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Induced Pluripotent Stem Cell Lines as a Model for Studying the Cellular Phase of Parkinson's Disease

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ABSTRACT: Parkinson's disease (PD) is a complex neurodegenerative disorder, characterized by the progressive loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) as a result of intraneural deposition of aggregated alpha-synuclein (aSyn) in Lewy bodies (LB). aSyn is an intrinsically-disordered protein, encoded by the SNCA gene, and is implicated in both familial and sporadic forms of PD. However, we still do not fully understand if and how aSyn causes cell dysfunction and death. Therefore, it is essential to develop and explore robust models for bridging the gap between preclinical research and clinical applications, creating platforms for testing hypotheses and assessing potential interventions. The emergence of patient-derived induced pluripotent stem cells (iPSCs) offers unique opportunities for investigating the cellular phase of PD and related synucleinopathies by enabling the systematic assessment of phenotypes in various cell types of relevance for disease. Moreover, advances in PD-derived iPSC technology also hold promise for cell replacement therapy and drug discovery efforts using pharmacological or genetic screening approaches. In this review, we focus on the application of aSyn iPSC models in PD research, summarizing their anticipated merits, challenges and present-day implementations. .

KEY WORDS: Parkinson's disease; alpha-synuclein; induced pluripotent stem cells; midbrain dopaminergic neurons; disease modeling

I. INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder afflicting growing number of people worldwide due to the ageing of the human population. PD is a complex, multisystem disorder characterized both by motor and non-motor clinical features. The four cardinal motor abnormalities (bradykinesia, rigidity, resting tremor, and postural instability) have routinely defined PD clinical diagnosis. However, PD is also associated with non-motor symptoms (e.g. REM sleep behavior disorder, autonomic dysfunctions) that often precede motor symptoms and significantly contribute to overall disease morbidity[1–6]. The hallmark pathological features of PD are the progressive loss

and degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), and the intraneural accumulation of alpha-synuclein (aSyn), a small protein encoded by the SNCA gene, in the form of Lewy bodies (LB) and Lewy neurites[1,7,8]. While the exact physiological function of aSyn is still unclear, different studies suggest that it may act as a molecular chaperone[9], play a role in both Ca^{+2} and dopamine homeostasis[10,11], and play a role in synaptic vesicles trafficking[11–13]. Under physiological conditions, aSyn is a natively unfolded protein which, under pathological conditions, can aggregate and form insoluble intracellular inclusions causing neurodegeneration[14–16]. In general, ~5-10% of PD cases are classified as familial, while the vast majority of cases represent sporadic PD. Most forms of PD share similar clinical and pathological features, although differences have been noted. The SNCA gene is associated with both familial and sporadic forms as point mutations and multiplications of the gene cause familial PD forms, while single nucleotide polymorphisms have been identified in idiopathic PD forms as susceptibility factors[17].

Given the increased prevalence of PD and our inability to effectively prevent onset or to modify disease progression, we urgently need to decipher the complex etiology of the disease in order to enable the development of novel therapeutic strategies[12,18,19].

II.

From biology to pathology: aSyn as a marker and target for intervention

aSyn is a 140 amino acid protein whose primary sequence can be divided into three main domains: (i) an N-terminal domain[1–60], which includes a multi-repeated hexameric motif (KTKEGV) and has alpha-helical propensity enabling lipid membrane-binding; (ii) a central domain[61–95], which is highly hydrophobic and represents the most aggregation-prone part due to conformational changes; and (iii) a C-terminal domain[96–140], which is characterized by a non-defined structure and enriched in negative charged and proline residues that plays a role in preventing aSyn self-aggregation[20]. aSyn is an intrinsically-disordered protein with a conformational plasticity that can adopt different conformations depending on the environmental context[21]. Physiologically, two forms of aSyn seem to coexist in a dynamic balance: the intrinsically disordered cytosolic monomer and (in large part) a membrane-bound and aggregation-resistant, helically folded tetramer[22,23]. However, tetramer destabilization and imbalances in the ratio folded tetramer:unfolded monomer may result in accumulation of pro-aggregating forms, as in LB pathology, where aSyn adopts a β-sheet conformation that further recruits monomers to form oligomers and amyloid fibrils[12,24].

aSyn is predominantly located at presynaptic nerve terminals, although initial studies pointed out its presence within the nucleus[25]. The occurrence of aSyn in the nucleus has been a matter of debate and its roles in the nucleus are still underappreciated. However, accumulating evidence, including from our own work, confirms the presence of aSyn in the nucleus of neuronal cells in human brain tissue as well as in animal models[25–32]. Consensus has not been reached regarding the role of nuclear aSyn (aSynNuc). Some studies demonstrated a protective role against stress, in maintaining genomic integrity as well as in DNA repair processes[33–36]. Other studies suggest a putative function in nucleocytoplasmic transport via the interaction with Ras-related nuclear protein (RAN), which is impaired by aSyn mutations[37]. On the other hand, evidence from both in vitro and in vivo suggests a detrimental role of aSynNuc highlighting its contribution to the pathogenesis of PD and other synucleinopathies[30,38,39]. Moreover, accumulation of aSynNuc can induce significant transcriptional dysregulation and epigenetic modifications which are linked to gliosis, increased inflammation, oxidative stress and mitochondrial dysfunction, DNA damage and cell cycle disruption as well as altered ribosomal RNA processing, ultimately accelerating cell senescence and neurodegeneration[27,31,40–43].

aSyn has emerged both as a biomarker and therapeutic target based on its central role in PD pathogenesis[44,45]. Ongoing efforts using aSyn seed amplification assays (aSyn-SAAs) were recently reported to detect seed-competent aSyn species in the CSF and to distinguish, with high sensitivity and specificity, healthy controls from prodromal and non-manifesting carriers, and from sporadic PD patients[46–48]. Given such biomarker advances and the fact that aSyn pathology is the gold standard for establishing the ultimate diagnosis, two recent studies proposed a major shift from a clinical to a biological definition of PD using different levels of 'biological' information[49,50]. The SynNeurGe classification system and the neuronal aSyn disease integrated staging system (NSD-ISS) were the two initial attempts to propose research criteria that may prove instrumental for guiding future clinical trials[51,52]. Nevertheless, it

is now necessary to harmonize such classification systems, and to refine them so that, one day, they may be used in the clinical practice[53].

aSyn PTMs, including phosphorylation, nitration, acetylation, O-GlcNAcylation, glycation, SUMOylation, ubiquitination, and C-terminal cleavage, may act as modifiers of both aSyn biology as well as pathological processes. As such, there is growing interest in assessing their potential as biomarkers of disease[54].

A substantial body of evidence suggests that alteration/reduction of aSyn aggregation might constitute a promising avenue for therapeutic intervention in PD. As a result, a plethora of anti-aggregation compounds, encompassing both small molecular entities, antibodies, and other macromolecular modalities, have been investigated for their potential to mitigate aSyn aggregation and its associated neurotoxicity[55,56]. Moreover, passive immunization is one of the major therapeutic approaches that have been attempted recently to target aberrant aSyn. Two monoclonal antibodies, Prasinezumab[57,58] (PRX002) and Cinpanemab[59,60] (BIIB054) that target C-terminal and N-terminal of aSyn respectively, have successfully passed phase I clinical trials. However, the outcomes of the phase II trials were negative. Currently, further studies with Prasinezumab are ongoing, and there is hope that some of the strategies being currently tested may prove beneficial[12,61].

Numerous cellular and animal models[62], with own strengths and limitations, have been established for modeling various aspects of PD. However, due to our limited understanding of the molecular underpinnings of PD, and to inherent limitations of model systems which fail to recapitulate important features of PD, we still need to continue to develop alternative models. In this context, induced pluripotent stem cell (iPSC) models hold a great promise due to their potential to provide a far greater supply of disease-relevant cells[63,64].

III.

Developing aSyn iPSC models

The emergence of induced pluripotent stem cells (iPSCs) technology led to a scientific breakthrough in PD modeling, affording the possibility of establishing cellular models of neurons from live PD patients. IPSCs refer to pluripotent stem cells that can be generated by introducing the four transcription factors OCT4, Sox2, Klf4, and c-Myc (Yamanaka factors) into adult somatic cells[65,66]. The delivery of Yamanaka factors is performed using both viral and non-viral based key techniques. For instance, viral vectors such as Sendai virus (SeV), measles virus and RNA virus-based episomal vector (REVec) system represent host genome integration-free options, showing superior differentiation potential and enhanced safety and genetic modification versatility, respectively[67–71]. Additionally, non-viral based approaches including episomal vectors, self-replicating RNA "srRNA" and nanoparticles delivery are gaining attention recently due to their potential to avert viral integration associated risks e.g. insertional mutagenesis[72–79].

Once reprogrammed, iPSCs can be differentiated into any cell type (e.g. dopaminergic neurons "DANs"); while maintaining the patient's complete genomic background and the capability of self-renewal. After the first successful establishment of PD iPSC models[80], as well as the first differentiation of iPSCs into DANs[81], revolutionary advances occurred in PD models derived from iPSCs, holding a great promise as a valuable tool not only in PD disease modeling, but also cell replacement therapy, and drug discovery[82–87].

Given the valuable contribution and the promising application of iPSCs in PD research as well as the pivotal role of aSyn in PD pathology, this review will highlight the use of aSyn iPSC models in PD encapsulating their potential, limitations and up-to-date applications.

Applying advanced viral and non-viral based approaches, researchers have established various aSyn iPSC lines (derived from patients with sporadic PD, SNCA duplication or triplication, point mutations (e.g. A30P, A53T, E46K, G51D)[63,88–93] which have been utilized in revealing underpinning PD molecular and cellular mechanisms, potential leveraging therapeutics and the role of aSyn in disease progression. Interestingly, episomal vectors and mRNA-based non-viral approaches were employed to generate iPSCs from patients with sporadic PD and missense G51D mutation, respectively[72,94]. Furthermore, genome-editing techniques such as CRISPR/Cas9 and zinc-finger nucleases (ZFNs) have been used to generate isogenic iPSC lines that varies in SNCA gene copies and aSyn expression levels, as well as point mutations' introduction or correction[92,95–102].

Finally yet importantly, iPSCs derived from healthy individuals have a therapeutic potential as well[103]. They serve as a baseline for comparison besides being crucial research models for decreasing experimental variability and improving reproducibility,

investigating non-pathological cellular processes and differentiation potential[87,99,104].

It is noteworthy that the generated iPSC lines must be validated whether they maintain pluripotency and differentiation potential to ensure their use in further downstream applications[72,99,101]. High-content screening and fluorescence-activated cell sorting (FACS) are examples of advanced screening approaches employed to not only select and validate iPSC clones' genetic correction, but to confirm the high quality of generated isogenic iPSCs[96]. (Table 1)

IV.
Applications of aSyn iPSC models in PD research

aSyn iPSC lines have been primarily differentiated into midbrain dopaminergic neurons (mDANs); the most affected neuronal subtype in PD. Studies demonstrate that either SNCA multiplication iPSCs or those from patients with SNCA mutations can still differentiate into DA neurons and their differentiation efficiency are not influenced by elevated SNCA dosage or aSyn overexpression. However, in some models with

TABLE 1. Examples of aSyn induced pluripotent stem cell models

iPSC model	Differentiated cells	Differentiation protocol	Phenotypic manifestations	Methods to induce/detect aSyn aggregation	Main research applications	Ref.
Triplication SNCA	mDANs	Dual SMAD inhibition	↑aSyn mRNA levels;↑aSyn protein expression and release	Not demonstrated	Disease modeling; Addressing epigenetic challenges	[63]
A53T SNCA; isogenic counterparts	(A9) mDANs	Dual SMAD inhibition	LB/neurite-like pathology; ↑Vulnerability to mitochondrial toxins; Mitochondrial dysfunction; ↑NS, OS & resultant apoptosis	Basal aSyn pathology; Mitochondrial toxins; NS, OS/ThT staining; Phosphorylation AB-based detection; WB	Disease modeling; studying gene-environment interactions; Identifying (MEF2C-PGC1α) pathway as a potential therapeutic target; HTS for drug discovery	[95]
Triplication SNCA; Triplication SNCA KD	DANs; NPCs	Neural induction from EBs & Dual SMAD inhibition	↑aSyn expression; Neurite outgrowth deficits; Delayed maturation; ↑Autophagic flux; Electrophysiological impairments	Not demonstrated	Disease modeling; Understanding neuronal differentiation; investigating bioenergetics dysfunctions; Genetic and epigenetic research	[105]
Triplication SNCA; A53T SNCA	pMac	pMacpre	↑Both intracellular & extracellular aSyn levels; Phagocytosis impairment; Cytokine dysregulation	Fibrils assembling from monomeric aSyn/ Fluorescent labeling and microscopy; Flow cytometry	Disease modeling; Exploring non-neuronal contributions to PD	[89]
A53T SNCA	DANs	Dual SMAD inhibition	aSyn & Tau aggregation; Compromised neuritic outgrowth; Axonal neuropathology; Defective synaptic connectivity; Dysregulated synaptic signaling genes' expression; Pathological phenotypes linked to PD-associated dementia	Basal aSyn pathology/ Proteinase K Treatment; ThS Staining; Fluorescence-based assay; WB	Disease modeling; Identifying SMs (NPT100-18A, NPT100-14A, ELN484228) targeting aSyn; Understanding synaptic connectivity; Investigating cellular stress responses	[110]
A53T SNCA; isogenic counterparts; E46K SNCA (hESCs)	(A9) mDANs	Dual SMAD inhibition	↑Accumulation of soluble aSyn in mitochondrial fractions; Impaired mitochondrial dynamics; Fragmented mitochondria; Pathology transmission	Basal aSyn pathology; Cardiolipin interaction & prolonged exposure/ WB analysis; FRET analysis; PLA; SR imaging	Disease modeling; Advancing aSyn immunotherapy; Exploring mitophagy; modeling disease transmission	[102]
Triplication SNCA; A53T SNCA	mDANs	Dual SMAD inhibition	↑Intracellular aSyn accumulation and extracellular release; Oligomeric aSyn pathology; mitochondrial dysfunction and aberrant morphology; ↑ER stress; Lipid metabolism disruption; Lysosomal dysfunction	PLA; MSD; Image acquisition	Disease modeling; Exploring bioenergetic and metabolic Pathways	[90]
YOPD	mDANs	Modified dual SMAD inhibition protocol	Accumulation of soluble aSyn; ↑p-PKCα levels; Lysosomal & mitochondrial dysfunction; ↓Na^+ current	Not demonstrated	Developing a new diagnostic tool; Highlighting the potential of Phorbol Ester drugs as potential therapeutics; Inclusion criteria for mechanistic studies and clinical trials; Suggesting further animal model studies	[118]

TABLE 1. *(continue)*

iPSC model	Differentiated cells	Differentiation protocol	Phenotypic manifestations	Methods to induce/detect aSyn aggregation	Main research applications	Ref.
A30P SNCA; isogenic counterparts	vmDANs	Neural induction from EBs & Dual SMAD inhibition	Neuritic pathology; Mitochondrial dysfunction; ↓DAT gene expression; sporadic presence of astrocytes & ↑expression of radial GPCs; Impaired electrical activity	Not demonstrated	Disease modeling; Understanding energy deficits and vulnerability in PD; Application of high-throughput approaches (MEAs)	*[96]*
Duplication SNCA	mDANs; CPNs; NPCs	FGF8- and small molecule-based midbrain protocol; FGF2-based cortical protocol	↑aSyn pathology; HMW aSyn oligomers' formation; ↑ROS & protein nitration; ↑cell death; Metabolic dysfunction; Mitochondrial impairment	Basal aSyn pathology; ↑ROS/ Sequential protein extraction; WB; Denaturing SDS-PAGE; Phosphorylation AB-based detection	Disease modeling; Understanding neuronal vulnerability; Exploring genetic contributions	[107]
Triplication SNCA; A53T SNCA	mDANs	Modified midbrain FP-based protocol	LB-like pathology; Mitochondrial abnormalities; Vulnerability to mitochondrial damage	Combining seeding with PFFs and extended culture duration/Immunostaining; Phosphorylation AB-based detection	Disease modeling; Understanding genetic influences; Investigating mitochondrial dysfunction	*[88]*
Triplication SNCA	DANs	Dual SMAD inhibition	↑aSyn expression; Altered firing pattern & DA release; ↑Burst activity; ↓D2 receptor functionality	Not demonstrated	Disease modeling; Targeted therapies development (D2 receptor agonists); Gene expression analysis	*[111]*
Triplication SNCA; A53T SNCA; DJ-1 KO	DANs	Modified FP protocol	Nuclear fragmentation; pSYN-positive aggregates; Neuronal death	Treatment with exogenous de novo-generated polymorphs (fibrils or ribbons) or brain-amplified fibrils/Cell Fractionation & WB; FRET assay; BioID2; Confocal microscopy; Mass spectrometry	Disease modeling; Genetic studies & gene therapy; Biomarker discovery through understanding the specific proteins interacting with aSyn aggregates; Identifying potential drug targets (DJ-1)	*[112]*
Isogenic iPSCs panel from Triplication SNCA	mDANs	Neural induction from EBs & Dual SMAD inhibition	aSyn aggregation; mitochondrial dysfunctions & fragmented morphology; ↑OS; Ca^{+2} mishandling; Vulnerability to aSyn aggregation	Basal aSyn pathology; seeding with synthetic fibrils/Nanobody-based biosensor (FluoReSyn); Immunostaining; PLA; Near-IR fluorescence	Disease modeling; Investigating aSyn aggregation; Studying genetic modifiers; Identifying modulators of aSyn aggregates clearance (TAX1BP1)	*[98]*
Triplication SNCA; A53T SNCA; isogenic counterparts	mDANs	SMs-based	↑aSyn expression; Small β-sheet-rich oligomeric aggregates formation; Mitochondrial damage (↓ PTP opening threshold) & fragmentation; Lysosomal pathology; Ca^{+2} desregulation; ↑cell death	Basal aSyn pathology; Endogenous aggregate formation/SML & SR microscopy; ELISA	Disease modeling; Investigating protein aggregation; Exploring Ca^{+2} Dysregulation; Dissecting the temporal sequence of pathological events	*[7]*
A53T SNCA	DANs; NPCs	Neural induction from EBs & Dual SMAD inhibition	↑aSyn mRNA & protein levels; Synaptic defects & early synaptic dysfunction; Poor neuronal networks formation; Overlap with ND disorders	Not demonstrated	Disease modeling; Comparative transcriptomics analysis; Exploring ND components	*[140]*
Triplication SNCA (opto-a-syn); SNCA KO	mDANs; MOs	Neural induction from EBs & Dual SMAD inhibition	Pathological aSyn aggregates; ↓ TH^+ mDANs; ↑PD–related cytokines & chemokines (e.g. MIF)	OASIS; Using optogenetic proteins/Immunostaining & automated image analysis; AIS	Optogenetic control of protein aggregation; HTS & HCS; Exploring autophagy-dependent mechanisms & autophagic clearance promoting cpds (BAG956); Development of drug screening platforms	*[106]*
G51D SNCA	Ectodermal, mesodermal, & endodermal cells	Spontaneous (EBs) & Direct (STEMdiff™ Trilineage Differentiation Kit)	pathogenic c.G152A mutation in Exon 3 of SNCA gene	Not demonstrated	Disease modeling; Genetic and pathological hallmarks studies	*[94]*

TABLE 1. *(continue)*

iPSC model	Differentiated cells	Differentiation protocol	Phenotypic manifestations	Methods to induce/detect aSyn aggregation	Main research applications	Ref.
KOLF2 hiPSC line; Triplication SNCA; isogenic SNCA-2KO; SNCA-4KO	mDAOs; Chimeric mDAOs	Neural induction from EBs & Dual SMAD inhibition	↑aSyn expression; Rotenone sensitivity; DANs vulnerability associated with genes involved in synaptic signaling and cholesterol biosynthesis; Molecular dysfunctions; Translation & OS tolerance	Not demonstrated	Disease modeling; Single-cell transcriptomics; Understanding synaptic signaling and cholesterol biosynthesis; Investigating non-cell autonomous effects and idiopathic PD through chimera organoids	*[104]*
Sporadic PD	vmDANs	Modified FP protocol; Dual SMAD inhibition	↑Pathological aSyn expression with somatic localization; ↓Viability; ↑ROS; Mitochondrial abnormalities; Dysregulated autophagy; Altered neuronal electrophysiology	Immunocytochemistry & WB	Disease modeling; Understanding PD as a generalized disorder rather than a neuron-centric condition.; Providing a platform for biomarker discovery	*[141]*
Triplication SNCA	*mDAOs*	*Neural induction from EBs & Dual SMAD inhibition*	*LB-like inclusions; DANs loss; ↑Apoptosis; Neurite deterioration*	*Basal aSyn pathology; 3D culture system/IF staining & microscopy; Quantification of immunoreactive areas*	*Disease modeling; Exploring spatiotemporal LB-related events; Studying genetic contributions*	*[108]*
Triplication SNCA; isogenic SNCA-4KO	*DANs; FPPs*	*Modified protocol using Dopaminergic Neuron Differentiation Kit*	*↑aSyn expression; Maturation variability; Dysregulated DA release & firing activity; ↓TH neuronal expression*	*Not demonstrated*	*Disease modeling; Development of cell replacement & stem cell-based therapies*	*[97]*
A30P SNCA; A30P_ChR2	*DANs*	*Modified dual SMAD inhibition*	*↑aSyn release with increasing neuronal activity (pharmacologically or by optogenetic stimulation) & vice versa*	*Not demonstrated*	*Disease modeling; Pharmacological and optogenetic Modulation (bicuculline, CNQX, Ch-R2); Exploring a-syn propagation*	*[142]*

LEGEND – **mDANs**: Midbrain dopaminergic neurons; **LB**: Lewy body; NS: Nitrosative stress; **OS**: Oxidative stress; **ThT**: Thioflavin T; **AB**: Antibody; **DANs**: Dopaminergic neurons; **NPCs**: Neural progenitor cells; **EBs**: Embryoid bodies; **pMac**: Macrophages; **pMacpre**: Non-adherent macrophage precursors; **WB**: Western blotting; **HTS**: High-throughput screening; **ThS**: Thioflavin S; **SMs**: Small molecules; **FRET**: Fluorescence Resonance Energy Transfer; **PLA**: Proximity Ligation Assay; **SR**: Super- resolution; **ER**: Endoplasmic Reticulum; **MSD**: Meso Scale Diagnostic Human aSyn Kit; **p-PKCα**: Phosphorylated protein kinase Cα; **vmDANs**: Ventral midbrain dopaminergic neurons; **DAT**: Dopamine Active Transporter; **GPCs**: Glia progenitor cells; **MEAs**: Multielectrode arrays; **CPNs**: Cortical projection neurons; **HMW**: High molecular weight; **ROS**: Reactive oxygen species; **FP**: Floor plate; **PFFs**: Preformed fibrils; **DA**: Dopamine; **BioID2**: Proximity-dependent Biotin identification; **IR**: Infrared; **PTP**: Permeability Transition Pore; **SML**: Single-molecule localization; **ND**: Neurodevelopmental; **TH**: Tyrosine hydroxylase; **MIF**: Macrophage migration inhibitory factor; **OASIS**: Optogenetic Alpha-Synuclein Induction System; **AIS**: Aggregates Induction Score; **HCS**: High-content screening; **cpds**: Compounds; **mDAOs**: Midbrain dopaminergic organoids; **IF**: Immunofluorescence; **FPPs**: Floorplate progenitors; **CNQX**: Cyanquixaline; **ChR2**: Channelrhodopsin-2; ↑: Increased; ↓: Decreased

elevated aSyn expression as well as SNCA mutations, delayed neuronal maturation, compromised neurite growth, poor neuronal activity and increased neuronal death are observed[89,92,97,101,105–107]. Furthermore, increased aSyn expression and SNCA mutations in differentiated neurons are thought to be associated with aSyn aggregation and LB-like pathology; which attributes to mDANs distinct vulnerability resulting in various phenotypic alterations and cellular dysfunctions. These deficits include perturbed synaptic connectivity, mitochondrial dysfunction, calcium dysregulation, elevated endoplasmic reticulum (ER) stress, firing activity and dopamine release dysregulation[88,90,92,98,107–111]. Moreover, upon exposure of differentiated neurons to de novo or brain-amplified fibrils, accelerated aSyn aggregation (in a time and dose-dependent manner) as well as LB-like deposits exist[98,112]. Thereby, aSyn iPSCs and/or derived neurons have been considered valuable and essential models for advancing our understanding of aSyn pathology and propagation in PD through their capability in recapitulating disease-relevant phenotypes, elucidating related cellular dysfunctions and investigating the underlying molecular mechanisms and pathways involved[64,113–115]. Besides facilitating the study of aSyn toxicity, these models have been instrumental, in conjunction with other systems, for the development of personalized medicine and cell therapy strategies[104,116,117], and testing of therapeutic compounds[106,111,118,119] that help mitigate pathology and restore neuronal function.

Recent studies highlighted the prominent implications of midbrain organoids (MOs) as they can mimic

the spatial cellular interactions, hence offering a novel platform for studying the spatiotemporal dynamics of LB pathology and exploring therapeutic interventions in a more physiologically-relevant context using non-invasive approaches[82,93,104,106,108,114,120–122].

Other viable strategies include consolidating stem cell and gene therapy (e.g. knocking down mutant aSyn in iPSCs using shRNA)[123], OASIS (Optogenetic Alpha-Synuclein Induction System) platform for compound screening[106], the promising utilization of extracellular single-chain variable fragments (scFvs) in mitigating aSyn spread[124], rapid aSyn inclusionopathy iPSC models[125], and switching the focus of the therapeutic pipeline on lowering insoluble aSyn to be more on restoring its soluble levels[126]. (Table 1)

V.
Challenges and future directions

Despite the significant insights that iPSC models provide into PD research and treatment, they face challenges associated with recapitulating the multifactorial and heterogeneous nature of the disease[64,127–131]. These limitations reduce their effectiveness in capturing the full-spectrum of PD pathology thus hamper findings' applicability. As demonstrated in (Figure 1), obstacles include confined replication of disease complexity, reproducibility issues, limited predictive value, challenges in modeling aSyn pathology, technical and methodological challenges, genetic and cellular limitations, and ethical concerns[2,64,82,84,93,101,113,114,132–136].

Ongoing efforts have been crucial to refine aSyn iPSC models further in order to enhance their applicability in PD research. A recent body of research has proposed strategies to enhance models' fidelity and complexity, and alleviate temporal limitations and lack of consistency. Several procedures have been promising such as MOs, 3D bioprinting and scaffolding approaches, using multi-modal approaches including mechanistic and artificial intelligence models, rapid induction of aSyn inclusions, and seeding techniques[2,75,82,98,119,134,135,137–139]. Nevertheless, 3D organoid models are more complex and challenging to interpret. A recent study(108) discussed various obstacles that arise for an A53T SNCA-derived MO model, such as inconsistent LB-like pathology with incomplete morphogenesis, difficulties in detection, and limited maturity. They therefore suggested that additional maturation and prolonged culture may be necessary to develop a more complete LB-like pathology.

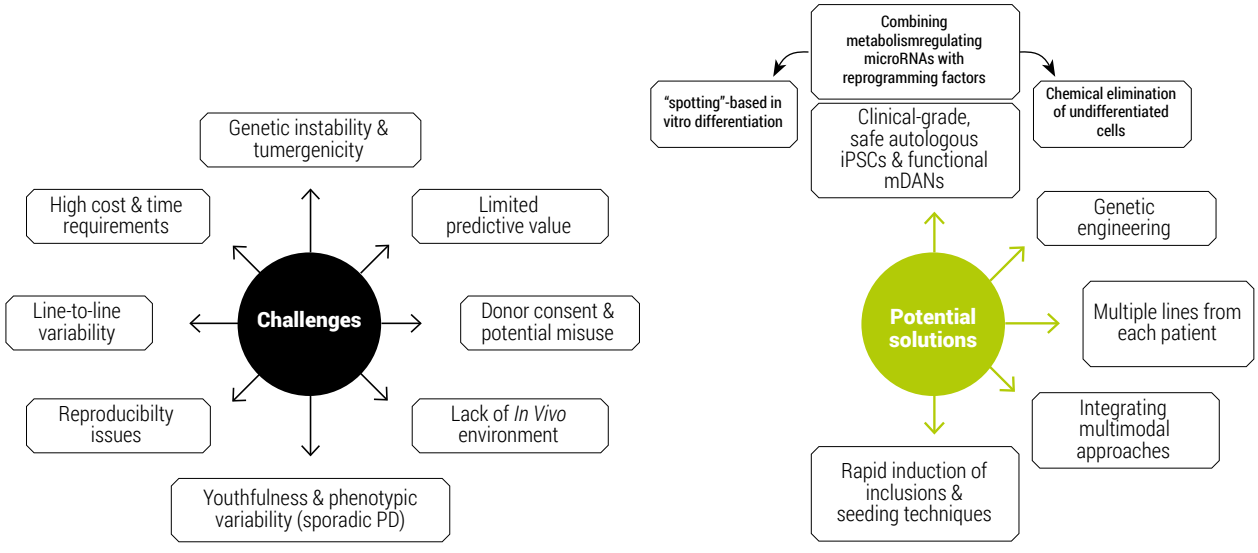

Fig 1. Main limitations of current iPSC models in PD and potential tackling approaches. The use of iPSC models in PD research offers significant potential but also comes with several technical, ethical, and genetic obstacles. Recent studies have proposed a range of methodologies e.g. genetic engineering, and multimodal integration to address substantial challenges.

VI. CONCLUSIONS AND OUTLOOK

Unlike the artificially-derived counterparts, iPSC systems maintain the native cell machinery and transcription feedback mechanisms. Moreover, genetic correction of iPSC lines followed by back transplantation into the same patient is opening up new routes in the development of personalized medicine and PD-directed therapies. In particular, the aSyn

iPSC models provide an exceptional platform for studying the crucial PD pathological features (such as LB formation and neuronal degeneration), discovering novel therapeutic targets, and testing various compounds that might contribute to mitigating the pathology.

To conclude, there is an urgent need for reliable models in PD context to promote further understanding of the disease mechanisms and early diagnosis, leading to the discovery of effective treatment options. The broad contribution made so far by iPSCs technology is recognized, and it surely represents a promising avenue into the future for managing PD. Ongoing refinements and technological developments will be constantly needed in order to take full advantage of iPSCs potentials.

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