (SPECT), 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 as that achieved using 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[81, 86]. Moreover, homotopic intranigrally grafted neurons showed target-specific innervation of appropriate neuroanatomical structures across distances of at least 10 mm in a rat model of PD[7, 88]. 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[82, 83].

Cumulatively, a large number of transplants of DA neurons derived from PSCs have now been reported in animal models of PD, and neither tumours nor uncontrolled proliferation have been reported from these newer protocols. However, there remains a need for caution as these approaches are translated to the clinic. 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[89-91]. 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

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, standardised manufacturing, the ability to be cryopreserved, and increased purity, allowing for 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 3 fetuses must be collected and transplanted to each hemisphere[153]. Moreover, the cells remain viable for only a short time in hibernation medium[154], meaning that all the material used for grafting one patient must be collected over a short period. The earlier fetal VM transplantation trials used tissue from surgical terminations of pregnancy[2], but now medical (non-surgical) terminations is often used in the clinics[155]. Although this change does not preclude tissue use, as fetal tissue is subject to carefully defined criteria, the collection is more challenging, the embryos often too young, and supply determines surgical transplantation date[67]. By contrast, pluripotent stem cells (hPSC)-derived DA neural progenitors can be produced in near-unlimited numbers, cryopreserved and used on demand.

Standardised manufacturing

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 vary between each patient: The age 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 processing[67]. 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 cells derivatives for transplantation is possible. A hPSC-derived product can be cryopreserved[6, 84], providing enormous advantages over previous approaches. Importantly, cryopreservation would enable 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 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 to humans.

Purity

The major concern with any stem cell-based therapy for PD is the risk of remaining pluripotent stem cells or other proliferative cell populations that can lead to tumours, teratoma formation or neural overgrowth after transplantation. Using stem cell-derived preparations allows for the use of cell purification strategies based on cell surface markers[5,156] and/or a thorough pre-clinical charactierization 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 (GID) that persisted in the absence of levodopa[51, 52]. This unwanted side effect has, at least in part, been linked with with activity of serotonergic neurons in the graft[97, 98]. Using well characterized cells for grafting, as is possible using hPSC-derived cell products, will enable exclusion of this type of cell in the graft, thus minimizing one of the factors associated with the risk of GID. 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 for a greater control of the precise cell doses delivered.

Surgery, dosing and distribution

Transplantable DA neuron progenitors can now be manufactured in near unlimited numbers from stem cells[78, 92, 93]. 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.

precise effects of immunosuppression. Therefore, potential lack of safety and the possibility of tumor 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[78, 92, 93].

Early clinical trial expectations

In 2018, a ground-breaking clinical trial of surgical transplantation of allogeneic hiPSC-derived DA neuron precursors into the putamen of individuals with PD launched in Japan[93, 94]. Further clinical trials of cell transplants in PD are anticipated to start in the near future[8, 78, 80]. 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 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, 82, 83, 88, 95], 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 pathways[96] 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, tremor, 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 a similar outcome as the best patients 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 dopaminergic drugs, consistent with the postulated mechanism of action of the engrafted cells.

Certain symptoms of PD respond less well and variably to dopaminergic medications, including speech difficulties, imbalance, and freezing of gait. In such cases, it is anticipated that response to

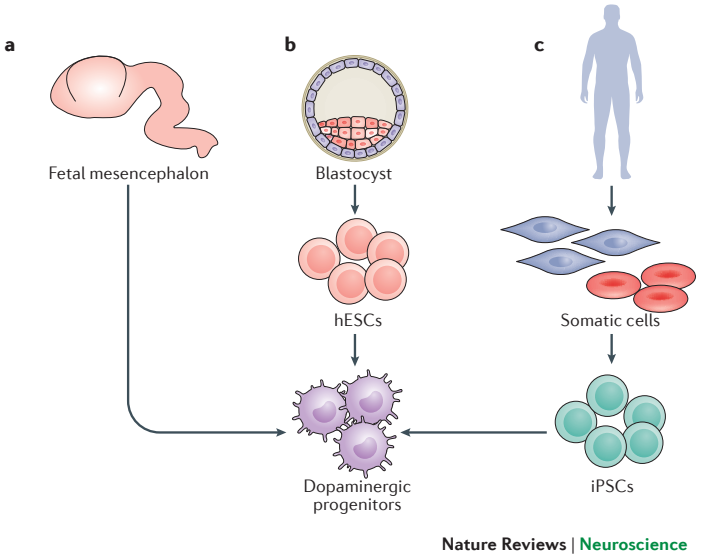

Fig.2 | **Cell sources being trialed for clinical cell replacement therapy in Parkinson disease. a** | Transplantable dopaminergic (DAergic) progenitors isolated from fetal ventral mesencephalon (VM) were first tested in 1980s and are also used in the ongoing TRANSEURO study. **b** | DAergic progenitors with equal functionality and potency can be isolated from human embryonic stem cells (hESCs) that are obtained from the inner cell mass of the pre-implantation 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 similar capacity to that of hESCs to form transplantable DAergic progenitors.

mesDA neuron replacement will reflect, at least partially, response to levodopa. One problem is that some symptoms, such as imbalance, may arise due 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 PD[55], 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 LIDs, 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 DAerigc drugs. However, a potential complicating factor is that GIDs were reported in a varying number of graft recipients in the open label trials and in 15% and 56% 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 serotonergic neurons present in the graft may have a role. Evidence in support of this proposed mechanism has been obtained from studies in grafted PD patients expressing GIDs using PET imaging. The GIDs could also be suppressed with drugs targeting the serotonergic system [97, 98]. The serotonin neurons develop caudal to but in close proximity and sometimes intermingled with the DA neurons[99] and because of this cannot be completely excluded when performing the dissection of fetal tissue. 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 serotonergic neurons is reduced. However it cannot be ruled out that other mechanisms may also contribute to the development GIDs.

One open question is whether the trialled hPSC-derived transplants will affect non-motor symptoms or the progression of the disease. The majority of non-motor symptoms in PD do not respond to levodopa treatment, and therefore might not be expected to experience meaningful improvement 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 benefit indirectly as a result of being able 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 (STN) and the globus pallidus pars interna (GPi) — integral parts of the indirect pathway, which is inhibited by DA inputs that are lost in PD. Interestingly, DBS provides relief not only from the motor symptoms but also from some of the non-motor symptoms and also improves quality of life[100, 101]. For example, the beneficial effects of STN-targeted DBS on sleep[102] 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 dopamine, 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 system[103] and the identifi-

cation of CNS lymphatic vessels connected to the deep cervical lymph nodes[104] have challenged this traditional view[105]. 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[85, 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 innervation[107]. In addition, PD compromises the blood–CNS barrier[108] 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 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 trials [110]. Drug toxicities, infections such as urinary tract infections, sepsis and pneumonia, as well as specific malignancies can be linked to immunosuppressive drugs[111]. 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 recipient[112], 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 what the optimal role of immunosuppression regimes.

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 dopamine (DA) neuron progenitors derived from stem cells (Table 1)[67, 78, 92, 93]. There is a clear rationale with insight into the mechanistic of action of the therapeutic hPSC-derived cells, as is recommended by International Society for Stem Cell Research guidelines for clinical translation[157]. Various aspects of clinical trial design, including the optimal cohort, dosing, outcome measures and follow-up periods should be accordingly defined. Below is a list of design considerations for clinical trials of mesencephalic DA neuron progenitors derived from human embryonic stem cells (hESCs) and induced PSCs. 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 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 under 60 years old[51]).
- In one study, 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 risk of GIDs51.
- Older patients are more likely to have comorbid and confounding disorders and therefore may be at higher risk from surgical intervention in addition to immunosuppressive drugs if used after transplantation[158].

PD stage

Individuals with early PD with localized pathology are predicted to be more likely to benefit67, but 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 favoring 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* (
- Gait*
- Freezing of gait*
- Balance*
- Adverse effects of medications such as dyskinesia, psychosis and orthostasis (indirect effects due to lower dose requirement)

*may vary between individuals, as it varies for levodopa response

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
- Sialorrhea
- 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 graft-induced dyskinesia, 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 duration of immunosuppression)

Secondary and exploratory outcomes

- Graft survival, growth and neurochemical function as reflected by neuroimaging, such as MRI, positron emission tomography using 18F-DOPA (fluorodopa) or DA transporter ligands, and single-photon emission computerized tomography57, 159
- 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

Table 1 | **Academic clinical cell transplantation trials in Parkinson disease**

Trial (NCT number)	Transplantations initiated	Donor cells (cryopreserved product)	Number of transplant recipient (age)	Disease duration	Disease severity	Primary endpoint
TRANSEURO* (0189839)[67]	Completed	Human fetal VM tissue (no)	11 (30–68 years)	2–13	Early to moderate	Efficacy
STEM-PD (NA)[93]	No	hESC-derived mesDA progenitors	8 (<70 years)	5–15	Moderate	Tolerability and feasibility
NYSTEM-PD (NA)[79]	No	hESC-derived mesDA progenitors	10 (45–72 years)	5–15	Severe	Safety, tolerability and feasibility
CiRA (NA)[94]	Yes	hiPSC-derived mesDA progenitors	5–10 (50–69 years)	>5	Severe	Safety and tolerability
Chinease Academy of Sciences (03119636)[160]	Yes	Stem cell-derived neural precursors	50 (50–80 years)	>5	Severe	Safety
Bundang CHA Hospital, Korea (01860794)	No	Human fetal VM neural precursors	15 (18–70 years)	NA	Severe	Safety and tolerability

Information on current and upcoming clinical transplantation trials is based on published data and clinical trial registration information on clinicaltrials.gov.The trials listed above are not blinded. *Trial includes randomization of participants to treatment groups. hESC, human embryonic stem cell; hiPSC, human induced-pluripotent stem cells; mesDA, mesencephalic dopamine; NA, not available; VM, ventral mesencephalon.

Similar 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 regimen[8]. 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, to use 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 transfer[113], it was the discovery of hiPSCs in 2007[69] that made the potential generation of HLA-matched and patient-specific hiPSCs for therapy a tangible target. This approach has proven successful in animal models of PD and is of intense interest for further clinical development[83].

Within 10 years of their discovery, the first hiPSC lines have already entered clinical trials for age-related macular degeneration[114] and PD[93, 94]. These trials are investigating the use of allogeneic grafts from donor iPSC lines that have been carefully characterized prior to transplantion 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 United States[115] 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 upon the major HLA proteins, immunosuppression may still be beneficial[85]. 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 a HLA match for only 79% of the 10,000 potential recipients of these iPSC-derived cells in the UK[116].

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 β2-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 immune-suppressive 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 for 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 in the expression of suicide genes such herpes simplex virus thymidine kinase (HSV-TK), that can be activated by the FDA approved TK-targeting drug ganciclovir[119-121].

Development of pathology in grafted cells.

Postmortem analysis of patients who have received fetal VM grafts has shown evidence of Lewy body pathology in the transplanted cells in some patients[48], leading to the hypothesis that pathology may spread from host to graft[122]. However, such pathology has not been observed in all studies[49, 54] and in the patients where pathology was observed, it affected only a small percentage of the DA cells[47, 123] 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 on 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 above) may risk accelerated pathology in theory in some cases. It can not be ruled out that cells sourced from individuals with PD may be more vulnerable to the pathological environment than cells from healthy donors, especially over long timeframes. Supporting this concept are multiple studies in which cells derived from patients' iPSCs have disturbances in cellular processes relevant to PD pathophysiology[124-130]. This concern extends beyond monogenetic forms of the disease, as disease-related pathology such as defects in mitophagy, autophagy as well as epigenomic and transcriptomic alterations have been detected also in DA neurons derived from people with sporadic PD[131-133].

From cell replacement to circuitry repair.

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[134,135]. 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 enabled monosynaptic connections of afferent neurons to and from the graft to be mapped in the 6-OHDA 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[88, 136]. 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 neurons[137]. These studies demonstrate that synaptic integration between host and transplanted neurons is a dynamic process,

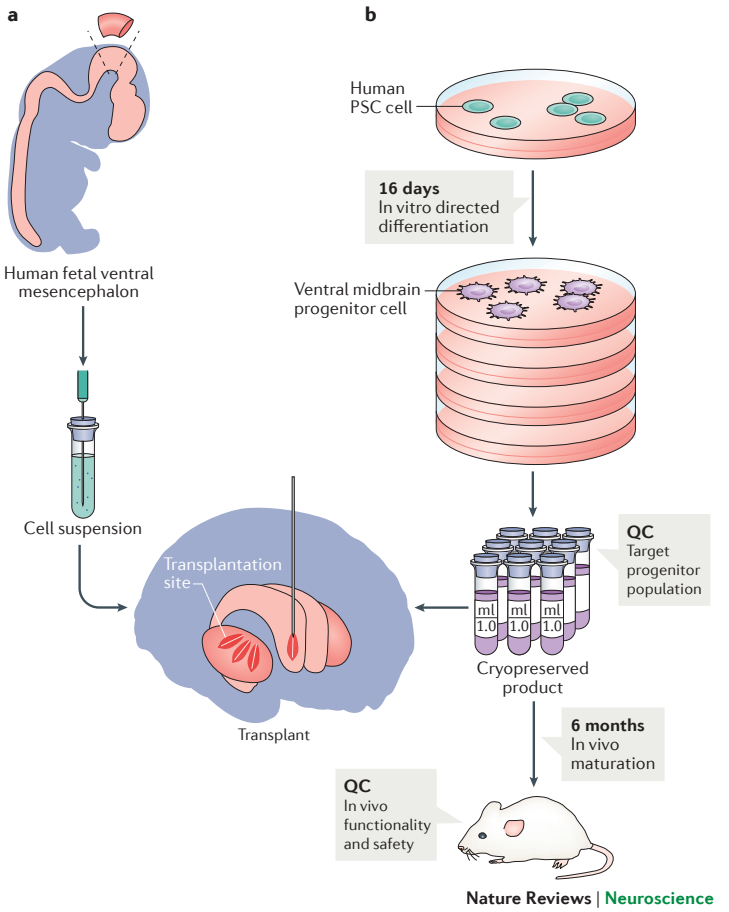

Fig.3 | **Advantages of PSCs versus fetal VM tissue as a cell source for transplantation: a** | Fetal cells can be isolated from the developing ventral mesencephalon (VM) and directly transplanted to the brain of patients with Parkinson disease. **b** | The use of pluripotent stem cells (PSCs) is currently being developed as a therapeutic approach that allows for 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 for extensive in vitro and in vivo testing for safety and efficacy prior to clinical transplantation.

starting as early as 6 weeks post-transplantation and maintained for at least 6 months, that occurs between re-constructed physiologically relevant circuits.

Functional connectivity in stem cell-derived grafts has been investigated using optogenetic and chemogenetic techniques that enable transplanted neurons to be switched 'on' or 'off' in vivo. In the 6-OHDA mouse model of PD, optogenetic inhibition of transplanted hESC-derived cells re-created pre-transplantation deficits in a spontaneous behavioural task, the corridor test, thus demonstrating that the behavioural recovery is indeed mediated via the transplanted cells86. 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 behaviours81. 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.

Extra-nigral 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 improved 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, 138].

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, 139, 140]. Lewy body pathology and loss of neurons in various specific locations have been found to correlate with particular non-motor symptoms[141]. 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 (RBD) and dysosmia. Cholinergic dysfunction and, in particular, loss of cholinergic neurons in the nucleus basalis of Meynert (NBM) has been implicated in PD dementia (PDD)[142], and has therefore been recently targeted, albeit with limited effects, in initial attempts to treat PDD using DBS[143]. Combination cell-replacement therapy including stem cell-derived cholinergic neurons could therefore be tested in the future to potentially help to 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 control[144].

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 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 DA 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 MRgFUS, and gene therapy.

However, this approach is not likely to have an effect on symptoms related to extra-nigral pathology. Extra-nigral networks could be 'pseudo-normalized' 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 upon PET biomarkers that quantify and map out existing DA inputs, will improve. In addition, adjunct interventions to improve 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 role of inflammation will need to be defined in these new cell-based interventions. When using patient-derived cells, 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 treatments for PD in the future.

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Author contributions

The authors all researched data for the article, provided substantial contributions 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 of 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 consultancies from US WorldMeds, Adamas Pharmaceuticals and Prevail Therapeutics.