Marei *et al.*
Cellular & Molecular Biology Letters (2023) 28:98
https://doi.org/10.1186/s11658-023-00504-2

Cellular & Molecular Biology Letters

REVIEW ARTICLE Open Access

Potential use of iPSCs for disease modeling, drug screening, and cell-based therapy for Alzheimer's disease

Hany E. Marei[1*], Muhammad Umar Aslam Khan[2,3] and Anwarul Hasan[3]

*Correspondence:
hanymarei@mans.edu.eg

[1] Department of Cytology and Histology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35116, Egypt
[2] Biomedical Research Center, Qatar University, 2713 Doha, Qatar
[3] Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University, Doha, Qatar

Abstract

Alzheimer's disease (AD) is a chronic illness marked by progressive cognitive decline and nervous system deterioration. At this time, there is no known medication that will stop the course of AD; instead, most of its symptoms are treated. The failure rates of clinical trials for new drugs remain high, highlighting the urgent need for improved AD modeling for improving understanding of the underlying pathophysiology of the disease and improving drug development. The development of induced pluripotent stem cells (iPSCs) has made it possible to model neurological diseases such as AD, providing access to an infinite number of patient-derived cells capable of differentiating neuronal fates. This advance will accelerate AD research and provide an opportunity to create more accurate patient-specific models of AD to support pathophysiological research, drug development, and the potential application of stem cell-based therapeutics. This review article provides a complete summary of research done to date on the potential use of iPSCs from AD patients for disease modeling, drug discovery, and cell-based therapeutics. Current technological developments in AD research including three-dimensional (3D) modeling, genome editing, gene therapy for AD, and research on the familial (FAD) and sporadic (SAD) forms of the disease are discussed. Finally, we outline the issues that need to be elucidated and future directions for iPSC modeling in AD.

Keywords: Alzheimer's diseases, Induced pluripotent stem cells, iPSCs, Disease modeling, Drug development, Mechanism of diseases, Regenerative medicine, Cell-based therapies

Overview of Alzheimer's disease

Alzheimer's disease (AD), a fatal condition, is a neurological disease characterized by progressively declining cognitive processes, such as memory and learning, and irreversible neurodegeneration [1]. According to van der Flier and Scheltens [2], AD is a major factor causing dementia, a clinical condition with pathological deterioration of multiple cognitive processes including cognition, language, and behavior. Over 46 million people worldwide suffer from dementia, and at least half of them have Alzheimer's disease. This number is expected to rise as the average lifespan rises. As the average age of the

population rises, AD will have a significant negative impact on individuals, families, and healthcare systems. Despite multiple investigations, identifying the underlying causes and possible treatments for Alzheimer's disease remains important. All treatments for AD focus on symptom relief and increased quality of life [3].

According to Stefani and Dobson [4], AD is a heterogeneous disorder with two distinct neuropathological features: development of intracellular neurofibrillary tangles (NFTs) and extracellular amyloid plaques. According to O'Brien and Wong [5], amyloid plaque deposits composed primarily of amyloid beta (Aβ) peptides are generated by proteolytic cleavage of the transmembrane amyloid precursor protein (APP), primarily in neurons. Aggregates of hyperphosphorylated tau protein constitute the majority of NFTs [6] (Fig. 1). NFTs and amyloid plaques were first identified more than 110 years ago, but their link to the cause of AD is still not understood [7]. First proposed in 1984, the amyloid cascade theory [8] is supported by extensive preclinical and clinical studies. Aβ is convincingly linked to the pathophysiology of AD. In mice models with AD mutations, human genetic investigations have successfully recapitulated age-related neurodegenerative elements of AD, delivering helpful molecular insights into cell-type-specific pathways of AD pathogenesis [9]. The difficulty in applying such findings from rodent studies to clinical trials involving AD patients highlights the requirement for more effective models [10]. Humans and rodents have distinctly different expressions and regulations of a number of key AD-associated proteins, which may have adversely affected the results [11, 12].

Recent studies have highlighted the importance of using human cells to model human neurodegenerative diseases, such as brain cells from iPSCs [9]. Research on modeling patient cells has increased dramatically since Takahashi and Yamanaka's discovery of induced pluripotent stem cells (iPSCs) in 2007 [1, 13]. These human iPSCs can successfully differentiate into a variety of different cell types, including cortical neurons [14, 15],

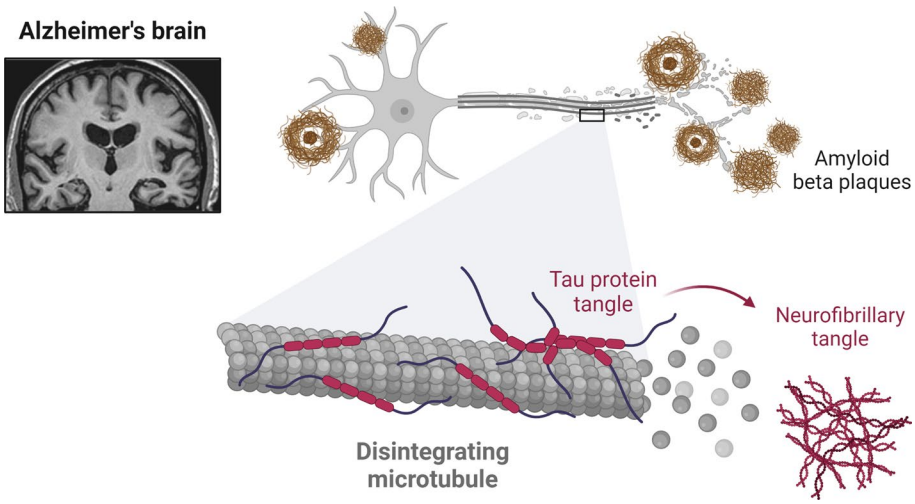

Fig. 1 Alzheimer's brain (disintegrating microtubule). The transmembrane amyloid precursor protein is cleaved by proteases to produce the β-amyloid (Aβ) peptides that make up the majority of the deposits that form amyloid plaques (APP). The bulk of NFTs are composed of aggregates of hyperphosphorylated tau protein

astrocytes [16–19], and oligodendrocytes [20]. Modeling of patient cells in vitro is not possible owing to the limitations of embryo-generated stem cells. Standard methods for mimicking neurodegenerative diseases in iPSCs include taking patient samples (often skin fibroblasts or polymorphonuclear cells (PMNCs) from whole blood) and reprogramming them using one of several methods [21]. Then, these cells are differentiated into a neurological fate and used as tools to study cellular pathology or to find and test potential therapeutics. More effectively extrapolating preclinical results from a range of neuropsychiatric and neurodegenerative disorders to relevant human populations is possible. This review emphasizes how iPSC technology, which is developing quickly, may be used to model AD. To gain molecular insights into the pathogenesis of AD, we also use iPSC-derived brain cell types. This highlights the potential possibility of utilizing iPSC technology for better translational investigations, such as AD modeling, drug discovery, and cell-based therapy.

Pathophysiology of AD

Along with the formation of extracellular amyloid plaques and intracellular neurofibrillary tangles containing hyperphosphorylated tau, the pathological indicators of AD also include widespread gliosis, synaptic dysfunction, and neuronal cell death (p-tau) [22]. According to Chow et al. [23] and Bernabeu-Zornoza et al. [24], Aβ peptides sequentially released from amyloid precursor protein (APP) by β-secretase and γ-secretase form amyloid plaques. α-Secretase and γ-secretase can also sequentially cleave APP, producing non-amyloidogenic fragments [23]. Since APP and β-secretase are highly expressed in neurons, most Aβ is produced in neurons [15] (Fig. 2). The most prevalent, Aβ42 and Aβ40 isoforms, which are the subject of AD study, are present in all Aβ species.

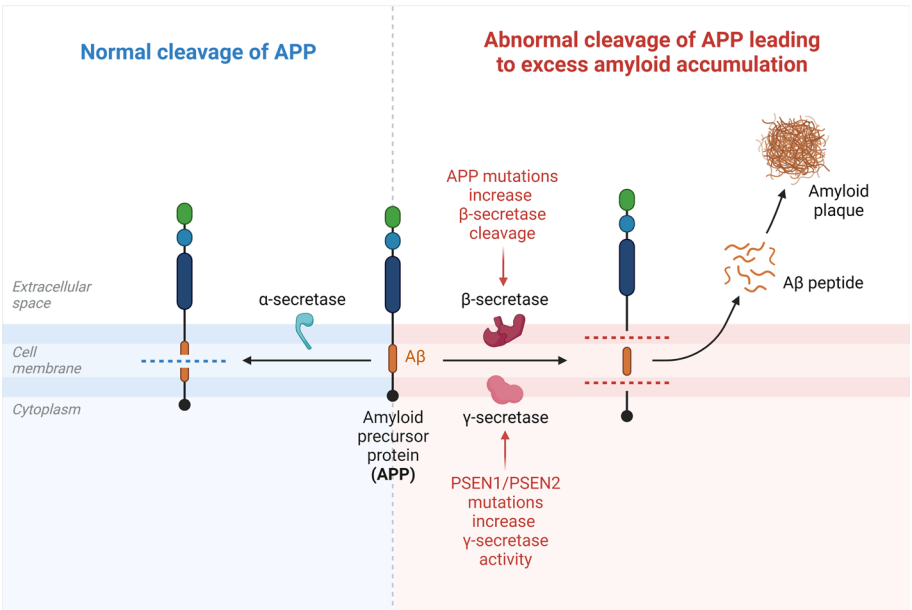

Fig. 2 Cleavage of amyloid precursor protein (APP). Aβ peptides, which are sequentially released from the amyloid precursor protein (APP) by β- and γ-secretase, are responsible for amyloid plaques. In addition, α-secretase and γ-secretase can sequentially cleave APP, yielding non-amyloidogenic fragments. Since APP and β-secretase are primarily expressed in neurons, most Aβ is produced in neurons

In human AD brains, these isoforms are present in amyloid plaques [25]. In contrast to other forms, Aβ-42 is formed in dense nuclear plaques in the brain parenchyma and has a high fibrosis rate and insolubility. Because it is more soluble, the most common form of Aβ, Aβ40, causes amyloid to accumulate in blood vessel walls and cause cerebral amyloid angiopathy (CAA). Reduced cerebrospinal fluid (CSF) Aβ42/Aβ40 ratio, suggesting reduced CSF-mediated Aβ clearance and increased buildup of amyloid plaques in the brain parenchyma, is a powerful diagnostic feature for AD [26]. This study shows that soluble Aβ42 oligomers impair glutamatergic neurotransmission, cause synaptic loss, and alter synaptic plasticity, and are thus more detrimental to AD patients than the Aβ protein found in amyloid plaques. [27] In addition to Aβ-induced toxicity, numerous investigations have demonstrated the molecular relevance of altered APP metabolism and loss of γ-secretase function as contributing to the pathogenesis of AD [28].

Tau pathology in Alzheimer's disease typically develops after Aβ pathology and may be brought on by Aβ [29]. The *MAPT* gene produces the microtubule-associated protein tau. Under physiological conditions, tau is essential for microtubule stabilization, regulation of microtubule assembly dynamics, and axonal transport [30]. Six tau isoforms are produced through alternative splicing of the *MAPT* gene exons 2, 3, and 10 [31]. Tau proteins with zero and two nucleotide repeats are generated by splicing exons 2 and 3, while tau proteins with three or four microtubule-binding domains are expressed by splicing exon 10 (3R or 4R dew) [32]. During the pathogenesis of AD, tau disease spreads like a prion and follows a stereotypical pattern. The integrity of this structure, which first develops in the locus coeruleus of the brainstem, is related to the neuropathology and cognitive function in AD patients [33]. Tau disease begins in the locus coeruleus of the entorhinal cortex and later extends to the hippocampus and neocortex [34]. Entorhinal cortical neurons expressing tungsten-1 are known to send toxic tau to hippocampal neurons [35].

Another important pathogenic aspect of AD is disturbance of the blood–brain barrier (BBB), and recent studies have shown that degradation of BBB pericytes contributes to neurovascular dysfunction and exacerbation of Aβ and tau pathology, a relation that was shown in Ref. [36]. Interestingly, AD is consistent with the deposition of Aβ, which can signal pericytes to constrict capillaries [37]. A lot of work has been done to create neurons from adult human brain pericytes to treat AD [38]. The use of patient- and control-specific iPSCs for disease modeling has been shown to be beneficial for disease modeling, drug screening, and cell-based therapeutics (Fig. 3). Early-onset familial AD (FAD) and sporadic AD (SAD) are the two main types of AD [39]. The *APP* gene and the *PSEN1* and *PSEN2* genes, which encode presenilin 1 and 2, respectively, are two examples of genes involved in Aβ synthesis that can be mutated and cause FAD [40]. The *APP* and *PSEN2* loci, respectively, contain roughly 30 and 20 recognized changes, and *PSEN1* has been associated with about 200 pathogenic variants [41]. All of these pathogenic FAD gene mutations result in an increase in overall Aβ42 or the Aβ42/Aβ40 ratio [42]. FAD accounts for 1–5% of all AD cases, and most AD cases are sporadic. Genome-wide association study (GWAS) has revealed over 40 genes associated with an increased risk of developing Alzheimer's disease, including the highly expressed glia-specific genes *APOE4*, *TREM2*, *ABCA7*, and *SORL1*. GWAS has also been used to identify the molecular mechanisms behind AD development [43]. These findings demonstrate that

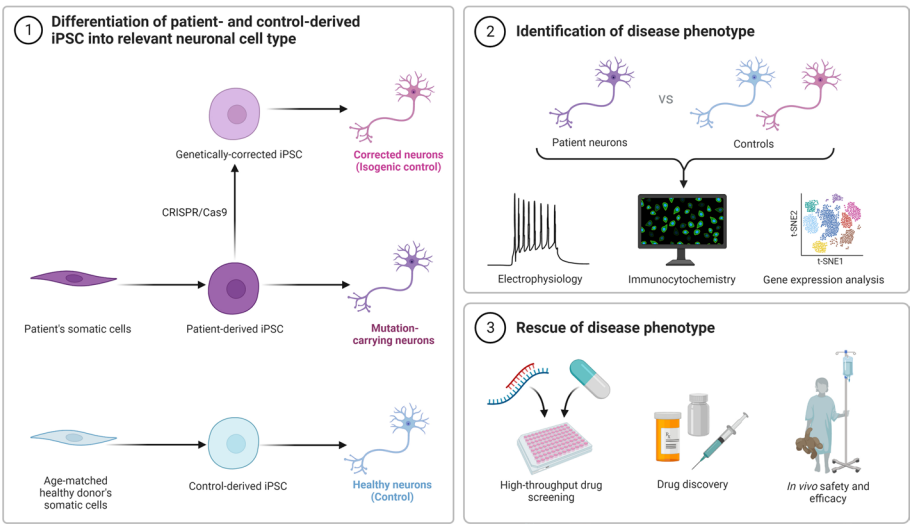

Fig. 3 Modeling AD disease using iPSCs. First is the development of suitable neuronal cell types derived from patient and control iPSCs. The next step is to describe the disease phenotype using various functional and genomic analyses. High-throughput drug discovery and screening might be performed on the patient and control iPSC-derived cells

astrocytes and microglia, among other noncell autonomic neuronal processes, largely contribute to the neurodegeneration in AD. According to Holtzmann et al. [39] and Selkoe and Hardy [44], amyloid pathology occurs at the onset of both FAD and SAD cases, followed by tau pathology and cognitive impairment.

According to Takahashi and Yamanaka [13], mouse and human fibroblasts can become pluripotent when four different exogenous transcription factors (Oct4, Sox2, cMyc, and Klf4) are overexpressed. At this stage, cells can develop into all kinds of somatic cells. To this point, most iPSC studies have focused on developing cell lines with FAD-associated mutations, as the monogenic pathogenesis of FAD makes this an interesting alternative to model AD from patient-derived cells. It is not unexpected that all induced pluripotent stem cell (iPSC) models of familial Alzheimer's disease (FAD) have been created by introducing mutations in the *APP*, *PSEN1*, or *PSEN2* genes. Generally, mutations have the effect of augmenting the production of Aβ, enhancing its tendency to aggregate, facilitating the creation of harmful aggregation structures, and influencing processes that encourage the generation of Aβ42, the primary isoform of Aβ implicated in the pathogenesis of Alzheimer's disease [45]. These genetic changes also result in reduced functionality of γ-secretase. The existence of these genetic modifications has been suggested to indicate supplementary paths to neurodegeneration and Alzheimer's disease (AD), which have not been extensively investigated [46]. Pathogenic mutations in *PSEN1* and *PSEN2* disrupt the catalytic subunit of γ-secretase., which also increase the Aβ42/Aβ40 ratio.

In addition, amyloid plaques and NFTs are produced by trisomy of chromosome 21, which causes Down's syndrome (DS). This is most likely the result of increased gene dosage, as chromosome 21 contains the gene for APP. Overall, owing to its monogenic nature, FAD is a perfect illness to simulate in patient-derived iPSCs and provides a well-defined and controllable etiology for the observed pathology [47].

iPSCs and AD modeling

According to Yagi et al., both secretion and the Aβ42/Aβ40 ratio are increased in developing neurons in *PSEN1* and *PSEN2* mutant FAD [48]. Israel et al. shortly thereafter described the generation of FAD iPSCs in which differentiated neurons from patients with both SAD and *APP* duplication displayed increased phosphorylated tau [49]. The Aβ42/Aβ40 ratio and total and phosphorylated tau generally contributed 1.2- to 5-fold greater AD pathogenesis, respectively, in subsequent studies using FAD-iPSC-derived models.

Moore et al. generated nerve cells from AD patients with mutations in *PSEN1* or *APP*, or trisomy 21, and used them to decipher theAβ/p-tau connections in vitro using iPSCs [50]. This study revealed a direct correlation between increased levels of total and phosphorylated tau and AD mutations (V717L mutation and *APP* duplication) that increase *APP* dosage. Additionally, they discovered that γ-secretase inhibition (GSI) markedly elevated total tau, whereas γ-secretase modulator (GSM), a substance that specifically disrupts γ-secretase APP processing activity, increased total tau. Li et al. discovered that DS neurons displayed a notable increase in protein p44 [51], a p53 tumor suppressor protein variant that has been discovered to produce cognitive deterioration similar to that of late aging and increased tau phosphorylation in mouse models when overexpressed [52].

Although replication of neurodegenerative changes in iPSC-derived nerve cells can be challenging, recent studies have demonstrated that there is a significant gene expression overlap and link between Aβ and tau species. This has demonstrated the value of using iPSCs to clarify the fundamental pathophysiology of AD in humans [53]. Another team demonstrated that neurons with *APP* and *PSEN1* mutations exhibited reduced general autophagy and lysosomal activity by blocking γ-secretase with γ-secretase inhibitors (GSI), and further suggested that FAD mutations are a direct cause of autophagy impairment [54]. It is important to note that healthy neurons exhibited mitochondrial dysfunction when extracellular vesicles from individuals with *PSEN1* mutations exhibited high Aβ42/Aβ40 ratios. Furthermore, lysosomal dysfunction caused by impaired autophagy resulted in increased pathogenic extracellular vesicles with high Aβ42/Aβ40 ratios [55].

iPSCs are an excellent cell source for studying pathogenic changes in human neurons associated with AD. Early studies showed that some of the key regulators and APP processing machinery were expressed in human iPSC-derived neurons, including β-secretase and γ-secretase, and a range of different APP and Aβ37-42 isoforms have been shown to be expressed at the N-terminus-truncated Aβ2-40 [56]. Additionally, in human iPSC-derived neurons, many tau isoforms, including 3R and 4R tau, display a developmental pattern [57]. In cortical neurons created from human iPSCs and mouse models of tauopathy, increased neuronal activity promotes the distribution of tau and favors the development of tau disease [58]. Low-density lipoprotein receptor-related protein 1 (LRP1) has recently been identified as a receptor that controls the endocytosis and spread of tau, as shown in human iPSC-derived neurons [59]. Human iPSC-derived cortical neurons and organoids with FAD mutations in *PSEN1* (PS1-DE9 and M146V mutations) and *APP* (KM670/671NL; Swedish mutations) exhibit abnormally increased electrical activity when compared with their isogenic WT controls [60]. HiPSCs were generated using dermal fibroblasts from AD patients harboring the *PSEN2* N141I

missense mutation. The N141I missense mutation was corrected through the utilization of genome editing technologies, resulting in the identification of iPSC colonies that exhibited recognition by pluripotent marker labeling [61].

Improved understanding of the molecular mechanisms behind AD pathogenesis has been achieved through the characterization of iPSC-derived neurons with FAD mutations. In animal models of AD, there is mounting evidence that Aβ causes aberrant tau production and accumulation [62], and this pathogenic characteristic may be reproduced in neurons made from iPSCs. The *APP* London mutation (V717I) causes aberrant *APP* cleavage and enhanced Aβ production in forebrain neurons made from iPSCs, which raises levels of total tau and p-tau [63]. These results demonstrate that tau pathology is an unfavorable effect of Aβ and that treating Aβ early in the course of AD development may be a successful therapeutic strategy. Research on iPSC-derived neural progenitor cells (NPCs) and neurons with FAD or SAD mutations/mutations, including hers, has shown that her FAD mutations in the *APP*, *PSEN1*, *PSEN2*, and *APOE* (APOE4) loci increase levels of Aβ-induced p-tau in wild-type (WT) neurons [64–66].

The importance of the 3D environment in recreating important clinical characteristics of AD is highlighted by the observation that human neural progenitor cells overexpressing FAD *APP* and *PSEN1* mutations have an increased Aβ42/Aβ40 ratio that encourages the production of neurofibrillary tangles in a 3D culture system [67, 68]. Furthermore, the development of brain organoids, which have served as representations for AD, can produce a 3D environment (Fig. 4). Using microglia made from iPSCs, several research studies have established a viable disease modeling method. However, there are considerable technological constraints to using these human microglia. For instance, it is challenging to study the interactions between various brain cell types and microglia in a controlled culture environment, and alterations in the microglia transcriptome are sensitive to medium composition [69]. iPSC-derived microglia are relevant to the study of AD, according to recent studies [70, 71]. However, because microglia and neurons have different embryonic origins, it can be challenging to discriminate between the two. Early on in the process of hematopoiesis, progenitors found in the yolk sac give rise to microglia, which are later produced by mesoderm that migrates to the neural tube [72]. Thus, microglial cells have a separate embryonic origin from neurons, astrocytes, and oligodendrocytes, which are formed from neuroectoderm and can be isolated from NPCs [73]. To increase human iPSC-derived microglia through lineage status analogous to hematopoietic progenitor cells (HPCs) in vitro, numerous approaches have been devised to provide key components for imitating microglial embryonic development [19, 74].

iPSC-derived microglia have also been used to explore the signaling processes of AD-related genes. For instance, AD is prevented by the PLCG2 functional gain-of-function variation P522R [75]. Recently, iPSC-derived TREM2- and PLCG2-deficient microglia were found to have similar clinical features, including increased lipogenesis, impaired phagocytosis, and decreased cell viability [76]. This study's use of genetically modified iPSC-derived microglia supported this finding by demonstrating that PLCG2 was required for downstream TREM2 signaling [76]. These iPSC-based research findings demonstrate that intrinsic microglial dysfunction and AD are related. Single-cell RNA sequencing (scRNA-seq) studies revealed that transplanted iPSC-derived microglia maintained their identity and had a range of gene

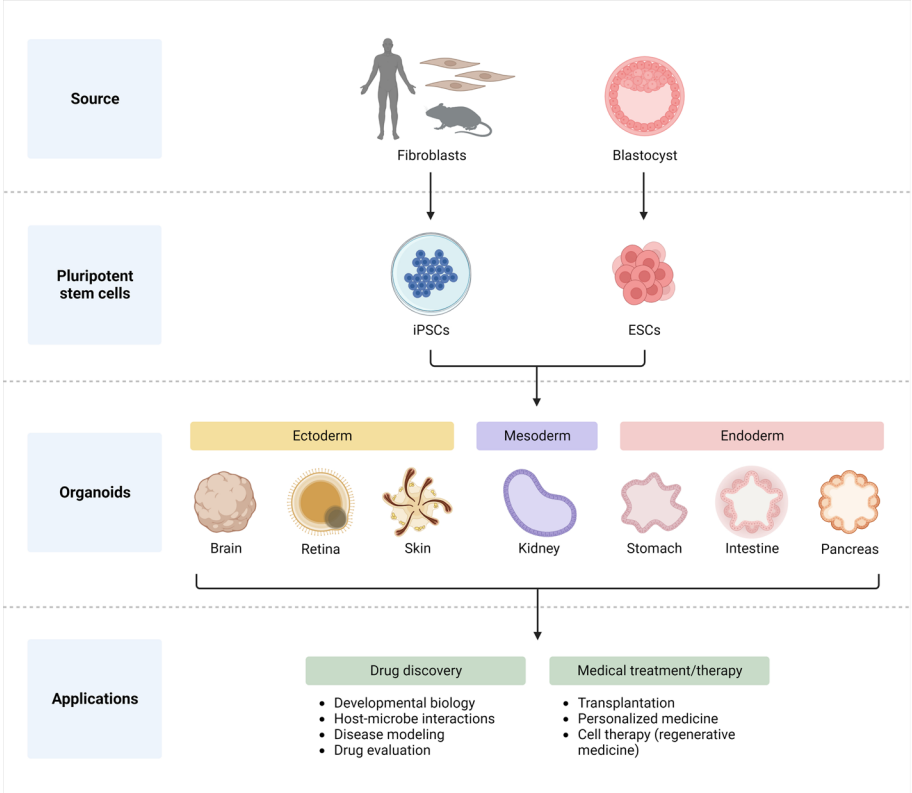

Fig. 4 Organoid generation from pluripotent stem cells. Blastocysts or somatic cells can be used to create pluripotent stem cells such as iPSCs and ESCs. These cells are differentiated into cell types that are embryologically separate and could be used to create organ-specific organoids. The application of the organoids for cell therapy, transplantation, customized medicine, and drug discovery follows

expression patterns that were strikingly similar to those of primary human microglia [77]. An equivalent model of microglial transplantation using human embryonic stem cells has also been reported [78]. Microglia derived from TREM2-deficient human iPSCs replicated key clinical features of TREM2-deficient human AD brains. These include defects in APOE phagocytosis and failure to surround amyloid plaques [79]. Furthermore, scRNA-seq studies have revealed that transplanted TREM2-deficient microglia failed to upregulate the human *DAM* gene. In a previous study, similar conclusions about the decreased function of TREM2 were reached [12]. In a different study, human iPSC-derived microglia from people with the TREM2 R47H mutation were implanted into neonatal mouse brains. This experiment showed decreased susceptibility to amyloid plaques and decreased lipid droplet formation [80], further highlighting TREM2's significance in the setting of AD. Together, these investigations indicate promise for disease modeling approaches using iPSC-derived microglia. Human iPSCs could be used to differentiate oligodendrocytes, and these cells were incorporated into brain organoids and successfully survived after being transplanted into the brains of myelin basic protein-deficient mice [81, 82]. An iPSC model of AD oligodendrocytes to study oligodendrocyte function during AD pathogenesis has yet to be published. Astrocytes can be distinguished from human iPSCs and have been used to study disease processes associated with AD [9, 19]. According

to several studies [74, 83], atrophy, increased Aβ secretion, altered inflammatory responses, aberrant calcium signaling, increased oxidative stress, and neural support are all linked to iPSC-derived astrocytes with *PSEN1* mutations. The morphology of astrocytes from APOE4-positive SAD patients is also changed, leading to an increase in the production of inflammatory cytokines, a decrease in the absorption of Aβ, a breakdown of lipid homeostasis, and an accumulation of lipid droplets [64, 84]. TNF-α released from microglia is capable of activating iPSC-derived astrocytes and interacts with microglia via complement C3 [19]. In addition, astrocytes secrete interleukin-3 (IL-3), which attracts microglia and activates them to eliminate Aβ and tau in response to stimuli associated with AD [19, 85]. Oligodendrocytes can be differentiated from human iPSCs, and these cells have been integrated into brain organoids and successfully survived after being injected into mouse brains lacking myelin basic protein [1, 2]. However, no published iPSC models of AD oligodendrocytes are currently available to investigate the role of oligodendrocytes in AD pathogenesis.

Human induced pluripotent stem (hiPS) cells undergo a process of cellular differentiation, resulting in the emergence of neuronal cells that exhibit the expression of the forebrain marker, Foxg1, as well as the neocortical markers, Cux1, Satb2, Ctip2, and Tbr1. The neuronal cells produced from induced pluripotent stem cells (iPSCs) also exhibited the expression of amyloid precursor protein, β-secretase, and γ-secretase components. Furthermore, these cells demonstrated the ability to secrete Aβ into the conditioned media. The generation of Aβ was hindered by the administration of a β-secretase inhibitor, a γ-secretase inhibitor (GSI), and a nonsteroidal antiinflammatory drug (NSAID). However, notable variations in the response to these three treatments were observed between the early and late stages of differentiation. During the first phase of differentiation, administration of GSI therapy resulted in a rapid rise in Aβ levels at lower doses (referred to as Aβ surge), followed by a significant decrease in Aβ production. The findings of this study suggest that the neuronal cells obtained from human induced pluripotent stem cells (hiPS cells) display functional β- and γ-secretases, which are known to be involved in the generation of Aβ. However, it is important to note that, to effectively screen anti-Aβ drugs utilizing these hiPS cell-derived neuronal cells, it is necessary to ensure an adequate level of neuronal development [86].

Wang et al. (2017) devised a resilient high-content screening assay for the purpose of identifying compounds that have the ability to reduce tau levels. In their study, they specifically focused on the Library of Pharmacologically Active Compounds (LOPAC) and successfully discovered adrenergic receptor agonists as a distinct class of compounds that exhibit the capability to decrease endogenous human tau. These methodologies facilitate the utilization of human neurons for conducting high-throughput screening of pharmaceutical compounds aimed at addressing neurodegenerative disorders [87]. In their study, Kondo et al. employed human-induced pluripotent stem cell (iPSC)-derived neurons, which possess the unique characteristic of human-specific drug responsiveness, in order to facilitate medication development targeted toward Alzheimer's disease (AD). Through the utilization of induced pluripotent stem cell (iPSC)-based screening of pharmaceutical compounds and employing chemical clustering techniques, the researchers were able to identify a specific combination of preexisting medications that exhibited a synergistic effect in enhancing the phenotypes associated with Aβ accumulation in cells

affected by Alzheimer's disease (AD) [88]. To provide insights into the genetic basis of Alzheimer's disease (AD), Kondo et al. (2023) successfully created models of AD using patient-derived cells with the aim of providing deeper understanding of the genetic factors that contribute to sporadic Alzheimer's disease (SAD) cases [89].

iPSCs and drug screening for AD

As part of the drug development process, various therapeutic targets are identified through intensive functional and genomic research. Drugs developed for different targets are examined through in vitro, in vivo, and toxicological studies to obtain meaningful preclinical data. Drug candidates qualify for clinical trials by providing preclinical evidence that is reviewed and approved after safety and efficacy assessments (Fig. 5). iPSCs isolated from AD patients are undoubtedly a powerful platform for identifying new drugs and interesting targets, but their acceptability and safety to people are often unpredictable [47]. Because iPSC-derived CNS cell types offer novel AD therapies, it remains to be seen whether they outperform current preclinical models in terms of translational efficacy. The field of drug development holds significant scientific significance in relation to the iPSC FAD model. The aforementioned investigations have demonstrated that GSI contributes to the understanding of the physiological mechanisms behind Alzheimer's disease (AD). It is noteworthy that GSI has been examined extensively in the context of generating and evaluating AD-induced pluripotent stem cells (iPSCs) [50, 82].

The ability of iPSC lines to respond to potential pharmacological therapies can be assessed on the basis of the mechanism of GSI preventing Aβ production [48, 54]. In addition, the therapeutic potential of GSIs, especially the latest, second-generation GSIs, has been investigated using iPSCs [90]. Although in vitro results of screening GSMs

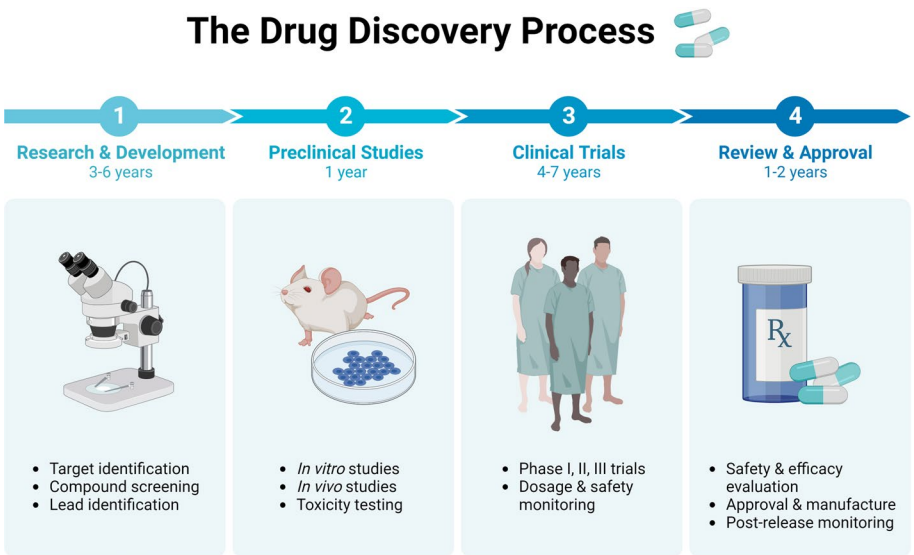

Fig. 5 The drug discovery process. The drug discovery process involves the identification of different therapeutic targets on the basis of extensive functional and genomic studies. Drugs designed to target different targets are tested in vitro and in vivo, and by toxicity testing to provide convincing preclinical evidence. The provision of preclinical evidence can qualify a candidate drug for clinical trials, followed by review and approval after evaluation of its safety and efficacy

for therapeutic potential with iPSCs were encouraging [48, 54, 90], the success of subsequent studies was limited by weak drug-like properties [91]. Using patient-derived iPSCs, drugs that do not manipulate γ-secretase have also been tested, with some success [92]. Tau in *APP* early mutant neurons was successfully reduced by Aβ antibodies, as seen by Muratore et al. [63]. The small molecule -butylidenephthalide, which is derived from chloroform extracts of *Angelica sinensis*, reduces total tau and phosphorylated tau levels in DS neurons, but neither Aβ-42 nor the Aβ-42/Aβ-40 ratio show any discernible decreases [93]. Additionally, when given to both FAD and SAD neurons, the natural polyphenol apigenin, which is present in many plants, demonstrated neuroprotective properties against inflammatory stress brought on by microglia [94]. Cholesterol metabolism has also been discovered as a possible druggable target for FAD, as *APP* FAD mutations result in elevated cholesterol esterase, which has been shown to affect both Aβ and tau [95].

Drug testing of patient-derived SAD iPSC models is an important research area as SAD accounts for more than 99% of all cases of AD [96–98]. Similar to FAD, GSI has been investigated as a validator for drug screening in SAD neurons. Another example of the diversity of pathophysiology identified in SAD cell lines come from Hossini et al., who performed GSI on two of their SAD cell lines and found reduced phosphorylated tau in only one of them [99]. Israel et al. demonstrated that phosphorylated tau and GSK-3 activity were decreased by γ-secretase inhibitors but not by GSI. This was a common feature of FAD-derived cells [49].

iPSCs and genome editing for AD

AD is still not treatable with effective targeted therapy, which is one of the causes of a significant public health burden. Genome engineering and induced pluripotent stem cells (iPSCs) are two revolutionary technologies being developed simultaneously that could change this. Investigating the underlying causes of disease and identifying therapeutic targets in AD is hampered by the largely inaccessible human central nervous system. Heterogeneous in vitro cell cultures and animal models shed light on the pathophysiological mechanisms underlying various neurological diseases, including AD. However, these models only partially reconstruct disease development and do not accurately reflect human physiology, metabolism, or homeostasis [100]. As a result, failure rates are high in both innovative therapeutics discovery and clinical trials for neurological disorders. Thus, iPSC patient-derived neurons provide a unique in vitro model for studying AD. They provide a limitless supply of genetically identical patient-derived cells that enable the study of disease-associated signaling pathways. They offer humanized models for testing new medicines, which might hasten their adoption. Additionally, they offer a trustworthy source of cells for cell replacement therapy in neurological conditions such as AD. Since their introduction, gene editing techniques have proven useful in creating in vitro disease models [103–107].

The discovery of the DNA-binding zinc finger nuclease (ZFN) technique boosted the effectiveness of genome editing in mammalian cells [108], which led to the creation of the first knockout rats [108]. Patient-derived iPSCs have been used to correct genetic mutations using ZFN-based genome editing [109] or to incorporate known disease-associated mutations into iPSCs produced from healthy people [110]. With the discovery of

transcription activator-like effector nucleases (TALENs), which have been shown to be valuable tools for the creation of animal models, genome editing technology was further refined [111]. TALENs have also been applied in neuropathy research by introducing disease-causing mutations into control iPSCs and/or reversing genetic mutations in patient-generated iPSCs [112]. This has increased confidence in developments regarding underlying mechanisms and therapeutic strategies.

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas9) technologies quickly developed after TALEN technology and have been shown to be capable of editing the mammalian cell genome in both culture and animal models [113]. Compared with ZFNs or TALENs, CRISPR/Cas9 uses different DNA cleavage and binding modules. However, to specifically bind target DNA sequences and activate Cas9, the CRISPR/Cas9 system depends on CRISPR RNA (crRNA), trans-activating RNA (transRNA), and a particular natural endonuclease. The CRISPR-based gene editing technique has demonstrated effectiveness for gene alteration, gene expression regulation, epigenetic regulation, and chromatin manipulation both at the single-gene level and in large-scale screening owing to its versatility and robustness [114]. Because of this, CRISPR-based technology has quickly taken over as the go-to technique for altering genomes, particularly in iPSC model systems. Furthermore, genome editing of control strains allows multiple variants to be studied simultaneously in the same genetic context. This may be more practical than assembling a substantial number of patient strains to explore related disease pathways [115].

Another study that corrected LRRK2 mutations revealed both LRRK2-dependent and LRRK2-independent effects that are probably genetically influenced and connected to different familial Parkinson's disease clinical presentations. It presents characteristics and varying degrees of severity [116]. Isogenic regulation can also indicate that some cell phenotypes depend on the genetic background even under monogenic conditions.

Different apolipoprotein E4 gene genotypes are associated with the risk of sporadic Alzheimer's disease (*APOE4*) [117]. In several studies, a patient's *APOE4* gene was converted to *APOE3* by iPSCs, while a neutral-risk gene (*APOE3*) was converted to *APOE4* (high risk) by using healthy individuals. This "rescue" of iPSC risk status from individuals prone to develop AD later in life impairs the inability of glial cells to clear extracellular Aβ and increases Aβ aggregates in cerebral organoids [118].

Understanding the pathophysiological pathways associated with disease-related gene alterations has been enabled by gene editing in iPSC systems. However, genome engineering can be combined with transcriptome studies to more thoroughly investigate the underlying causes of disease. To research AD in early-onset Down syndrome patients, CRISPR/Cas9 was utilized to remove the extra copy of *APP* from the T21 lineage, and inducible CRISPRa was employed to boost *APP* gene expression [119]. Levels of the *APP* gene have been found to be associated with Aβ formation, but not with other cellular traits associated with AD such as apoptosis. The use of CRISPR screening to uncover disease pathways is discussed in greater detail below. In AD, certain brain areas seem to be particularly impacted by the development of Aβ plaques. Brain areas in AD imply vulnerability, and neurons from patient-derived iPSCs carrying *APP* mutations were differentiated with either a caudal (hindbrain) or rostral (forebrain) destiny. Forebrain neurons displayed more severe tau reaction [120]. A study of the effect of the *APOE4*

genotype in microglia [74] showed that SAD is more likely to affect some cell types than familial AD. Utilizing the pluripotency of iPSCs could help identify potential illness causes and tissue-specific treatment options. Oikarie et al. investigated the impact of familial AD mutations in *PSEN1* on the development of the blood–brain barrier (BBB) by generating induced brain endothelial cells (iBECs) from patient-derived and isogenic lines [121]. Mutant iBECs showed abnormal expression of adherin and tight junction proteins. This could be a novel way to improve CNS medication delivery in AD because AD and isogenic iBECs responded differently in iBEC cultures.

In a separate study focused on familial AD (*APP*), 200 heterozygous disease-causing mutations in presenilin isoforms (*PSEN1* and *PSEN2*) and amyloid precursor protein were screened using the CRISPR/Cas9 system [111]. Cortical neurons generated from multiple, genomically altered iPSC lines were subjected to transcriptomic and translational analyses, which revealed that AD family mutations in two distinct genes are connected to the endocytic/endosomal trafficking pathways previously linked to late-onset AD. They turned out to have overlapping effects. By demonstrating that the genesis of familial and sporadic AD may share a network of cellular and molecular changes, our finding offers a shared therapeutic objective. In light of this, combining CRISPR KO and CRISPR KI screening methods with iPSC-based illness modeling may enhance comprehension of pathophysiological signaling networks and direct therapeutic strategies for neurological diseases.

As indicated above, new developments in electrophysiology and transcriptome analysis have demonstrated that, even after prolonged culture, iPSC-derived neurons only represent late stages of fetal development [122, 123]. This is acceptable for early-onset and/or highly penetrant monogenic disorders with cell-autonomous phenotypes, but it is challenging to identify in vitro late-onset phenotypes or those in which environmental variables play a significant role. There is still concern that these will not be accurately duplicated. Progesterone, telomere shortening, expression, direct differentiation, pharmacological signaling, and other mechanisms can inhibit this when reprogramming has not taken place [124]. A number of factors, such as reprogramming-induced epigenetic alterations and genomic instability, background genetic dispersion, and variations in differentiation propensity, contribute to the innate diversity and heterogeneity of iPSC-derived neurons [125].

To study juvenile Alzheimer's disease in a patient with Down's syndrome, excess copies of *APP* from T21 strain were removed using CRISPR/Cas9 and *APP* gene expression was boosted using inducible CRISPRa [119, 126]. The use of iPSC-based disease models for both Mendelian and more complicated neurological illnesses has been transformed by genome editing. The enhanced accuracy of CRISPR gene editing, promoter regulation, and epigenome editing, along with an individualized patient-derived iPSC model system, may result in a paradigm change in how neurological illnesses are seen and treated.

iPSC cell-based therapy for AD

Since the discovery of iPSCs, innovative techniques utilizing cells produced from iPSCs have provided crucial insights into the pathogenesis of AD and prospective AD therapies. A number of recent studies using animal models have shown that cell replacement therapy can help alleviate disease conditions and improve cognitive performance. In

the following section, we review the advantages, disadvantages, applicability, potential use in clinical settings, and safety and ethical considerations of cell replacement therapy (Fig. 6).

In clinical studies on AD, disease-modifying treatments have been explored extensively, but nearly all of them were abandoned in phase 3 trials because they either did not show any cognitive benefit or had severe adverse effects (https://Clinicaltrials.gov). Acuranumab, a human IgG1 antibody, recently received accelerated FDA approval for the treatment of all stages of dementia. However, there has been a lot of debate about the FDA's approval of aducanumab for AD. This is due to the fact that aducanumab was excluded from several clinical trials and did not show cognitive advantages in phase 3 trials [127]. These extracellularly focused strategies may rarely restore all of the damaged neurons. The binding characteristics of lecanemab, aducanumab, and gantenerumab to various Aβ species were investigated using inhibitory enzyme-linked immunosorbent assay (ELISA), immunodepletion, and surface plasmon resonance techniques. All three antibodies showed modest affinity for binding monomers. Nevertheless, it should be noted that lecanemab and aducanumab had very low affinity toward monomers, while gantenerumab showed comparatively higher binding affinity. Lecanemab exhibited a notable characteristic in that it demonstrated a binding strength that was ten times greater toward protofibrils as compared with fibrils. Aducanumab and gantenerumab had higher affinity for binding to fibrils compared with protofibrils [128]. The findings of this research demonstrate the distinct binding profiles exhibited by lecanemab, aducanumab, and gantenerumab, which could potentially elucidate the reported clinical outcomes pertaining to the efficacy and adverse effects associated with these antibodies [129]. There is recent evidence that the classical amyloid hypothesis might not fully reflect all aspects of AD and that, for example, tau pathology even precedes the

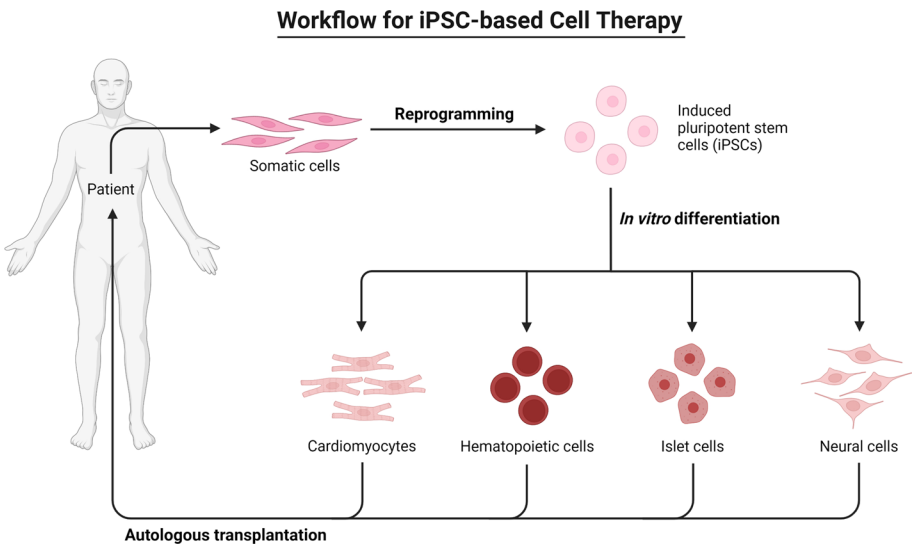

Fig. 6 Workflow for iPSC-based cell therapy. Somatic cells undergo iPSC reprogramming. Following differentiation of iPSCs into various cell types utilizing various particular methods, the patient receives an autologous transplant

formation of plaques, in such a way that the tau pathology is still benign and "boosted" by plaque formation [130–132].

Therapies using stem cells to replace missing or defective neurons can alleviate these problems and make them functional. This has sped up the creation of improved stem cell treatments [13].

Since 1995 [133], when mesenchymal stromal cells (MSCs) were initially utilized as therapeutic agents in clinical trials, cellular therapy has drawn interest worldwide [134]. MSCs have the benefit of being easily accessible from tissue sources and can be generated in great numbers by utilizing straightforward culture techniques [135, 136]. MSC transplantation in AD animal models has been shown to be both safe and effective, according to a meta-analysis study. MSCs have recently been used extensively in preclinical animal studies of AD to cure or palliate symptoms. Human umbilical cord (hUC)-MSCs were found to target hyperphosphorylated tau and improve synaptic plasticity in a senescence-accelerated mice model of Alzheimer's disease. Hepatocyte growth factor (HGF) is secreted to promote structural and functional repair of damaged brain cells [137]. MenSCs made from human menstruation have been marketed as a potential AD treatment to lessen the AD pathology in an AD model mice [138].

According to a recent study, transplantation of dental-pulp-derived MSCs can improve cognitive function and raise hippocampal neuronal activity, pointing to possible therapeutic uses in Alzheimer's disease [139]. In a 3xTg-AD animal model, transplantation of bone marrow-derived MSCs (BM-MSCs) was also able to reduce the inflammatory response and tau phosphorylation [140]. Notably, a different study demonstrated that, as compared with BM-MSCs, human neural crest-derived nasal turbinate stem cells dramatically enhanced cognitive function and decreased A42 levels in a 5xFAD mouse model [141]. In mouse models of AD, transplantation of MSCs, or MSC-conditioned medium (MSC-CM), may improve mitochondrial function and reduce mitochondrial oxidative stress, representing a potentially effective therapeutic strategy [142].

Immune reaction may be brought on by MSC transplantation. Extracellular vehicles (EVs) created from MSCs might be a different strategy because they can pass through the BBB and mimic the advantages of MSCs [143]. MSC-derived EVs have been shown to improve cognitive function and reduce AD pathology when transplanted into animal models of the disease [144, 145]. According to one study, giving MSC-derived EVs to patients caused a shift from the proinflammatory to antiinflammatory phenotype of macrophages, which may have an impact on immunological responses and neuroprotection [146]. Another study found that MSC-EVs could prevent hippocampal neuronal loss in her AD mice from being exacerbated by her Aβ42-induced synaptic dysfunction [147, 148]. Notably, exosomes from MSCs reduced Aβ production by modulating α- and β-secretase expression and induced neuronal death by elevating miR-223 levels. According to Liu et al. [149], lateral ventricle injection of BMSC-derived exosomes can lessen cognitive impairments in a mouse model of sporadic AD. Restoring the brain's depleted NSC pool can restore function to a malfunctioning cerebrum, which suggests a viable treatment strategy [150]. One neurodegenerative disease that benefits from the use of multipotent self-renewing cells is AD. They can essentially form the three major cell types of the nervous system: neurons, astrocytes, and oligodendrocytes. This is markedly different compared with lineage-specific brain progenitors. The capability of

MSCs to differentiate into bona fide, functional neural cells is highly doubted, and the upregulation of certain, isolated neuron-specific proteins should not be regarded as successful (trans)differentiation. While beneficial effects of MSC transplantation have been observed in various diseases, even without the generation of functional cell types in disease, these are mostly attributed to supportive effects of the transplanted cells, as correctly cited, by EVs or other trophic factors [149, 150].

Human olfactory bulb (OB)-derived NSCs (OB-NSCs) have previously been shown to have the ability to survive, proliferate, differentiate, and correct cognitive and motor deficits associated with AD and PD rat models, respectively [151–155]. Recently, it has been proposed to use carbon nanotubes (CNTs) to enhance NSC differentiation and survival after in vivo transplantation. To test whether CNTs may enhance human OB-NSCs' therapeutic potential for treating cognitive impairments and neurodegenerative lesions, we co-engrafted CNTs and human OBNSCs in a rat model of trimethyltin (TMT) neurodegeneration. According to the results of the current work, TMT-induced rat neurodegeneration model cognitive impairments and neurodegenerative alterations might be reversed by engrafting human OBNSCs-CNTs. Additionally, the engrafted OBNSCs appeared to be supported by the CNTs, boosting their propensity to develop into neurons as opposed to glia cells. The current study's findings demonstrate that CNTs can significantly boost human OBNSCs' therapeutic potential, making this novel therapeutic paradigm a possible option for cell-based therapy of numerous neurodegenerative illnesses [156].

According to Zhang et al. [157], hNSC transplantation can improve memory in P301L mice by significantly reducing aberrant tau aggregation by controlling a number of proteins, mostly those involved in neurogenesis and long-term potentiation. It is interesting to note that intranasal transplantation of hNSCs can improve conditions similar to AD, as well as finally reverse the cognitive impairment of AD model mouse by boosting adult hippocampus neurogenesis [158]. As an alternative to NSCs, extracellular vesicles can be used since they have antioxidant, antiinflammatory, and antiapoptotic capabilities that are similar to those of NSCs [159]. Using EVs obtained from various iPSC-derived brain cell types, You et al. [160] found that astrocyte-specific EV-enriched hub modules may contribute to AD pathology and cognitive decline. Additional studies using NSC-derived EVs demonstrated improvements in cognitive deficits, synaptic activity, mitochondrial function, and inflammatory responses in AD mouse models [161]. Human embryonic stem cells (hESCs) are one of the safest sources of stem cells for transplantation therapy, notwithstanding the ethical issues they bring up. Medial ganglionic eminence (MGE)-like progenitor cells derived from hESCs have the potential to cure neurological diseases, according to Liu et al. [162]; when transplanted into AD animal models, iPSCs pretreated with ESC protein extracts have been demonstrated to decrease Aβ plaque development and exacerbate cognitive impairments. Furthermore, transplantation of thymic epithelial progenitor cells (TEPs) generated from $APP^{-/-}$ ESCs may provide a new therapeutic option for AD patients. Peripheral delivery of immune and matrix regulatory cells (IMRC) generated from human ESCs has also shown promise as a potential therapy for AD.

By stimulating neuronal development and real-time tracking of NSCs in vivo, encapsulated nanoparticles can be administered into NSCs in animal models to alleviate Aβ

deposition and cognitive deficits brought on by neurodegeneration. Notably, 6-month results continue to demonstrate improvements in learning and memory deficiencies [163].

In SAMP8 mice, Daz-Moreno et al. [164] found that intracranial injection of antiaging compounds could prevent hippocampal damage caused by pathological aging. These results may shed light on the problems that stem cell transplantation has in maintaining long-term efficacy. This has been used in many studies to enhance the potential neuroprotective effects, including limiting proliferation, resuming neurogenesis, and improving long-term transplant survival [165].

Cell replacement therapies for AD are currently being tested in humans; the majority of these therapies use MSCs from various sources. Stem cell therapy for AD is not yet in phase 3 clinical trials. Using the findings from the initial trial, the effectiveness, tolerance, and safety of transplanting were evaluated. Allogeneic human umbilical cord MSCs (hUCB-MSCs) were injected into the right precuneus and hippocampus of her patients with mild to moderate AD in a phase 1 clinical experiment carried out in South Korea in 2015. This trial investigated the treatment's effectiveness and safety. Safety, survival, and tolerability goals were met for all primary and secondary endpoints [166]. In addition, a case study using intrathecal injection of autologous MSCs demonstrated significant improvement in clinical symptoms in two patients and overall glucose metabolism in the brain as determined by ^{18}F-fluorodeoxyglucose positron emission tomography (PET) imaging [167]. These effective paradigms imply that MSCs have a minimal risk of side effects and are suitable for widespread usage in upcoming AD clinical trials. According to previous studies [168], patients with Alzheimer's disease exhibit region-specific basal forebrain cholinergic system depression (BFCS). The utilization of cholinergic cell-based transplantation as a therapeutic strategy might become a reality thanks to advancements in stem cell biology.

According to two investigations, model animals' cognitive function was greatly enhanced by transplanting both human fetal basal forebrain cholinergic cells and human chorion-derived basal forebrain cholinergic progenitor cells [169]. A description of the excitatory and inhibitory imbalance that served as an example of the pathophysiology of Alzheimer's disease has been provided. This theory implies that the main focus for improving cognitive function in AD patients may be the GABAergic system [170]. Shrestha et al. [171] transplanted human GABAergic interneuron progenitor cells made from hESCs into the hippocampus of rodents and discovered that the transplanted interneurons were better developed and had intricate dendrites. In mice models of neurodegeneration, neurogenic transcription factors or RNA-binding proteins have been shown to transform glial cells into functioning neurons [172]. Moreover, newly generated neurons have the ability to be innervated, repopulated, and ameliorate movement deficits in PD models [173].

In AD mice models, direct reprogramming of astrocytes and neuroglia 2 (NG2) cells results in functioning neurons [174]. In addition, there is proof that microglia can convert into neurons in vivo [175]. *ApoE*, *TREM2*, and *CD33* have been identified as key genes involved in the intermediate state of disease-associated microglia (DAM, also referred to as microglial neurodegenerative phenotype) by the most recent single-cell RNA sequencing studies of microglia from AD-transgenic (Tg) mice [176].

Delivery of cell therapy to the brain has been demonstrated to trigger an immunological response in models of Parkinson's disease. They demonstrated that using MHS-matched grafts greatly reduced immune responses when compared with using non-MHS-matched grafts, but immunological responses did not seem to be totally avoided. Immune rejection is thus a significant problem in the treatment of AD cells. Major histocompatibility complex (MHC) matching has been demonstrated in animal experiments to improve graft survival following organ transplantation [177]. Surprisingly, transplanted cells can be modified in vitro to reduce intracellular immunogenicity using genome editing engineering, or used as vectors to enhance the immunological milieu in vivo, and can dramatically reduce the risk of immune rejection [178].

When transplanted into nonhuman primate models, MHC-matched allografts have been found to decrease immune rejection and increase survival [179]. Beta 2-microglobulin (*B2M*) gene knockout and interference with human leukocytes antigen A (HLA-A) and B (HLA-B) may also lessen the immunogenicity of stored allografts [179].

To evaluate the effectiveness of individualized treatments, disease-in-a-dish models with patient-specific data can be created using patient-derived autologous cells. Genetic alterations or modifications can render transplanted cells resistant or refractory to disease pathologies prior to transplantation. No adverse effects were observed in this area from studies of human immunodeficiency virus (HIV) and acute lymphoblastic leukemia (ALL) [180]. This preventive approach focuses on the preclinical stages of AD, when only a few damaged brain cells need to be repaired. The problem with this method is that the overall medical procedure is expensive and time consuming. Chronic illnesses such as Alzheimer's disease, on the other hand, might not call for the quick synthesis of premade cells like other acute illnesses do. Prioritizing this design will boost reprogramming efficiency and safety while lowering expenses [181–183].

Conclusions

Future multilineage techniques and stem cell models may be able to detect early interactions between genes and molecules and developmental abnormalities in cells before they eventually become dysfunctional and die in AD. Disease models generated from iPSCs possess a level of detail that allows us to determine the neurological underpinnings of disease states and carefully examine the mechanisms behind the development and progression of such diseases. Combining stem cell-derived models improves the accuracy of detecting early immune cell changes and determining their contribution to AD pathogenesis. Future research should improve many issues related to stem cells. A fundamental problem is the immaturity of stem cell-derived cell types, which complicates the handling of these cells after transplantation into patients. By combining transplanted cells with a cell engineering toolkit that can target endogenous loci or disrupt gene expression at specific loci without altering therapeutic efficacy, transplanted cells can be immunogenic and genetically modified. HLA-matched cell banks are commonly used because gene editing can be used to reduce the immunogenicity of transplanted cells. Cell therapy can now be used for AD clinical research by delivering neurotrophic factors, replacing lost cells, promoting endogenous neurogenesis, modulating inflammatory responses, and altering the host microenvironment. Stem cell therapy combined with precision medicine is probably the most efficient treatment for AD. Using

hiPSC-derived models as a predictive platform could accelerate the development of precision medicine and "clinical trials in a dish," making AD therapeutics more likely to be effective. Currently, there are no proven methods for AD stem cell therapy, and it is still in its early stages. Given the many failures of AD treatment trials, we believe that stem cell-based AD treatments will shock us in the near future.

Abbreviations

AD	Alzheimer's disease
iPSCs	Induced pluripotent stem cells
FAD	Familial AD
SAD	Sporadic AD
NFTs	Neurofibrillary tangles
Aβ	Amyloid β
APP	Amyloid precursor protein gene
p-Tau	Hyperphosphorylated tau
β-Secretase	Beta-secretase
γ-Secretase	Gamma-secretase
α-Secretase	Alpha-secretase
CAA	Cerebral amyloid angiopathy
CSF	Cerebrospinal fluid
MAPT	Microtubules associated protein
BBB	Blood–brain barrier
PSEN1	Presenilins 1 gene
PSEN2	Presenilins 2 gene
GWAS	Genome-wide association study
APOE4	Apolipoprotein E gene
TREM2	Triggering receptor expressed on myeloid cells 2 gene
ABCA7	ATP-binding cassette sub-family A member 7 gene
SORL1	Sortilin-related receptor, L (*DLR* class) gene
OCT4	Octamer-binding transcription factor 4 gene
SOX2	Sex determining region Y-box 2 gene
KLF4	Kruppel-like factor 4 gene
DS	Down's syndrome
GSM	γ-Secretase modulator
GSI	γ-Secretase inhibitors
LRP1	Low-density lipoprotein receptor-related protein 1
HiPSCs	Human induced pluripotent stem cells
WT	Wild-type
HPCs	Hematopoietic progenitor cells
PLCG2	1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2
scRNA-seq	Single-cell RNA sequencing
ZFN	Zinc finger nuclease
TALENs	Transcription activator-like effector nucleases
CRISPR	Clustered regularly interspaced short palindromic repeats
Cas9	CRISPR-associated protein
crRNA	CRISPR RNA
transRNA	Trans-activating RNA
iBECs	Induced brain endothelial cells
MSCs	Mesenchymal stem cells
hUC-MSCs	Human umbilical cord mesenchymal stem cells
HGF	Hepatocyte growth factor
MenSCs	Menstruation stem cells
BM-MSCs	Bone marrow mesenchymal stem cells
EVs	Extracellular vesicles
OB-NSCs	Human olfactory bulb (OB)-derived NSCs
CNTs	Carbon nanotubes
hESCs	Human embryonic stem cells
MGE	Medial ganglionic eminence
TEPs	Thymic epithelial progenitor cells
IMRC	Immune and matrix regulatory cells
hUCB-MSCs	Human umbilical cord MSCs
BFCS	Basal forebrain cholinergic system depression
NG2	Neuroglia 2 gene
MHC	Major histocompatibility complex
B2M	Beta 2-microglobulin gene
HLA-A	Human leukocytes antigen A gene
HLA-B	Human leukocytes antigen B gene

HIV	Human immunodeficiency virus
ALL	Acute lymphoblastic leukemia

Acknowledgements
We acknowledge Biorender for provision of some templates used for the figures.

Author contributions
H.E.M. wrote the manuscript and prepared the figures, A.H. revised the manuscript, M.U.A.K. revised the manuscript. All authors read and approved the final manuscript.

Funding
Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Availability of data and materials
All data are available in the manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors have provided their consent for publication.

Competing interests
The authors declare no conflict of interests.

Received: 26 August 2023 Accepted: 20 October 2023
Published online: 30 November 2023

References

1. Tarawneh R, Holtzman DM. The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment. Cold Spring Harb Perspect Med. 2012;2(5): a006148.
2. van der Flier WM, Scheltens P. Epidemiology and risk factors of dementia. J Neurol Neurosurg Psychiatry. 2005;76(suppl 5):v2-7.
3. Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. F1000Research. 2018;7:1161.
4. Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med. 2003;81(11):678–99.
5. O'brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci. 2011;34:185–204.
6. Metaxas A, Kempf SJ. Neurofibrillary tangles in Alzheimer's disease: elucidation of the molecular mechanism by immunohistochemistry and tau protein phospho-proteomics. Neural Regen Res. 2016;11(10):1579.
7. Bondi MW, Edmonds EC, Salmon DP. Alzheimer's disease: past, present, and future. J Int Neuropsychol Soc. 2017;23(9–10):818–31.
8. Glenner GG, Wong CW, Quaranta V, Eanes ED. The amyloid deposits in Alzheimer's disease: their nature and pathogenesis. Appl Pathol. 1984;2(6):357–69.
9. Penney J, Ralvenius WT, Tsai LH. Modeling Alzheimer's disease with iPSC-derived brain cells. Mol Psychiatry. 2020;25(1):148–67.
10. Ceyzériat K, Zilli T, Millet P, Frisoni GB, Garibotto V, Tournier BB. Learning from the past: a review of clinical trials targeting amyloid, tau and neuroinflammation in Alzheimer's disease. Curr Alzheimer Res. 2020;17(2):112–25.
11. Hernández F, Merchán-Rubira J, Vallés-Saiz L, Rodríguez-Matellán A, Avila J. Differences between human and murine tau at the N-terminal end. Front Aging Neurosci. 2020;12:11.
12. Zhou Y, Song WM, Andhey PS, Swain A, Levy T, Miller KR, et al. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. Nat Med. 2020;26(1):131–42.
13. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–72.
14. Shi Y, Kirwan P, Smith J, Robinson HP, Livesey FJ. Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. Nat Neurosci. 2012;15(3):477–86.
15. Zhang Y, Pak C, Han Y, Ahlenius H, Zhang Z, Chanda S, et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. Neuron. 2013;78(5):785–98.
16. Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R, et al. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. Proc Natl Acad Sci. 2013;110(12):4697–702.

17. Hallmann AL, Araúzo-Bravo MJ, Mavrommatis L, Ehrlich M, Röpke A, Brockhaus J, et al. Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein. Sci Rep. 2017;7(1):1–10.
18. Zhao J, Davis MD, Martens YA, Shinohara M, Graff-Radford NR, Younkin SG, et al. APOE ε4/ε4 diminishes neurotrophic function of human iPSC-derived astrocytes. Hum Mol Genet. 2017;26(14):2690–700.
19. Guttikonda SR, Sikkema L, Tchieu J, Saurat N, Walsh RM, Harschnitz O, et al. Fully defined human pluripotent stem cell-derived microglia and tri-culture system model C3 production in Alzheimer's disease. Nat Neurosci. 2021;24(3):343–54.
20. Ehrlich M, Mozafari S, Glatza M, Starost L, Velychko S, Hallmann AL, et al. Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. Proc Natl Acad Sci. 2017;114(11):E2243–52.
21. Malik N, Rao MS. A review of the methods for human iPSC derivation. Pluripotent Stem Cells. 2013;23–33.
22. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011;1(1): a006189.
23. Chow VW, Mattson MP, Wong PC, Gleichmann M. An overview of APP processing enzymes and products. Neuromolecular Med. 2010;12(1):1–12.
24. Bernabeu-Zornoza A, Coronel R, Palmer C, Monteagudo M, Zambrano A, Liste I. Physiological and pathological effects of amyloid-β species in neural stem cell biology. Neural Regen Res. 2019;14(12):2035.
25. Masters CL, Selkoe DJ. Biochemistry of amyloid β-protein and amyloid deposits in Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(6): a006262.
26. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65(4):403–13.
27. Benilova I, Karran E, De Strooper B. The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci. 2012;15(3):349–57.
28. Kametani F, Hasegawa M. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. Front Neurosci. 2018;25.
29. Stancu IC, Vasconcelos B, Terwel D, Dewachter I. Models of β-amyloid induced Tau-pathology: the long and "folded" road to understand the mechanism. Mol Neurodegener. 2014;9(1):1–14.
30. Mietelska-Porowska A, Wasik U, Goras M, Filipek A, Niewiadomska G. Tau protein modifications and interactions: their role in function and dysfunction. Int J Mol Sci. 2014;15(3):4671–713.
31. Trabzuni D, Wray S, Vandrovcova J, Ramasamy A, Walker R, Smith C, et al. MAPT expression and splicing is differentially regulated by brain region: relation to genotype and implication for tauopathies. Hum Mol Genet. 2012;21(18):4094–103.
32. Park SA, Ahn SI, Gallo JM. Tau mis-splicing in the pathogenesis of neurodegenerative disorders. BMB Rep. 2016;49(8):405.
33. Jacobs HI, Becker JA, Kwong K, Engels-Domínguez N, Prokopiou PC, Papp KV, et al. In vivo and neuropathology data support locus coeruleus integrity as indicator of Alzheimer's disease pathology and cognitive decline. Sci Transl Med. 2021;13(612):eabj2511.
34. Clavaguera F, Hench J, Goedert M, Tolnay M. Invited review: prion-like transmission and spreading of tau pathology. Neuropathol Appl Neurobiol. 2015;41(1):47–58.
35. Delpech JC, Pathak D, Varghese M, Kalavai SV, Hays EC, Hof PR, et al. Wolframin-1–expressing neurons in the entorhinal cortex propagate tau to CA1 neurons and impair hippocampal memory in mice. Sci Transl Med. 2021;13(611):eabe8455.
36. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14(3):133–50.
37. Nortley R, Korte N, Izquierdo P, Hirunpattarasilp C, Mishra A, Jaunmuktane Z, et al. Amyloid β oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. Science. 2019;365(6450):eaav9518.
38. Karow M, Camp JG, Falk S, Gerber T, Pataskar A, Gac-Santel M, et al. Direct pericyte-to-neuron reprogramming via unfolding of a neural stem cell-like program. Nat Neurosci. 2018;21(7):932–40.
39. Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. Sci Transl Med. 2011;3(77):77sr1.
40. Haass C, Kaether C, Thinakaran G, Sisodia S. Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med. 2012;2(5): a006270.
41. Lanoiselée HM, Nicolas G, Wallon D, Rovelet-Lecrux A, Lacour M, Rousseau S, et al. APP, *PSEN1*, and PSEN2 mutations in early-onset Alzheimer disease: a genetic screening study of familial and sporadic cases. PLoS Med. 2017;14(3): e1002270.
42. Fernandez MA, Klutkowski JA, Freret T, Wolfe MS. Alzheimer presenilin-1 mutations dramatically reduce trimming of long amyloid β-peptides (Aβ) by γ-secretase to increase 42-to-40-residue Aβ. J Biol Chem. 2014;289(45):31043–52.
43. Tábuas-Pereira M, Santana I, Guerreiro R, Brás J. Alzheimer's disease genetics: review of Novel Loci associated with disease. Curr Genet Med Rep. 2020;8(1):1–16.
44. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016;8(6):595–608.
45. Weggen S, Beher D. Molecular consequences of amyloid precursor protein and presenilin mutations causing autosomal-dominant Alzheimer's disease. Alzheimers Res Ther. 2012;4(2):1–14.
46. Kelleher RJ III, Shen J. Presenilin-1 mutations and Alzheimer's disease. Proc Natl Acad Sci. 2017;114(4):629–31.
47. Lee C, Willerth SM, Nygaard HB. The use of patient-derived induced pluripotent stem cells for Alzheimer's disease modeling. Prog Neurobiol. 2020;192: 101804.
48. Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, et al. Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum Mol Genet. 2011;20(23):4530–9.
49. Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, et al. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature. 2012;482(7384):216–20.

50. Moore S, Evans LD, Andersson T, Portelius E, Smith J, Dias TB, et al. APP metabolism regulates tau proteostasis in human cerebral cortex neurons. Cell Rep. 2015;11(5):689–96.
51. Li M, Pehar M, Liu Y, Bhattacharyya A, Zhang SC, O'Riordan KJ, et al. The amyloid precursor protein (APP) intracellular domain regulates translation of p44, a short isoform of p53, through an IRES-dependent mechanism. Neurobiol Aging. 2015;36(10):2725–36.
52. Pehar M, Ko MH, Li M, Scrable H, Puglielli L. P44, the 'longevity-assurance' isoform of P53, regulates tau phosphorylation and is activated in an age-dependent fashion. Aging Cell. 2014;13(3):449–56.
53. Lagomarsino VN, Pearse RV II, Liu L, Hsieh YC, Fernandez MA, Vinton EA, et al. Stem cell-derived neurons reflect features of protein networks, neuropathology, and cognitive outcome of their aged human donors. Neuron. 2021;109(21):3402–20.
54. Hung CO, Livesey FJ. Altered γ-secretase processing of APP disrupts lysosome and autophagosome function in monogenic Alzheimer's disease. Cell Rep. 2018;25(13):3647–60.
55. Eitan E, Hutchison ER, Marosi K, Comotto J, Mustapic M, Nigam SM, et al. Extracellular vesicle-associated Aβ mediates trans-neuronal bioenergetic and Ca2+-handling deficits in Alzheimer's disease models. NPJ Aging Mech Dis. 2016;2(1):1–11.
56. Bergström P, Agholme L, Nazir FH, Satir TM, Toombs J, Wellington H, et al. Amyloid precursor protein expression and processing are differentially regulated during cortical neuron differentiation. Sci Rep. 2016;6(1):1–14.
57. Sposito T, Preza E, Mahoney CJ, Setó-Salvia N, Ryan NS, Morris HR, et al. Developmental regulation of tau splicing is disrupted in stem cell-derived neurons from frontotemporal dementia patients with the 10+ 16 splice-site mutation in MAPT. Hum Mol Genet. 2015;24(18):5260–9.
58. Wu JW, Hussaini SA, Bastille IM, Rodriguez GA, Mrejeru A, Rilett K, et al. Neuronal activity enhances tau propagation and tau pathology in vivo. Nat Neurosci. 2016;19(8):1085–92.
59. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. Nature. 2020;580(7803):381–5.
60. Ghatak S, Dolatabadi N, Trudler D, Zhang X, Wu Y, Mohata M. Mechanisms of hyperexcitability in Alzheimer's disease hiPSC-derived neurons and cerebral organoids vs isogenic controls. Elife. 2019;8:e50333.
61. Marei HE, Althani A, Afifi N, Hasan A, Caceci T, Pozzoli G, et al. Generation of gene edited hiPSC from familial Alzheimer's disease patient carrying N141I missense mutation in presenilin 2. Stem Cell Res. 2021;56: 102552.
62. Bloom GS. Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol. 2014;71(4):505–8.
63. Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Benjamin LN, et al. The familial Alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. Hum Mol Genet. 2014;23(13):3523–36.
64. Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, et al. APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. Neuron. 2018;98(6):1141–54.
65. Yang J, Zhao H, Ma Y, Shi G, Song J, Tang Y, et al. Early pathogenic event of Alzheimer's disease documented in iPSCs from patients with *PSEN1* mutations. Oncotarget. 2017;8(5):7900.
66. Bassil R, Shields K, Granger K, Zein I, Ng S, Chih B. Improved modeling of human AD with an automated culturing platform for iPSC neurons, astrocytes and microglia. Nat Commun. 2021;12(1):1–21.
67. Park J, Wetzel I, Marriott I, Dréau D, D'Avanzo C, Kim DY, et al. A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat Neurosci. 2018;21(7):941–51.
68. Kwak SS, Washicosky KJ, Brand E, von Maydell D, Aronson J, Kim S, et al. Amyloid-β42/40 ratio drives tau pathology in 3D human neural cell culture models of Alzheimer's disease. Nat Commun. 2020;11(1):1–14.
69. Hasselmann J, Coburn MA, England W, Velez DXF, Shabestari SK, Tu CH, et al. Development of a chimeric model to study and manipulate human microglia in vivo. Neuron. 2019;103(6):1016–33.
70. Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CH, Newman SA, et al. iPSC-derived human microglia-like cells to study neurological diseases. Neuron. 2017;94(2):278–93.
71. Hasselmann J, Blurton-Jones M. Human iPSC-derived microglia: a growing toolset to study the brain's innate immune cells. Glia. 2020;68(4):721–39.
72. Ginhoux F, Lim S, Hoeffel G, Low D, Huber T. Origin and differentiation of microglia. Front Cell Neurosci. 2013;7:45.
73. Csobonyeiova M, Polak S, Zamborsky R, Danisovic L. Recent progress in the regeneration of spinal cord injuries by induced pluripotent stem cells. Int J Mol Sci. 2019;20(15):3838.
74. Konttinen H, Ohtonen S, Wojciechowski S, Shakirzyanova A, Caligola S, Giugno R, et al. *PSEN1*ΔE9, APPswe, and APOE4 confer disparate phenotypes in human iPSC-derived microglia. Stem Cell Rep. 2019;13(4):669–83.
75. Sims R, Van Der Lee SJ, Naj AC, Bellenguez C, Badarinarayan N, Jakobsdottir J, et al. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. Nat Genet. 2017;49(9):1373–84.
76. Andreone BJ, Przybyla L, Llapashtica C, Rana A, Davis SS, van Lengerich B, et al. Alzheimer's-associated PLCγ2 is a signaling node required for both TREM2 function and the inflammatory response in human microglia. Nat Neurosci. 2020;23(8):927–38.
77. Svoboda DS, Barrasa MI, Shu J, Rietjens R, Zhang S, Mitalipova M, et al. Human iPSC-derived microglia assume a primary microglia-like state after transplantation into the neonatal mouse brain. Proc Natl Acad Sci. 2019;116(50):25293–303.
78. Fattorelli N, Martinez-Muriana A, Wolfs L, Geric I, De Strooper B, Mancuso R. Stem-cell-derived human microglia transplanted into mouse brain to study human disease. Nat Protoc. 2021;16(2):1013–33.
79. McQuade A, Kang YJ, Hasselmann J, Jairaman A, Sotelo A, Coburn M, et al. Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. Nat Commun. 2020;11(1):1–17.

80. Claes C, Danhash EP, Hasselmann J, Chadarevian JP, Shabestari SK, England WE, et al. Plaque-associated human microglia accumulate lipid droplets in a chimeric model of Alzheimer's disease. Mol Neurodegener. 2021;16(1):1–11.
81. Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, et al. Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods. 2018;15(9):700–6.
82. Marton RM, Miura Y, Sloan SA, Li Q, Revah O, Levy RJ, et al. Differentiation and maturation of oligodendrocytes in human three-dimensional neural cultures. Nat Neurosci. 2019;22(3):484–91.
83. Elsworthy RJ, King MC, Grainger A, Fisher E, Crowe JA, Alqattan S, et al. Amyloid-β precursor protein processing and oxidative stress are altered in human iPSC-derived neuron and astrocyte co-cultures carrying presenillin-1 gene mutations following spontaneous differentiation. Mol Cell Neurosci. 2021;114: 103631.
84. Sienski G, Narayan P, Bonner JM, Kory N, Boland S, Arczewska AA, et al. APOE4 disrupts intracellular lipid homeostasis in human iPSC-derived glia. Sci Transl Med. 2021;13(583):eaaz4564.
85. McAlpine CS, Park J, Griciuc A, Kim E, Choi SH, Iwamoto Y, et al. Astrocytic interleukin-3 programs microglia and limits Alzheimer's disease. Nature. 2021;595(7869):701–6.
86. Yahata N, et al. Anti-Aβ drug screening platform using human iPS cell-derived neurons for the treatment of Alzheimer's disease. PLoS ONE. 2011;6(9):e25788.
87. Wang C, et al. Scalable production of iPSC-derived human neurons to identify tau-lowering compounds by high-content screening. Stem Cell Rep. 2017;9(4):1221–33.
88. Kondo T, et al. iPSC-based compound screening and in vitro trials identify a synergistic anti-amyloid β combination for Alzheimer's disease. Cell Rep. 2017;21(8):2304–12.
89. Kondo T, et al. CDiP technology for reverse engineering of sporadic Alzheimer's disease. J Human Genet. 2023;68(3):231–5.
90. Mertens J, Stüber K, Wunderlich P, Ladewig J, Kesavan JC, Vandenberghe R, et al. APP processing in human pluripotent stem cell-derived neurons is resistant to NSAID-based γ-secretase modulation. Stem Cell Rep. 2013;1(6):491–8.
91. Liu Q, Waltz S, Woodruff G, Ouyang J, Israel MA, Herrera C, et al. Effect of potent γ-secretase modulator in human neurons derived from multiple presenilin 1–induced pluripotent stem cell mutant carriers. JAMA Neurol. 2014;71(12):1481–9.
92. Bursavich MG, Harrison BA, Blain JF. Gamma secretase modulators: new Alzheimer's drugs on the horizon? J Med Chem. 2016;59(16):7389–409.
93. Chang CY, Chen SM, Lu HE, Lai SM, Lai PS, Shen PW, et al. N-butylidenephthalide attenuates Alzheimer's disease-like cytopathy in Down syndrome induced pluripotent stem cell-derived neurons. Sci Rep. 2015;5(1):1–7.
94. Balez R, Steiner N, Engel M, Muñoz SS, Lum JS, Wu Y, et al. Neuroprotective effects of apigenin against inflammation, neuronal excitability and apoptosis in an induced pluripotent stem cell model of Alzheimer's disease. Sci Rep. 2016;6(1):1–16.
95. van der Kant R, Langness VF, Herrera CM, Williams DA, Fong LK, Leestemaker Y, et al. Cholesterol metabolism is a druggable axis that independently regulates tau and amyloid-β in iPSC-derived Alzheimer's disease neurons. Cell Stem Cell. 2019;24(3):363–75.
96. Joshi A, Ringman JM, Lee AS, Juarez KO, Mendez MF. Comparison of clinical characteristics between familial and non-familial early onset Alzheimer's disease. J Neurol. 2012;259(10):2182–8.
97. Piaceri I, Nacmias B, Sorbi S. Genetics of familial and sporadic Alzheimer's disease. Front Biosci-Elite. 2013;5(1):167–77.
98. Ryan NS, Nicholas JM, Weston PS, Liang Y, Lashley T, Guerreiro R, et al. Clinical phenotype and genetic associations in autosomal dominant familial Alzheimer's disease: a case series. Lancet Neurol. 2016;15(13):1326–35.
99. Hossini AM, Megges M, Prigione A, Lichtner B, Toliat MR, Wruck W, et al. Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. BMC Genomics. 2015;16(1):1–22.
100. Young JE, Fong LK, Frankowski H, Petsko GA, Small SA, Goldstein LS. Stabilizing the retromer complex in a human stem cell model of Alzheimer's disease reduces TAU phosphorylation independently of amyloid precursor protein. Stem Cell Rep. 2018;10(3):1046–58.
101. Hu YB, Dammer EB, Ren RJ, Wang G. The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. Transl Neurodegener. 2015;4(1):1–10.
102. Mecozzi VJ, Berman DE, Simoes S, Vetanovetz C, Awal MR, Patel VM, et al. Pharmacological chaperones stabilize retromer to limit APP processing. Nat Chem Biol. 2014;10(6):443–9.
103. Barral S, Kurian MA. Utility of induced pluripotent stem cells for the study and treatment of genetic diseases: focus on childhood neurological disorders. Front Mol Neurosci. 2016;9:78.
104. Kinch MS. An analysis of FDA-approved drugs for neurological disorders. Drug Discov Today. 2015;20(9):1040–3.
105. Smithies O, Koralewski MA, Song KY, Kucherlapati RS. Homologous recombination with DNA introduced into mammalian cells. In: Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor Laboratory Press; 1984. p. 161–70.
106. Hasty P, Rivera-Perez J, Chang C, Bradley A. Target frequency and integration pattern for insertion and replacement vectors in embryonic stem cells. Mol Cell Biol. 1991;11(9):4509–17.
107. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, et al. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Mol Cell Biol. 2001;21(1):289–97.
108. Geurts AM, Cost GJ, Freyvert Y, Zeitler B, Miller JC, Choi VM, et al. Knockout rats via embryo microinjection of zinc-finger nucleases. Science. 2009;325(5939):433–433.
109. Korecka JA, Talbot S, Osborn TM, de Leeuw SM, Levy SA, Ferrari EJ, et al. Neurite collapse and altered ER Ca2+ control in human Parkinson disease patient iPSC-derived neurons with LRRK2 G2019S mutation. Stem Cell Rep. 2019;12(1):29–41.

110. Verheyen A, Diels A, Reumers J, Van Hoorde K, Van den Wyngaert I, van Outryve DC, et al. Genetically engineered iPSC-derived FTDP-17 MAPT neurons display mutation-specific neurodegenerative and neurodevelopmental phenotypes. Stem Cell Rep. 2018;11(2):363–79.
111. Tesson L, Usal C, Ménoret S, Leung E, Niles BJ, Remy S, et al. Knockout rats generated by embryo microinjection of TALENs. Nat Biotechnol. 2011;29(8):695–6.
112. Akiyama T, Suzuki N, Ishikawa M, Fujimori K, Sone T, Kawada J, et al. Aberrant axon branching via Fos-B dysregulation in FUS-ALS motor neurons. EBioMedicine. 2019;45:362–78.
113. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013;153:910–8.
114. Adli M. The CRISPR tool kit for genome editing and beyond. Nat Commun. 2018;9:1911.
115. Kwart D, Gregg A, Scheckel C, Murphy EA, Paquet D, Duffield M, et al. A large panel of isogenic APP and *PSEN1* mutant human iPSC neurons reveals shared endosomal abnormalities mediated by APP β-CTFs, not Aβ. Neuron. 2019;104(2):256–70.
116. Nickels SL, Walter J, Bolognin S, Gérard D, Jaeger C, Qing X, et al. Impaired serine metabolism complements LRRK2-G2019S pathogenicity in PD patients. Parkinsonism Relat Disord. 2019;67:48–55.
117. Raman S, Brookhouser N, Brafman DA. Using human induced pluripotent stem cells (hiPSCs) to investigate the mechanisms by which Apolipoprotein E (APOE) contributes to Alzheimer's disease (AD) risk. Neurobiol Dis. 2020;138: 104788.
118. Meyer K, Feldman HM, Lu T, Drake D, Lim ET, Ling KH, et al. REST and neural gene network dysregulation in iPSC models of Alzheimer's disease. Cell Rep. 2019;26(5):1112–27.
119. Ovchinnikov DA, Korn O, Virshup I, Wells CA, Wolvetang EJ. The impact of APP on Alzheimer-like pathogenesis and gene expression in down syndrome iPSC-derived neurons. Stem Cell Rep. 2018;11(1):32–42.
120. Muratore CR, Zhou C, Liao M, Fernandez MA, Taylor WM, Lagomarsino VN, et al. Cell-type dependent Alzheimer's disease phenotypes: probing the biology of selective neuronal vulnerability. Stem Cell Rep. 2017;9(6):1868–84.
121. Oikari LE, Pandit R, Stewart R, Cuní-López C, Quek H, Sutharsan R, et al. Altered brain endothelial cell phenotype from a familial Alzheimer mutation and its potential implications for amyloid clearance and drug delivery. Stem Cell Rep. 2020;14(5):924–39.
122. Velasco S, Kedaigle AJ, Simmons SK, Nash A, Rocha M, Quadrato G, et al. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. Nature. 2019;570(7762):523–7.
123. Logan S, Arzua T, Yan Y, Jiang C, Liu X, Yu LK, et al. Dynamic characterization of structural, molecular, and electrophysiological phenotypes of human-induced pluripotent stem cell-derived cerebral organoids, and comparison with fetal and adult gene profiles. Cells. 2020;9(5):1301.
124. Ziff OJ, Patani R. Harnessing cellular aging in human stem cell models of amyotrophic lateral sclerosis. Aging Cell. 2019;18(1): e12862.
125. Soldner F, Jaenisch R. iPSC disease modeling. Science. 2012;338(6111):1155–6.
126. McTague A, Rossignoli G, Ferrini A, Barral S, Kurian MA. Genome editing in iPSC-based neural systems: from disease models to future therapeutic strategies. Front Genome Ed. 2021;3: 630600.
127. Jaffe S. US FDA defends approval of Alzheimer's disease drug. The Lancet. 2021;398(10294):12.
128. Shi M, et al. Impact of anti-amyloid-β monoclonal antibodies on the pathology and clinical profile of Alzheimer's disease: a focus on aducanumab and lecanemab. Front Aging Neurosci. 2022;14:870517.
129. Söderberg L, Johannesson M, Nygren P, Laudon H, Eriksson F, Osswald G, et al. Lecanemab, aducanumab, and gantenerumab—binding profiles to different forms of amyloid-beta might explain efficacy and side effects in clinical trials for Alzheimer's disease. Neurotherapeutics. 2023;20(1):195–206.
130. Braak H, Del Tredici K. Neuroanatomy and Pathology of Sporadic Alzheimer's Disease Preface. Neuroanatomy and pathology of sporadic Alzheimer's disease (2015): VII+.
131. De Strooper B, Karran E. The cellular phase of Alzheimer's disease. Cell. 2016;164(4):603–15.
132. Karran E, De Strooper B. The amyloid hypothesis in Alzheimer disease: new insights from new therapeutics. Nat Rev Drug Discovery. 2022;21(4):306–18.
133. Lazarus H, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI. Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. Bone Marrow Transplant. 1995;16(4):557–64.
134. Galipeau J, Sensébé L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell Stem Cell. 2018;22(6):824–33.
135. Cha MY, Kwon YW, Ahn HS, Jeong H, Lee YY, Moon M, et al. Protein-induced pluripotent stem cells ameliorate cognitive dysfunction and reduce Aβ deposition in a mouse model of Alzheimer's disease. Stem Cells Transl Med. 2017;6(1):293–305.
136. Qin C, Lu Y, Wang K, Bai L, Shi G, Huang Y, et al. Transplantation of bone marrow mesenchymal stem cells improves cognitive deficits and alleviates neuropathology in animal models of Alzheimer's disease: a meta-analytic review on potential mechanisms. Transl Neurodegener. 2020;9(1):1–20.
137. Jia Y, Cao N, Zhai J, Zeng Q, Zheng P, Su R, et al. HGF mediates clinical-grade human umbilical cord-derived mesenchymal stem cells improved functional recovery in a senescence-accelerated mouse model of Alzheimer's disease. Adv Sci. 2020;7(17):1903809.
138. Zhao Y, Chen X, Wu Y, Wang Y, Li Y, Xiang C. Transplantation of human menstrual blood-derived mesenchymal stem cells alleviates Alzheimer's disease-like pathology in APP/PS1 transgenic mice. Front Mol Neurosci. 2018;11:140.
139. Zhang XM, Ouyang YJ, Yu BQ, Li W, Yu MY, Li JY, et al. Therapeutic potential of dental pulp stem cell transplantation in a rat model of Alzheimer's disease. Neural Regen Res. 2021;16(5):893.
140. Neves AF, Camargo C, Premer C, Hare JM, Baumel BS, Pinto M. Intravenous administration of mesenchymal stem cells reduces Tau phosphorylation and inflammation in the 3xTg-AD mouse model of Alzheimer's disease. Exp Neurol. 2021;341: 113706.

141. Lim JY, In Park S, Park SA, Jeon JH, Jung HY, Yon JM, et al. Potential application of human neural crest-derived nasal turbinate stem cells for the treatment of neuropathology and impaired cognition in models of Alzheimer's disease. Stem Cell Res Ther. 2021;12(1):1–18.
142. Zhang Z, Sheng H, Liao LI, Xu C, Zhang A, Yang Y, et al. Mesenchymal stem cell-conditioned medium improves mitochondrial dysfunction and suppresses apoptosis in okadaic acid-treated SH-SY5Y cells by extracellular vesicle mitochondrial transfer. J Alzheimers Dis. 2020;78(3):1161–76.
143. Nakano M, Fujimiya M. Potential effects of mesenchymal stem cell derived extracellular vesicles and exosomal miRNAs in neurological disorders. Neural Regen Res. 2021;16(12):2359.
144. Chen YA, Lu CH, Ke CC, Chiu SJ, Jeng FS, Chang CW, et al. Mesenchymal stem cell-derived exosomes ameliorate Alzheimer's disease pathology and improve cognitive deficits. Biomedicines. 2021;9(6):594.
145. Sha S, Shen X, Cao Y, Qu L. Mesenchymal stem cells-derived extracellular vesicles ameliorate Alzheimer's disease in rat models via the microRNA-29c-3p/BACE1 axis and the Wnt/β-catenin pathway. Aging. 2021;13(11):15285.
146. Losurdo M, Pedrazzoli M, D'Agostino C, Elia CA, Massenzio F, Lonati E, et al. Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. Stem Cells Transl Med. 2020;9(9):1068–84.
147. de Godoy MA, Saraiva LM, de Carvalho LR, Vasconcelos-dos-Santos A, Beiral HJ, Ramos AB, et al. Mesenchymal stem cells and cell-derived extracellular vesicles protect hippocampal neurons from oxidative stress and synapse damage induced by amyloid-β oligomers. J Biol Chem. 2018;293(6):1957–75.
148. Wei H, Xu Y, Chen Q, Chen H, Zhu X, Li Y. Mesenchymal stem cell-derived exosomal miR-223 regulates neuronal cell apoptosis. Cell Death Dis. 2020;11(4):1–11.
149. Liu Y, Weick JP, Liu H, Krencik R, Zhang X, Ma L, et al. Medial ganglionic eminence–like cells derived from human embryonic stem cells correct learning and memory deficits. Nat Biotechnol. 2013;31(5):440–7.
150. Shu H, Guo Z, Chen X, Qi S, Xiong X, Xia S, et al. Intracerebral transplantation of neural stem cells restores manganese-induced cognitive deficits in mice. Aging Dis. 2021;12(2):371.
151. Marei HE, Althani A, Afifi N, Abd-Elmaksoud A, Bernardini C, Michetti F, et al. Over-expression of hNGF in adult human olfactory bulb neural stem cells promotes cell growth and oligodendrocytic differentiation. PLoS ONE. 2013;8(12): e82206.
152. Marei HE, Ahmed AE, Michetti F, Pescatori M, Pallini R, Casalbore P, et al. Gene expression profile of adult human olfactory bulb and embryonic neural stem cell suggests distinct signaling pathways and epigenetic control. PLoS ONE. 2012;7(4): e33542.
153. Marei HE, Althani A, Rezk S, Farag A, Lashen S, Afifi N, et al. Therapeutic potential of human olfactory bulb neural stem cells for spinal cord injury in rats. Spinal Cord. 2016;54(10):785–97.
154. Marei HE, Farag A, Althani A, Afifi N, Abd-Elmaksoud A, Lashen S, et al. Human olfactory bulb neural stem cells expressing hNGF restore cognitive deficit in Alzheimer's disease rat model. J Cell Physiol. 2015;230(1):116–30.
155. Marei HE, Lashen S, Farag A, Althani A, Afifi N, Rezk S, et al. Human olfactory bulb neural stem cells mitigate movement disorders in a rat model of Parkinson's disease. J Cell Physiol. 2015;230(7):1614–29.
156. Marei HE, Elnegiry AA, Zaghloul A, Althani A, Afifi N, Abd-Elmaksoud A, et al. Nanotubes impregnated human olfactory bulb neural stem cells promote neuronal differentiation in Trimethyltin-induced neurodegeneration rat model. J Cell Physiol. 2017;232(12):3586–97.
157. Zhang HA, Yuan CX, Liu KF, Yang QF, Zhao J, Li H, et al. Neural stem cell transplantation alleviates functional cognitive deficits in a mouse model of tauopathy. Neural Regen Res. 2022;17(1):152.
158. Lu MH, Ji WL, Chen H, Sun YY, Zhao XY, Wang F, et al. Intranasal transplantation of human neural stem cells ameliorates Alzheimer's disease-like pathology in a mouse model. Front Aging Neurosci. 2021;13: 650103.
159. Upadhya R, Madhu LN, Attaluri S, Gitaí DLG, Pinson MR, Kodali M, et al. Extracellular vesicles from human iPSC-derived neural stem cells: miRNA and protein signatures, and anti-inflammatory and neurogenic properties. J Extracell Vesicles. 2020;9(1):1809064.
160. You Y, Muraoka S, Jedrychowski MP, Hu J, McQuade AK, Young-Pearse T, et al. Human neural cell type-specific extracellular vesicle proteome defines disease-related molecules associated with activated astrocytes in Alzheimer's disease brain. J Extracell Vesicles. 2022;11(1): e12183.
161. Apodaca LA, Baddour AAD, Garcia C, Alikhani L, Giedzinski E, Ru N, et al. Human neural stem cell-derived extracellular vesicles mitigate hallmarks of Alzheimer's disease. Alzheimers Res Ther. 2021;13(1):1–18.
162. Liu J, Hou Z, Wu J, Liu K, Li D, Gao T, et al. Infusion of hESC derived Immunity-and-matrix regulatory cells improves cognitive ability in early-stage AD mice. Cell Prolif. 2021;54(8): e13085.
163. Huang C, Gan D, Fan C, Wen C, Li A, Li Q, et al. The secretion from neural stem cells pretreated with lycopene protects against tert-butyl hydroperoxide-induced neuron oxidative damage. Oxid Med Cell Longev. 2018;2018.
164. Díaz-Moreno M, Armenteros T, Gradari S, Hortigüela R, García-Corzo L, Fontán-Lozano Á, et al. Noggin rescues age-related stem cell loss in the brain of senescent mice with neurodegenerative pathology. Proc Natl Acad Sci. 2018;115(45):11625–30.
165. Ma D, Zhao L, Zhang L, Li Y, Zhang L, Li L. Icariin promotes survival, proliferation, and differentiation of neural stem cells in vitro and in a rat model of Alzheimer's disease. Stem Cells Int. 2021;2021.
166. Kim HJ, Seo SW, Chang JW, Lee JI, Kim CH, Chin J, et al. Stereotactic brain injection of human umbilical cord blood mesenchymal stem cells in patients with Alzheimer's disease dementia: a phase 1 clinical trial. Alzheimers Dement Transl Res Clin Interv. 2015;1(2):95–102.
167. Vaquero J, Zurita M, Mucientes J, Pascual ML, Fernández-Mateos C, Garcia E, et al. Intrathecal cell therapy with autologous stromal cells increases cerebral glucose metabolism and can offer a new approach to the treatment of Alzheimer's type dementia. Cytotherapy. 2019;21(4):428–32.
168. Kilimann I, Grothe M, Heinsen H, Alho EJL, Grinberg L, Amaro E Jr, et al. Subregional basal forebrain atrophy in Alzheimer's disease: a multicenter study. J Alzheimers Dis. 2014;40(3):687–700.
169. Mohammadi A, Maleki-Jamshid A, Sanooghi D, Milan PB, Rahmani A, Sefat F, et al. Transplantation of human chorion-derived cholinergic progenitor cells: a novel treatment for neurological disorders. Mol Neurobiol. 2019;56(1):307–18.

170. Bi D, Wen L, Wu Z, Shen Y. GABAergic dysfunction in excitatory and inhibitory (E/I) imbalance drives the pathogenesis of Alzheimer's disease. Alzheimers Dement. 2020;16(9):1312–29.
171. Shrestha S, Anderson NC, Grabel LB, Naegele JR, Aaron GB. Development of electrophysiological and morphological properties of human embryonic stem cell-derived GABAergic interneurons at different times after transplantation into the mouse hippocampus. PLoS ONE. 2020;15(8): e0237426.
172. Qian H, Kang X, Hu J, Zhang D, Liang Z, Meng F, et al. Reversing a model of Parkinson's disease with in situ converted nigral neurons. Nature. 2020;582(7813):550–6.
173. Zhou H, Su J, Hu X, Zhou C, Li H, Chen Z, et al. Glia-to-neuron conversion by CRISPR-CasRx alleviates symptoms of neurological disease in mice. Cell. 2020;181(3):590–603.
174. Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell. 2014;14(2):188–202.
175. Matsuda T, Irie T, Katsurabayashi S, Hayashi Y, Nagai T, Hamazaki N, et al. Pioneer factor NeuroD1 rearranges transcriptional and epigenetic profiles to execute microglia-neuron conversion. Neuron. 2019;101(3):472–85.
176. Krasemann S, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity. 2017;47(3):566–81.
177. Morizane A, Kikuchi T, Hayashi T, Mizuma H, Takara S, Mawatari A, et al. MHC matching improves engraftment of iPSC-derived neurons in non-human primates. Nat Commun. 2017;8(1):1–12.
178. Hendriks WT, Warren CR, Cowan CA. Genome editing in human pluripotent stem cells: approaches, pitfalls, and solutions. Cell Stem Cell. 2016;18(1):53–65.
179. Xu H, Wang BO, Ono M, Kagita A, Fujii K, Sasakawa N, et al. Targeted disruption of HLA genes via CRISPR-Cas9 generates iPSCs with enhanced immune compatibility. Cell Stem Cell. 2019;24(4):566–78.
180. Xu L, Wang J, Liu Y, Xie L, Su B, Mou D, et al. CRISPR-edited stem cells in a patient with HIV and acute lymphocytic leukemia. N Engl J Med. 2019;381(13):1240–7.
181. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med. 2019;25(4):554–60.
182. Patsch C, Challet-Meylan L, Thoma EC, Urich E, Heckel T, O'Sullivan JF, et al. Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. Nat Cell Biol. 2015;17(8):994–1003.
183. Serrano-Pozo A, Das S, Hyman BT. APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. Lancet Neurol. 2021;20(1):68–80.

Publisher's Note