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Brain organoids in Parkinson's disease drug development: Human-specific models for translational discovery

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Parkinson's disease (PD) poses a major unmet therapeutic challenge, with most drug candidates failing in clinical translation despite promising animal model data. Human induced pluripotent stem cell-derived midbrain organoids recapitulate key PD pathological hallmarks – including dopaminergic neuron loss, α-synuclein aggregation, and neuroinflammation – in a genetically defined, human-specific context. This review summarizes drug screening studies in midbrain organoids across genetic, toxin-based, and α-synuclein preformed fibril models. We highlight therapeutic interventions that rescue PD phenotypes and discuss the personalized medicine potential of patient-derived organoids. We also critically assess current limitations and outline how artificial intelligence integration and assembloid platforms are advancing organoid-based drug discovery towards regulatory acceptance.

Keywords: organoids; Parkinson's disease; translational models; drug development

Introduction

Today's increasingly ageing population has an unmet need to tackle age-associated diseases. Among these, neurodegenerative diseases, such as Parkinson's disease (PD), are a rapidly growing global health burden, with cases projected to rise substantially over the coming decades and the associated health-care costs increasing accordingly.[(p1)]

Several recent reviews have examined brain organoids in the context of neurodegenerative disease more broadly, covering Alzheimer's disease, PD, and amyotrophic lateral sclerosis (ALS), with emphasis on gene editing and artificial intelligence (AI)-driven analysis.[(p2),(p3),(p4)] Two further reviews have focused specifically on midbrain organoids in PD, critically evaluating disease recapitulation and discussing areas for future development.[(p5),(p6)]

The present review is distinguished by its exclusive focus on drug screening experiments in PD midbrain organoids, its systematic coverage of genetic, toxin-based, and α-synuclein preformed fibril (PFF) models, and its direct comparison of organoid and animal model systems as emerging enablers of translational drug discovery.

Despite decades of research on PD, therapeutic progress has been limited, and many drug candidates that show promise in animal models fail in clinical trials.[(p7),(p8)] From 1999 until 2019, in 357 clinical trials, 152 compounds were studied and tested, with an overall success rate of 14.9%.[(p9)] However, this figure is an overestimation due to the inclusion of approved reformulations and new-indication approvals for existing symptomatic agents. More recent data from 2024 report 107 drug candidates in 136 active Phase I–III clinical trials, with most still in Phase II and only 12% reaching Phase III.[(p10)] The drugs that succeed are widely used for symptom management, such as levodopa and dopamine agonists. They often provide substantial motor improvement, especially in the early years of treatment; however, they do not halt or reverse neurodegeneration, highlighting the ongoing need for disease-modifying therapies.[(p11)]

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It is well known that animal systems do not fully replicate human brain physiology, genetic diversity, or disease progression.[p12],[p13],[p14],[p15],[p16] By contrast, human induced pluripotent stem cell (iPSC)–derived brain organoids offer a more physiologically relevant platform by recapitulating key aspects of human neural architecture, cell–cell interactions, and patient-specific genetic backgrounds.[p17],[p18],[p19] Organoids demonstrate clear neurodegeneration phenotypes and allow the investigation of early disease molecular mechanisms owing to their embryonic-like developmental state, while more mature cultures reveal advanced phenotypes, such as protein aggregation.[p20],[p21],[p22],[p23],[p24] Moreover, it has been shown that organoids robustly recapitulate PD phenotypes across independent organoid generation rounds or batches, providing evidence of their reproducibility.[p25] These characteristics make brain organoids appealing not only for target validation, but also for therapeutics development and neurotoxicity studies. Furthermore, these properties improve the predictive value of preclinical testing and facilitate the identification of effective therapeutic candidates for neurodegenerative disorders when compared with traditional animal or 2D cellular models.[p26],[p27],[p28],[p29],[p30],[p31],[p32],[p33]

Notably, there have been recent efforts to phase out animal testing. The 2022 US Food and Drug Administration (FDA) Modernization Act 2.0 removed the formal mandatory requirement for animal testing prior to clinical trials, encouraging the inclusion of human-relevant *in vitro* data in preclinical studies.[p34] Following that, on April 10, 2025, a strategic plan was announced to replace animal testing in the development of monoclonal antibodies and biologic therapies – including vaccines, cell and gene therapies, and therapeutic proteins – with New Approach Methodologies (NAMs).[p35] NAMs include human-derived models, such as human organoids, organ-on-chip systems, and computational models, that are designed to assess human drug safety and efficacy more reliably than animal models.[p36],[p37]

Organoids are already widely used for drug screening and toxicology testing across multiple organs, offering more human-predictive models for safety and efficacy assessment. Over the past decade, organoid research has expanded rapidly, with publications growing from fewer than 20 per year before 2012 to over 1,000 annually by 2022, indicating a sharp increase that has continued in recent years.[p38] Moreover, some drugs have already entered clinical trials based primarily on preclinical data from human-derived models such as organoids or organ-on-a-chip systems.[p39] Nevertheless, such cases still remain rare and are assessed individually rather than representing a standard preclinical procedure.

Considering the human brain's particularities and differences from animal models, organoids should be part of the pipeline of drug development for neurodegenerative diseases. At the moment, organoid-based drug testing and toxicology screening have been most extensively validated in oncology to evaluate patient-specific drug sensitivity *in vitro*, and therefore guide personalized treatment decisions. Examples include patient-derived models of metastatic gastrointestinal cancer that have been used to screen anticancer drug libraries, demonstrating the ability to predict actual patient treatment responses and clinical trial outcomes.[p40] Similar studies have been done on breast, kidney, and other types of cancers.[p41],[p42],[p43] Furthermore, functional hepatic organoids have successfully been deployed to evaluate drug-induced liver injury, accurately isolating the unique toxic markers of hepatotoxic compounds where traditional cell cultures often fail.[p44] Despite this progress, the application of organoids as primary preclinical platforms for neurodegenerative diseases remains comparatively underdeveloped.

In this review, we summarize available studies demonstrating drug effects, focusing on PD phenotype amelioration in human-specific organoids. We emphasize the translational value of these human-specific organoids as part of NAMs for evaluating drug efficacy and mechanism (Table 1, Figure 1). Importantly, PD is a highly heterogeneous disease, and current therapies are largely symptomatic, with limited efficacy across patient populations. Organoids offer a unique opportunity to address this heterogeneity by enabling patient-specific drug screening, testing multiple therapeutic strategies on individualized models, and identifying interventions that are most likely to be effective for a given genetic or cellular background.

Midbrain organoids for PD research: Generation and features

Brain organoids are three-dimensional (3D), stem-cell-derived, *in vitro* cultures that are capable of self-organization, which is driven by region-specific cell fate commitment. They are able to recapitulate key aspects of human brain development and neuronal functionality in terms of electrophysiology and neurotransmitter release.[p20],[p45],[p46],[p47] Furthermore, their transcriptomic and epigenetic signatures recapitulate the early development of the human brain.[p19],[p48]

For PD research, the focus is on cell patterning towards a ventral midbrain fate to generate dopaminergic neurons of the substantia nigra, the primary neuronal population affected in PD. This process generally involves stepwise differentiation of human pluripotent stem cells (hPSCs), including iPSCs and embryonic stem cells (ESCs), towards a neural lineage. Neural induction is first achieved by directing hPSCs towards a neuroectodermal fate through inhibition of the bone morphogenetic protein (BMP) and transforming growth factor-β (TGFβ) signalling pathways. Subsequent ventral midbrain patterning is induced by activation of canonical WNT and sonic hedgehog (SHH) signalling, promoting the specification of floor plate progenitors. In many protocols, these patterning steps occur within 3D aggregates derived from hPSCs, formed as embryoid bodies.[p49],[p50] Alternatively, neuroectodermal progenitors can first be generated as an intermediate population.[p20],[p46],[p51] Subsequent midbrain organoid generation from these prepatterned progenitors is robust and allows observation of reproducible disease-relevant phenotypes.[p25],[p46]

Over time, midbrain-specific organoids mature, developing cellular and molecular characteristics of the human midbrain, including the presence of tyrosine hydroxylase (TH)-positive dopaminergic neurons, astrocytes, and oligodendrocytes, and exhibit spontaneous neuronal activity.[p46],[p50] Notably, longer cultures of midbrain organoids demonstrate the presence of

TABLE 1

Summary of therapeutic interventions tested in organoid models for PD phenotype amelioration.

Refs	Organoid model	PD model	Molecule/ intervention	Target/mechanism	Effect/outcome
Kurniawan *et al.*, 2025	Midbrain organoids	Healthy midbrain organoid + *LRRK2*-G2019S microglia	Oxamic acid	Inhibition of lactate dehydrogenase, aiming to reduce glycolysis and mTOR-dependent metabolic reprogramming	Decreased microglial inflammation, reducing levels of TNFα; improved dopaminergic neuron survival
Frattini *et al.*, 2025	Patient-derived midbrain organoids	*GBA1*-L444P	Ambroxol	GCase enhancer	Restored GCase protein levels and increased its enzymatic activity; reduced levels of misfolded GCase; restored dopaminergic neuron levels; reduced Lewy body-like fibrillary α-synuclein deposition
Frattini *et al.*, 2025	Patient-derived midbrain organoids	*GBA1*-L444P	GZ667161	Glucosylceramide synthase inhibitor	Reduced glucosylceramide levels; reduced levels of misfolded GCase; reduced Lewy body-like fibrillary α-synuclein deposition
Zagare *et al.*, 2025	Patient-derived midbrain organoids	*GBA1*-N370S	FOXO1-ASOs	Blocking of FOXO1 protein translation	Improved dopamine release; increased dopaminergic neuron levels and cellular viability
Zagare *et al.*, 2025	Patient-derived midbrain organoids	*GBA1*-N370S	Pioglitazone	Modulation of metabolic/insulin signalling	Improved dopamine release; increased dopaminergic neuron levels
Zhang *et al.*, 2024	Midbrain organoids	α-Synuclein PFF treatment	Tilorone	Inhibition of α-synuclein uptake/ propagation	Reduced a-synuclein fibril internalization; reduced apoptosis, and stabilization of inflammation profile
An *et al.*, 2024	Patient-derived midbrain organoids	*PRKN* (1 bp del; ex3–4 del); *LRRK2*-G2019S	Levodopa	Dopaminergic pathway activation	Increased dopamine release
Kim *et al.*, 2023	Midbrain organoids	Optogenetic induction of α-synuclein aggregation	BAG956	Autophagic clearance enhancement of α-synuclein aggregates	Reduced pathological α-synuclein levels; increased survival of dopaminergic neurons
Mendes-Pinheiro *et al.*, 2023	Midbrain organoids	6-OHDA treatment	MSC secretome	Neurotrophic/neuroprotective effects	Rescued dopaminergic neuron loss; reduced their fragmentation/neurodegeneration
Jarazo *et al.*, 2022	Patient-derived midbrain organoids	*PINK1*-p.Q456X; *PINK1*-p.I368N	2-HP-β-CD	Autophagy modulation	Normalized PD-associated proteomics signature; restored dopaminergic neuron levels
Boussaad *et al.*, 2020	Midbrain organoids	*PARK7*-p.E64D	Splicing modulator RECTAS + PB	Corrects splicing defect in DJ-1	Rescued dopaminergic neuron loss

Abbreviations: 2-HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; 6-OHDA, 6-hydroxydopamine; ASO, antisense oligonucleotide; GCase, glucocerebrosidase; MSC, mesenchymal stem cell; PB, phenylbutyrate; PD, Parkinson's disease; PFF, preformed fibril; RECTAS, rectifier of aberrant splicing; TNFα, tumour necrosis factor-α.

neuromelanin, a hallmark of human substantia nigra dopaminergic neurons, providing evidence of their maturation and nigrostriatal identity.[p23],[p46],[p50] Importantly, organoids generated from patient iPSCs provide a personalized platform for disease investigation and drug screening based on the patient's genetic background. In addition, organoids harbouring PD-associated mutations in genes such as *GBA1, SNCA, LRRK2, RHOT1,* and *PINK1* recapitulate key pathological hallmarks of the disease, which include α-synuclein aggregation, mitochondrial dysfunction, oxidative stress, and progressive dopaminergic neuron vulnerability.[p20],[p21],[p22],[p24],[p33],[p52],[p53],[p54]

A growing body of research centres around producing midbrain organoids from cells carrying mutations in the major PD-associated genes. *LRRK2*-G2019S organoids display reduced TH^+ neuron complexity, increased FOXA2 expression, and altered gene expression profiles recapitulating early-onset neuronal dysfunction.[p20] Organoids from PD patients with heterozygous mutations in the *GBA1* gene demonstrate glucocerebrosidase (GCase) deficiency, lipid dyshomeostasis, and Lewy-body-like α-synuclein inclusions.[p21],[p23] *SNCA* triplication organoids (3 × *SNCA*) exhibit progressive autophagy failure and elevated phosphorylated α-synuclein (pS129) levels, establishing an iPSC organoid model for gain-of-function *SNCA* pathology.[p54] *PARK7*/DJ-1-p.E64D organoids model oxidative stress and TH^+ neuron loss rescuable by splicing correction.[p31] *RHOT1*/MIRO1-p.R272Q organoids display mitochondrial bioenergetics disruption and calcium dysregulation,[p53] while *PINK1* mutant organoids recapitulate mitophagy defects and mitochondrial

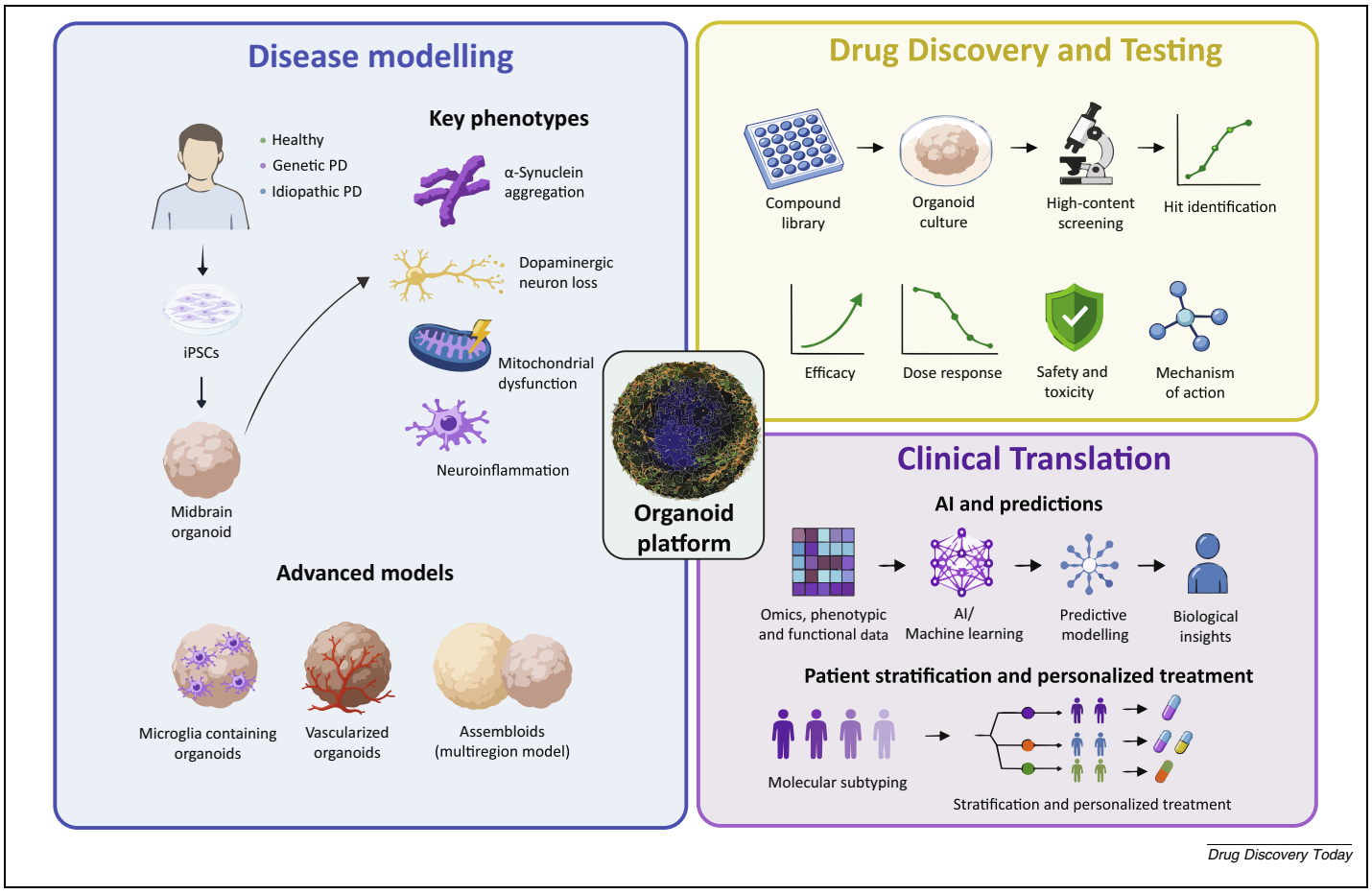

FIGURE 1

Summary of organoid applications in Parkinson's disease (PD) modelling and therapeutic development. Patient-derived organoids can be used to generate personalized *in vitro* models that recapitulate key PD phenotypes. These models enable disease mechanism studies, drug screening, and biomarker discovery. In addition, organoids serve as data-rich platforms for predictive medicine, facilitating patient stratification, therapeutic response prediction, and the development of personalized treatment strategies.

fragmentation.[p32] Moreover, recent advances have enabled the incorporation of microglia and vascular-like structures into organoids, further improving physiological relevance and enabling the investigation of neuroinflammatory pathways as well as the role of vasculature functionality in PD progression.[p55],[p56]

Organoid models of Parkinson's disease and applications in drug discovery

Rescue of dopaminergic neuron loss

Human midbrain organoids have been successfully used to recapitulate PD-associated cellular phenotypes, such as dopaminergic neuron loss, neuroinflammation, altered metabolism, and accumulation of pathological α-synuclein forming aggregate-like structures.[p21],[p23],[p32],[p33],[p52] Several studies also demonstrate rescue of the observed phenotypes and provide a platform to evaluate potential therapeutic interventions (Table 1).

For example, Boussaad *et al*. showed that the c.192G>C pathogenic variant in *PARK7* induced aberrant exon 3 skipping, resulting in reduced levels of correctly spliced full-length mRNA and decreased DJ-1 protein expression.[p31] Reduced DJ-1 levels in PD models have been shown to increase oxidative stress, impair mitochondrial function, and enhance the vulnerability of dopaminergic neurons.[p57],[p58] Similarly, Boussaad and colleagues observed significantly reduced levels of TH^+ dopaminergic neurons in midbrain organoids generated from CRISPR/Cas9-engineered neuronal progenitors harbouring the pathogenic variant in the *PARK7* gene. They showed that this selective TH^+ dopaminergic neuron loss can be reversed in a dose-dependent manner by combining treatment with 1 mM phenylbutyrate (PB) and the splicing modulator RECTAS (rectifier of aberrant splicing). Both molecules were selected from literature-based evidence as potential rescue strategies with a synergetic effect. Overall, the study demonstrates that correcting RNA mis-splicing and its downstream consequences can ameliorate disease-associated phenotypes in patient-based midbrain models of PD.

Another study by Jarazo *et al*. showed that patient-derived midbrain organoids with *PINK1* mutations demonstrated an altered proteomics profile, suggesting dysregulation of metabolism, autophagy, inflammation, and cell cycle and cell survival pathways. After midbrain organoid treatment with 2-hydroxypro pyl-β-cyclodextrin (2-HP-β-CD) – a known modulator of

autophagy that causes an increase in nuclear translocation of transcription factor EB (*TFEB*), a master regulator of lysosomal biogenesis and autophagy[p59] – the proteomics profile was ameliorated, and midbrain organoids showed restored levels of TH^+ dopaminergic neurons.[p32]

Using a toxin-induced model to recapitulate dopaminergic degeneration, Mendes-Pinheiro *et al.* observed reduced TH^+ dopaminergic neuron levels and increased fragmentation after organoid treatment with 6-hydroxydopamine (6-OHDA). This study showed that this PD model is responsive to bone-marrow-derived mesenchymal stem cell (MSC) secretome treatment, and its neuroprotective and neuroregulatory effects rescued TH^+ dopaminergic neuron levels and reduced their fragmentation.[p60]

While the above studies primarily assessed dopaminergic neuron survival, other work has focused on the functional restoration of dopaminergic signalling, as well as assessing dopaminergic neuron functionality.

An *et al.* demonstrated a sensitive electrochemical sensing platform for detecting the response of midbrain organoids. They observed functional deficits in dopamine release in PD patient midbrain organoids, which were restored by levodopa, confirming dopaminergic pathway responsiveness and the utility of their platform.[p61]

Similarly, addressing dopaminergic dysfunction in a genetic context, Zagare *et al.* demonstrated impaired dopamine release, decreased dopaminergic neuron numbers, and an altered lipid profile in *GBA1*-N409S variant carriers (GBA-PD).[p33] In addition, this study demonstrated the application of organoids in studying comorbidities by investigating the role of type 2 diabetes-associated insulin resistance in PD development. The authors showed that increased insulin sensitivity restores cellular viability in GBA-PD midbrain organoids. In addition, treatment with the anti-diabetic drug pioglitazone not only improved dopaminergic neuron survival, but also enhanced dopamine secretion. Further, they identified *FOXO1* as a downstream effector of impaired insulin signalling in GBA-PD, and demonstrated that targeted antisense oligonucleotide (ASO)-mediated downregulation of FOXO1 protein significantly increased dopaminergic neuron numbers, dopamine release, and cellular viability.

Microglia-mediated neuroinflammation

Advanced organoid models incorporating patient-derived microglia enable the study of neuroinflammatory mechanisms in PD and their impact on neuronal survival.

Kurniawan *et al.* observed that *LRRK2*-G2019S-affected microglia were proinflammatory, secreting elevated levels of tumour necrosis factor-α (TNFα) and exhibiting metabolic changes to sustain their activity.[p52] Further, this study showed that midbrain organoids experienced selective TH^+ dopaminergic neuron loss upon TNFα treatment, as well as when co-cultured with microglia harbouring the *LRRK2*-G2019S pathogenic variant, reflecting key features of neuroinflammation in PD.[p52] Treatment with the metabolic modulator oxamic acid, aimed at inhibiting glycolysis as the main metabolic player fuelling microglial inflammatory activation, restored levels of dopaminergic neurons in midbrain organoids co-cultured with *LRRK2*-G2019S microglia.

Overall, this study demonstrates that advanced midbrain organoid models incorporating microglia provide a robust platform to investigate microglial roles in PD and enable the modulation of their functionality and metabolism within a physiologically relevant context, rather than in traditional 2D culture systems.

Targeting α-synuclein pathology

In addition to neuronal loss and inflammation, α-synuclein aggregation is a central pathological hallmark of PD, and it has been successfully modelled and targeted in organoid systems. Kim *et al.* used optogenetic induction of α-synuclein aggregation in human iPSC-derived midbrain organoids, resulting in pathological α-synuclein accumulation and PD-like cellular phenotypes.[p29] This was reversed by the molecule BAG956 through enhanced autophagic clearance of α-synuclein aggregates.

Zhang *et al.* challenged organoids with α-synuclein PFFs, resulting in pathological α-synuclein aggregation and apoptosis.[p30] They used the antiviral drug tilorone to inhibit α-synuclein uptake and propagation, reducing aggregation and stabilizing neuroinflammation and metabolic signature. Tilorone was a promising candidate because of its lysosomotropic and immune-modulating properties, which can inhibit α-synuclein endocytosis and aggregate propagation.[p62]

Finally, targeting lysosomal dysfunction in *GBA1*-associated PD, Frattini *et al.* reported that patient-derived *GBA1*-L444P organoids endogenously accumulated α-synuclein and displayed characteristic *GBA1* mutation features, such as reduced GCase enzyme activity, endoplasmic reticulum (ER) retention of mutant GCase, and increased levels of its substrate, glucosylceramide. Mutant GCase is prone to misfolding and is retained in the ER, compromising chaperone-mediated autophagy and lysosomal function, resulting in impaired α-synuclein clearance and promoting its accumulation. The authors used ambroxol, a GCase enhancer currently in clinical trials for PD, which was able to increase the protein amount and enzymatic activity of GCase, resulting in a reduction of α-synuclein aggregate numbers. Additionally, they found that GZ667161, a glucosylceramide (GlcCer) synthase inhibitor, effectively reduced GlcCer levels and also decreased α-synuclein aggregate levels in *GBA1*-L444P mutant midbrain organoids.

Collectively, these studies show that human midbrain organoids can reproduce PD-related molecular and cellular phenotypes – including α-synuclein aggregation, dopaminergic neuron vulnerability, lysosomal dysfunction, and neuroinflammation – and serve as a versatile platform for testing interventions that rescue these disease-relevant phenotypes (Table 1). Together, these data support the application of midbrain organoids as a valuable preclinical platform for therapeutic compound screening.

Adapted models

Although genetic iPSC models represent the predominant approach in midbrain organoid-based drug discovery, complementary strategies using exogenous neurotoxins or pathogenic α-synuclein PFFs model the sporadic and propagation-associated aspects of PD that are difficult to recapitulate with

mutation-based models. Exposure of midbrain organoids to mitochondrial complex I inhibitors, such as the rotenone and neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) (the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)) or the dopaminergic neurotoxin 6-OHDA, induces dopaminergic neuron loss and mitochondrial dysfunction analogous to environmental risk factor-based PD.[p60],[p63],[p64],[p65] These models are particularly useful for identifying neuroprotective compounds in the absence of a defined genetic background and for testing mitochondria-targeted therapies.

Furthermore, midbrain organoids can be seeded with fluorescently labelled or sonicated α-synuclein PFFs, which are internalized by neurons and trigger the phosphorylation (pS129) and accumulation of endogenous α-synuclein, providing a model of Lewy body-like pathology propagation.[p30] This system was used to evaluate the antiviral compound tilorone, which was found to inhibit PFF uptake, reduce pS129-α-synuclein levels, and reverse PFF-induced proteomic disruptions including lipid homeostasis networks.

Electrochemical sensing platforms have been developed for real-time, non-invasive monitoring of dopamine release from healthy and PD-patient organoids, providing a quantitative pharmacodynamic readout for *LRRK2* kinase inhibitor drug screening that complements immunofluorescence-based endpoints.[p66] Similarly, single-cell electrochemical cytometry in iPSC-derived neurons and organoids from young-onset PD patients has revealed vesicular catecholamine storage deficits as a novel drug target, with amantadine and phorbol ester treatment partially rescuing the phenotype.[p67]

Current limitations

Despite their translational promise, midbrain organoids present unresolved limitations that must be acknowledged to contextualize reported findings and guide future development.[p68],[p69] Standard midbrain organoid protocols lack a vascular network, resulting in diffusion-limited core necrosis in organoids exceeding ~1 mm diameter and in the absence of endothelial cell-mediated blood–brain barrier (BBB) properties. This limits long-term culture viability and the testing of compounds requiring active vascular transport. Vascular integration strategies, including co-culture with brain vascular organoids and microfluidic perfusion systems, are under active development. Similarly, conventional midbrain organoids lack immune cells. Microglia can be added as co-cultures, but cells from the adaptive immune system are still lacking from current models.

The throughput of current midbrain organoid protocols remains below what is required for primary drug discovery screens of large compound libraries (thousands of molecules). Miniaturization to 96- and 384-well formats and automated organoid production systems are being developed, but require further optimization.

In addition, inter-line differences must be considered when grouping multiple lines within the same experimental category. Although they might reflect biologically relevant individual variability, they also introduce heterogeneity that can complicate data interpretation. Therefore, robust quality control metrics and standardized characterization procedures should be routinely applied to distinguish technical variation from biological heterogeneity.

Addressing these limitations will be essential for translating the promise of midbrain organoids into reliable, scalable preclinical tools.

Discussion and future perspectives

In this review, we shed light on evidence that organoids are capable of recapitulating complex features of the human brain, and therefore complex diseases such as PD. Recent studies using human-derived brain organoids, particularly midbrain-specific models, have demonstrated significant potential for preclinical drug testing in PD. These organoids successfully recapitulate midbrain-specific dopaminergic neuron populations and key PD hallmarks, loss of nigrostriatal dopaminergic neurons, α-synuclein aggregation, neuroinflammation, and other PD-associated phenotypes, allowing researchers to evaluate the efficacy of candidate therapeutics in a human-relevant system. In this review, we highlight investigations that have reported the rescue of PD-associated phenotypes following treatment with selected compounds, demonstrating the ability of organoid platforms to model drug responses and mechanism-of-action *in vitro*.

While not diminishing the value of 2D cultures and animal models, organoids offer several opportunities, including reduced ethical concerns, recapitulation of human biology, and physiological relevance, making them a relevant platform for human drug screening. By enabling patient-specific disease modelling, mechanistic studies, and assessment of therapeutic efficacy and safety, organoids provide a human-relevant tool for the development of therapeutics and personalized treatments for PD. This is a well-established concept in oncology, but it is emerging in neurodegenerative diseases.[p43]

Beyond individual drug responses, midbrain organoids hold promise as tools for patient stratification in clinical trial design. Generating organoids from patients representing distinct genetic PD subtypes, such as those with lysosomal dysfunction (*GBA1*), defective mitochondrial quality control (*PINK1/PRKN*), or neuroinflammatory dysregulation (*LRRK2*), could define subpopulations sharing common molecular vulnerabilities. Organoid-derived phenotypic and omic profiles could then guide enriched enrolment, increasing the likelihood of treatment response. This mirrors the developments in precision oncology, where patient-derived organoids have informed chemotherapy selection. By combining mechanistic insights with personalized efficacy testing, organoids provide a powerful platform to guide the development of targeted and precision therapies for PD.

Beyond conventional organoids, advances in 3D neural culture systems have led to the development of assembloids, generated through the fusion of multiple region-specific organoids, to model interactions between distinct brain regions. In the context of PD, midbrain–striatum assembloids are particularly interesting, because they can recapitulate key features of the nigrostriatal pathway, enabling the study of dopaminergic neuron projections and circuit dysfunction.[p70] Assembloid models have also provided important insights into the inter-regional propagation of pathological α-synuclein. For example, midbrain–hindbrain assembloids successfully demonstrate the spread of α-synuclein

pathology between interconnected neuronal populations, recapitulating a key feature of PD progression that cannot be captured in isolated organoid cultures.[p71]

Further increasing in physiological complexity, chimeroids – in which human iPSC-derived neurons are integrated into living rodent brain tissue – represent an emerging platform for studying individual susceptibility to neurotoxic insults and human-specific disease mechanisms in a vascularized, mature neural context. A recent publication demonstrated that chimeroids can capture human-specific vulnerability patterns that are not accessible in purely *in vitro* or conventional animal models.[p72] Although chimeroids introduce new complexities (such as ethical considerations, human–animal chimera regulations, and reduced throughput), they could occupy an important niche as validation platforms for lead compounds emerging from organoid primary screens.

The rapid advancement of human-derived organoid models is reshaping preclinical drug development and disease modelling, with emerging applications in regenerative medicine, where organoid-based therapies are being investigated for functional tissue replacement therapy.[p73] Despite all this progress, the regulatory frameworks for integrating these models as complementary or even primary tools for testing drug safety and efficacy remain underdeveloped. However, the first steps towards adapting the regulatory framework were the FDA Modernization Act 2.0 (2022) and the April 2025 US Department of Health and Human Services (HHS)/FDA NAM strategic plan. According to these guidelines, organoid data can be submitted as primary or supplementary evidence for Investigational New Drug (IND) applications, with the level of evidence required being determined on a case-by-case basis. For a NAM to be accepted as a substitute for animal studies in neurodegenerative disease indications, regulators expect evidence of: (i) assay qualification using reference compounds with known *in vivo* activity; (ii) inter-laboratory reproducibility across at least two independent sites; (iii) defined quality control criteria applied to organoid preparations; and (iv) a mechanistic rationale linking organoid readouts to clinically measurable outcomes. A recent publication provides a useful conceptual grounding, proposing that NAMs should be species-specific and human-relevant, a criterion that organoids readily satisfy.[p74]

Concrete steps towards routine acceptance include: the formation of organoid-specific working groups within European Medicines Agency (EMA) and FDA; the publication of standardized midbrain organoid protocol guidance, and the integration of midbrain organoid data into multi-platform NAM packages alongside organ-on-chip and *in silico* models to provide converging lines of evidence, rather than isolated end points. Successful incorporation of organoid-derived data into regulatory submissions will require standardized protocols, clear validation criteria, and agreement on relevant end points for disease modelling. Ensuring reproducibility and data harmonization across laboratories will be essential to build regulatory confidence.

In PD drug development, midbrain-specific organoids could play a central part in evaluating dopaminergic neuron survival, α-synuclein pathology, and neuroinflammatory responses, ultimately guiding the design of safer and more effective therapeutics. The extraction of disease-related features will be increased by the use of AI and machine learning, but these tools will also contribute to addressing two key challenges: variability and throughput. For variability, deep learning classifiers trained on live-imaging data sets can distinguish disease from healthy phenotypes at early time points, despite intrinsic organoid heterogeneity.[p75] For throughput, convolutional neural networks trained on high-content images of stained organoid sections can automate TH^+ neuron counting, α-synuclein aggregate quantification, and morphology classification at scales that current analysis cannot match. Multi-parametric AI models integrating transcriptomic, proteomic, and imaging readouts from drug-treated organoids hold the potential to predict drug response trajectories, identify combination therapy synergies, and stratify patients by predicted therapeutic sensitivity, positioning organoids as central nodes in a data-rich, AI-enabled drug discovery pipeline.

These findings underscore the value of organoids and other NAMs as predictive preclinical models that bridge the gap between conventional animal studies and clinical outcomes. By enabling human-specific assessments of drug safety and efficacy, these models can accelerate the identification and optimization of therapeutics, inform patient-specific treatment strategies, and support regulatory adoption of human-relevant testing approaches. Collectively, current organoid-based studies illustrate a promising shift towards accurate, efficient, and translationally relevant preclinical research for PD and potentially other neurodegenerative disorders.

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Declaration of generative AI use

During the preparation of this work, the authors used OpenAI's ChatGPT (GPT-5.5) and Anthropic's Claude (Sonnet 5) for language editing purposes, including improving sentence structure, clarity, and conciseness. In addition, Figure 1, which summarizes the potential applications of midbrain organoids discussed in this review, was created using the OpenAI tool. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interests

J.S. is a co-inventor on a patent covering the generation of midbrain organoids (WO2017060884A1). J.S. and J.J. are co-founders and shareholders of the company OrganoTherapeutics, which

makes use of midbrain organoid technology. A.Z. is a lead scientist in OrganoTherapeutics.

CRediT authorship contribution statement

Alise Zagare: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Javier Jarazo:** Writing – review & editing, Supervision, Conceptualization. **Jens Schwamborn:** Writing – review & editing, Supervision, Conceptualization.

Data availability

No data was used for the research described in the article.

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