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Moving from the Dish to the Clinical Practice: A Decade of Lessons and Perspectives from the Pre-Clinical and Clinical Stem Cell Studies for Alzheimer's Disease

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Abstract. To date, there is no definitive treatment for Alzheimer's disease (AD). The realm of stem cells is very promising in regenerative medicine, particularly neurodegenerative disorders. Various types of stem cells have been used in multiple trials on AD models, trying to find an innovative management of this disease. In this systematic review, we trace the published preclinical and clinical data throughout the last decade, to show how much knowledge we gained so far in this field and the future perspectives of stem cells in AD treatment.

Keywords: Alzheimer's disease, clinical trails, neurodegenerative diseases, preclinical trials, stem cells

INTRODUCTION

Since the development of the cell theory by Mattias Schleiden then Theodor Schwann in 1839, and after Rudulf Virchow popularized the slogan: Omnis cellula e cellula (All cells come from cells) in 1855, cell division has been considered the most fundamental process in the development of living organisms [1]. The cell undergoes diverse processes throughout development that ensures maintenance of organs and tissues integrity [2, 3].

In order to sustain a balance between cell loss and replacement, cells should be capable of self-renewal as well as differentiation. Stem/progenitor cells first described in 1964, lying at the heart of this process as the functional units of regeneration, are clonogenic populations that can give rise to various cell lineages [4, 5]. As their therapeutic potential has already been demonstrated in different settings, stem cells novel applications for clinical and preclinical trials are of great interest, which will hopefully enable the promise of regenerative medicine to be accomplished [6, 7].

Currently, the incurable neurodegenerative diseases reached a staggering prevalence [8]. For example, in the United States, the prevalence of Alzheimer's disease (AD) is estimated at 4.7 million cases and is expected to continue increasing dramatically [9]. The lack of definitive treatment to such

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disorders made it imperative for new innovative forms of management, to ameliorate their crippling effects [10].

AD is the most common form of neurodegenerative dementia accounting for about 60% to 70% of cases. AD is associated with several distinct neuropathological features, mainly extracellular amyloid-beta peptide (Aβ) plaques, intracellular neurofibrillary tangles due to deposition of hyperphosphorylated tau protein, and neuron loss with degeneration of the temporal lobe, parietal lobe, and parts of the frontal cortex and cingulate gyrus. Clinically, it shows a progressive impairment of learning, memory, language abilities, disorientation, behavioral problems, body functions decline, and ultimately death. Several hypotheses mentioned risk factors related to AD development such as genetics, neurotransmitters malfunction, and environmental factors, including head trauma and others. Currently, treatment for AD is only symptomatic and there are no disease-modifying therapies [11–13].

As a proof of concept, stem cells appeared to be promising in the field of AD. The multifactorial complex nature of AD pathogenesis, in addition to the limited efficacy of the current pharmacological and immunological strategies, increased the necessity for novel methods to tackle the disease. This led to the application of various forms of stem cell lines in numerous studies, ranging from basic lab research to preclinical studies and clinical trials, hoping for an innovative stem cell treatment for AD [14].

Due to the actively evolving nature of both stem cell biology and neurogenesis research, we performed a state-of-the-art review tracing preclinical and clinical studies that dealt with stem cells therapeutic role in AD. Here, we aimed to highlight crucial questions as: How far did we progress? What are the challenges we still have? Which cell lines are more promising? Are we ready for a definitive treatment?

METHODS

This is a state-of-the-art review of published data regarding potential use of stem cells in AD treatment.

Inclusion and exclusion criteria

Our inclusion criteria entailed all scientific peer-reviewed original articles (preclinical and clinical trials) published from 2006 until February 2016. As exclusion criteria, we omitted all bench side studies, reviews, editorials, communications, opinions, letters, news, and reports. Also, any study that used concomitant treatment with stem cells or entailed indirect cell use (e.g., cells' medium, endogenous stem cell population activation or inflammatory mediators derived from the cells) was excluded. This systematic review focused on direct cells application studies only. We used several electronic databases (PubMed, Google Scholar, Elsevier ScienceDirect, SpringerLink and PsycINFO) and the keywords "STEM CELLS, NEURODEGENERATIVE DISEASES, ALZHEIMER'S, PRECLINICAL, CLINIAL TRIALS" for search. In addition, we searched the http://www.clinicaltrials.gov website to compare the results of our search with the existing ongoing or finished clinical trials (Table 2). All the results were double checked to ensure they meet the mentioned criteria.

It is should be noted that almost all trials regarding the use of the pluripotent stem cells are bench side studies. Thus, we decided to include some bench side studies in a short paragraph on the topic about pluripotent cells to be more comprehensive regarding all the potentially beneficial stem cells types.

Data handling

Different levels of categorizations were done. We divided the articles into clinical and preclinical, then we further categorized them according to four important parameters (variables): year of publication, AD model (animal or human subjects), type of stem cells used, and route of cells administration. Published articles from the same research group that present different data regarding a research trial are treated as separate articles.

RESULTS

Our search yielded 65 articles published between 2006 and 2016 fulfilling our criteria (Fig. 1), in addition to 13 listed clinical trials on http://clinicaltrials.gov (listed in Table 2). One of the studies compared the use of two separate groups of animals with two different routes of cells injection each with its own control group (4 groups in total), therefore this study was considered as two independent studies. Collectively after adjustment, 66 studies were further analyzed (Table 1). Only one clinical study was found, while the remaining studies were all preclinical ones.

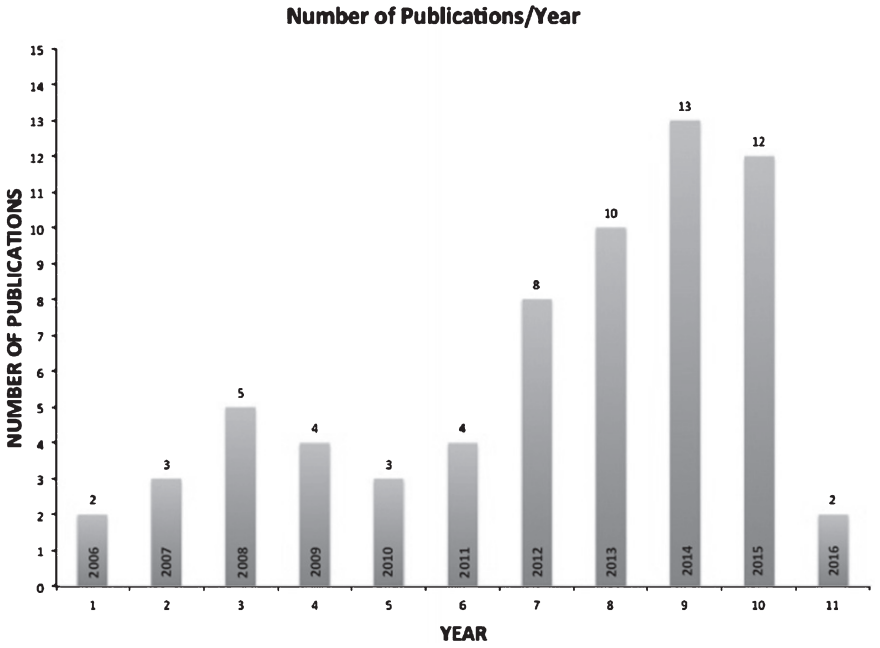

Fig. 1. Histogram comparing number of publications per each year starting from 2006 until early 2016.

DISCUSSION

Cell types

Lines

Studies have used different types of stem cells for transplantation (Fig. 2). We found that the most used cell types were mesenchymal stem cells (MSCs, 37.9%) and neuronal stem cells (NSCs, 36.4%).

Mesenchymal stem cells. The MSCs are the most studied adult stem cell type since their discovery by Friedenstein and co-workers, especially in age-related disorders [15]. First identified in the bone marrow [16], MSCs are currently being harvested from almost all tissues, such as adipose tissue [17], olfactory bulb [18], placenta [19], cord blood [20], and amniotic fluid [21]. Upon transplantation, MSCs showed the capacity to rapidly home to ischemic sites [22]. They have long proven ability to trigger immune responses via microglia activation in various AD transgenic mice models [23]. In a very elegant study, MSCs have been shown to modulate cytokines and brain inflammation in AβPP/PS1 mice, and authors could prove for the first time the negative correlation between MSC induced microglial activation with high anti-inflammatory cytokines expression on one side and Aβ deposits decrease and tau phosphorylation reduction, mostly in the hippocampus, on the other side [24]. In another debatable study, it was shown that MSCs direct transplantation into the hippocampus can improve cognition in C57BL/6 mice, as well as augmentation of hippocampal neurogenesis, allegedly through *Wnt* signaling pathway, thus enhancing endogenous repair [25]. MSCs that overexpress the vascular endothelial growth factor was shown to improve neovascularization and the clearance of Aβ, ultimately leading to memory and learning deficits recovery in 2xTg-AD animals [26]. However, a major concern in the study was that in older animals (12 months), the condition was worsened by the accentuated neurodegeneration caused by the AD genotype. Moreover, the robust effect of the VEGF in improving social memory deficit seen in younger animals was attenuated in older animals and no reverse of the impairment of the social recognition memory was accomplished at that age.

In an interesting study set to investigate the potential role of MSCs in treating early AD, high-risk pre-dementia AβPP mice models were treated with MSC from the bone marrow. Two months post injection, a reduction in Aβ deposits and changes in key proteins required for synaptic transmission were detected, suggesting a potential early interventional therapy in prodromal AD by bone marrow-MSC transplantation [27].

Table 1
Summary of evidence regarding the use of stem cells in Alzheimer's disease

	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
1	Preclinical	MSCs	IC	Human MSCs reduced the potentially neurotoxic 56 kDa Aβ oligomers, increased the endogenous neurogenesis in the SVZ, and maintained the glutamate transport systems in the entorhinal cortex, thus leading to a smaller working memory deficit in AD models.	Ruzicka et al., [110]
2	Preclinical	UCBCs	IV	UCBCs reach several tissues including the brain following a single IV treatment. Low IV dose of UCBCs led to eventual loss of these cells in key organs, such as the spleen, thus the use of multiple low-dose infusions in AD models may be necessary to prolong the therapeutic benefit of these cells.	Ehrhart et al., [66]
3	Clinical	MSCs	IC	Stereotactic administration of MSCs into the hippocampus and precuneus of human subjects was feasible, safe, and well tolerated. A phase 1 clinical trial.	Kim et al., [61]
4	Preclinical	MSCs	IV	Wharton Jelly-derived cells led to a significant reduction in Aβ deposition and soluble Aβ levels, with a significantly increased expression of the anti-inflammatory cytokine IL-10 and down-regulation of pro-inflammatory cytokines IL-1β and TNF-α. Thus, improving the spatial learning and alleviated the memory decline.	Xie et al., [111]
5	Preclinical	BMMNCs	IV	Transplantation of BMMCs suppressed neuronal loss and reduced Aβ deposition in the brain, leading to restoration of memory impairment of DAL transgenic mice to almost the same level as in wild-type mice.	Kanamaru et al., [28]
6	Preclinical	iPSCs derived neural cells	IC	ChAT-positive neurons and alpha7 nicotinic acetylcholine receptor (α7nAChR)-positive neurons were significantly increased in the cortex and hippocampus with significantly increased number of GABA receptor-positive neurons of both human origin and mouse origin compared with those in the vehicle-injected mouse cortex.	Fujiwara et al., [112]
7	Preclinical	MSCs	IC	Repeated administration of MSCs markedly promoted the expression of synaptic vesicle markers, including synaptophysin, which are usually downregulated in patients with AD.	Kim et al., [113]
8	Preclinical	NSCs	IC	Optimal time frame of cells injection was crucial to obtain maximal therapeutic effects that can restore functional deficits or stop the progression of AD.	Kim et al., [41]
9	Preclinical	NSCs	IC	NSCs transplantation resulted in significant decrease in activation of GFAP, Iba-1, TLR4 and TLR4 pathway-related agents (MyD88, TRIF, P38 MAPK, and NF-κB P65) with decreased expression of pro-inflammatory mediators (IL-1, IL-6, TNF-α and PGE2).	Zhang et al., [39]
10	Preclinical	NSCs	IC	Number of mitochondria and expression of mitochondria-related proteins, specifically the mitochondrial fission factors [dynamin-related protein 1 (Drp1) and fission 1 (Fis1)] and the mitochondrial fusion factor optic atrophy 1 (OPA1), were significantly increased with NSCs transplantation.	Zhang et al., [40]

(*Continued*)

Table 1
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	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
11	Preclinical	NSCs	IC	NSCs transplantation reduced tau phosphorylation via Trk-dependent Akt/GSK3β signaling, down-regulated Aβ production through an Akt/GSK3β signaling-mediated decrease in BACE1, and decreased expression of inflammatory mediators through deactivation of microglia that was mediated by cell-to-cell contact, secretion of anti-inflammatory factors. It also facilitated synaptic plasticity and anti-apoptotic function via trophic supplies.	Lee et al., [114]
12	Preclinical	NSCs	IC	Human NSCs cells can migrate and differentiate into immature neurons and glia and significantly increase synaptic and growth-associated markers. Transplantation ameliorated context and spatial learning and memory impairments in transgenic models of AD.	Ager et al., [42]
13	Preclinical	NSCs	IC	After NSCs transplantation, the number of basal forebrain cholinergic neurons, hippocampal synaptophysin, and AchE-positive fibers were all significantly larger than the control group.	Chen et al., [115]
14	Preclinical	NSCs	IC	Stereological analysis of engrafted Olfactory Bulb NSCs eight weeks post transplantation revealed a 1.89 fold increase with respect to the initial cell population, indicating a marked ability for survival and proliferation. In addition, 54.71 ± 11.38%, 30.18 ± 6.00%, and 15.09 ± 5.38% of engrafted cells were identified by morphological criteria suggestive of mature neurons, oligodendrocytes and astrocytes, respectively.	Marei et al., [18]
15	Preclinical	MSCs	IC	MSC-VEGF treatment favored the neovascularization and diminished senile plaques in hippocampal specific layers. Consequently, the treatment was able to provide behavioral benefits and reduce cognitive deficits by recovering the innate interest to novelty and counteracting memory deficits in AD transgenic animals.	Garcia et al., [26]
16	Preclinical	MSCs	IN	Transplanted cells showed predominant distribution within the olfactory bulb and brainstem.	Danielyan et al., [72]
17	Preclinical	MSCs	IV	Placenta derived cells attenuated the expression of AβPP, BACE1, and Aβ, as well as the activity of β-secretase and γ-secretase. In addition, they inhibited the activation of glia cells and the increased expression of inducible nitric oxide synthase and cyclooxygenase-2.	Yun et al., [19]
18	Preclinical	MSCs	IV	Bone Marrow MSCs were able to home at the injured brains and produced significant increase in the number of positive cells for ChAT, as well as selective AD indicator-1 (seladin-1), and nestin gene expression. Histopathological examination indicated that they could also remove beta-amyloid plaques from hippocampus.	Salem et al., [116]
19	Preclinical	ADSCs	IV	ADSCs labeled with LEO-LIVE-Magnoxide were still detected *in vivo* 12 days after injection.	Ha et al., [117]
20	Preclinical	NSCs	IC	Aβ plaque loads were reduced not only in the hippocampus and subiculum adjacent to engrafted NSCs, but also within the amygdala and medial septum, areas that receive afferent projections from the engrafted region.	Blurton-Jones et al., [76]

(*Continued*)

Table 1
(*Continued*)

	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
21	Preclinical	NSCs	IC	Nissl staining revealed that the number of neurons in the hippocampus of a transgenic model of AD (3×Tg mice) receiving NSCs was significantly greater than the control group, indicating that new neurons were generated. NSC transplantation can improve spatial learning and memory via neuronal regeneration.	Chen et al., [118]
22	Preclinical	ADSCs	IC	ADSCs transplantation enhanced neurogenic activity in the SVZ with reduced oxidative stress and alleviated cognitive impairment in the mice. Based on these findings, it was proposed that ADSCs enhances endogenous neurogenesis in both the subgranular and subventricular zones.	Yan et al., [119]
23	Preclinical	MSCs	IV	Combined MSCs and erythropoietin markedly reduced lipopolysaccharide mediated cell damage in AD model, leading to effective relief of AD pathology.	Khairallah et al., [120]
24	Preclinical	ADSCs	IV & IC	IV or IC transplanted human ADSCs greatly improved the memory impairment and the neuropathology.	Chang et al., [14]
25	Preclinical	NSCs	IC	NSC-induced neurons highly expressed SYN and growth associated protein-43 (GAP-43) in hippocampal areas.	Gu et al., [121]
26	Preclinical	NSCs	IC	The improvement in cognitive function was correlated with enhanced long-term potentiation (LTP) and an increase in the neuron expression of proteins related to cognitive function: N-methyl-D-aspartate (NMDA) 2B unit, SYP, protein kinase C ζ subtypes (PKCζ), tyrosine receptor kinase B (TrkB) and BDNF.	Zhang et al., [122]
27	Preclinical	NSCs	IC	The expression of SYN and growth-associated protein-43 (GAP-43) in Tg-NSC mice, 8 weeks after transplantation, were significantly improved. This was confirmed by the increase in the number of synapses in Tg-NSC mice as observed via electron microscopy.	Zhang et al., [123]
28	Preclinical	NSCs	IC	Transplantation of transgenic IL-1 receptor antagonist (IL-1ra TG) NSCs one month before the neurobehavioral disturbances completely rescued the brain and significantly increased the number of endogenous hippocampal cells expressing the plasticity-related molecule BDNF.	Ben-Menachem-Zidon et al., [124]
29	Preclinical	MSCs	IV & IC	MSCs transplantation improved spatial memory in bulbectomized mice with AD-type neurodegeneration and no cases of malignant transformation were noted.	Bobkova et al., [125]
30	Preclinical	MSCs	IC	In transplanted models, M2-like microglial activation was significantly increased, and the expression of anti-inflammatory cytokine associated with M2-like microglia, IL-4, was also increased, whereas the expression of pro-inflammatory cytokines associated with classic microglia (M1-like microglia), including IL-1β and TNF-α was significantly reduced.	Yang et al., [20]

(*Continued*)

Table 1
(*Continued*)

	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
31	Preclinical	NSCs	IC	No difference was detected in total number of Aβ plaques between NSC and control groups after transplantation. Also, there was no difference in the number of thioflavin-S-positive (TS+) plaques between both groups after eight weeks of NSCs transplantation. However, engrafted NSCs showed partial chemotaxis toward Aβ plaques.	Zhang et al, [126]
32	Preclinical	UCBCs	IV	UCBC therapy correlated with decreased cognitive impairment, Aβ levels/Aβ plaques, amyloidogenic AβPP processing, and reactive microgliosis after a treatment of 6 or 10 months.	Darlington et al., [48]
33	Preclinical	MSCs	IC	Single IC injection of bone marrow-MSCs resulted in significant decrease in the cerebral Aβ deposition sustained up to 2 months. Expression of dynamin 1 and Synapsin 1, key pre-synaptic proteins associated with synaptic transmission, were considerably enhanced in the brains of AD mice sustained beyond 2 months.	Bae et al., [27]
34	Preclinical	ADSCs	IC	ADSC transplantation dramatically reduced Aβ deposition and significantly restored the learning/memory function in AβPP/PS1 transgenic mice with more activated microglia.	Ma et al., [52]
35	Preclinical	MSCs	Intracardiac	Systemic transplantation of autologous T regulatory cells (Tregs) significantly ameliorate the cognition and reduced the Aβ plaque deposition and the levels of soluble Aβ, accompanied with significantly decreased levels of activated microglia and systemic inflammatory factors.	Yang et al., [20]
36	Preclinical	MSCs	IV	Six weeks after the IV injection of MSCs, mice models showed evidence of improved spatial learning, which significantly correlated with the observation of fewer Aβ plaques in brain. Furthermore, the level of pro-inflammatory cytokines, IL-1 and TNF-α, was lower while the anti-inflammatory cytokines, IL-10 and transforming growth factor-β, was higher in MSC-injected mice the controls. These effects lasted until 12 weeks after injection.	Kim et al., [21]
37	Preclinical	MSCs	IV	At 13 and 14 days, MSC transplantation group showed significantly improved spatial learning and memory ability than controls. 14 days after transplantation in rat hippocampus, PKH26-positive cells could be found consistent with DAPI staining. PKH26-positive cells in animal models of AD were significantly more than those in the normal control group.	Li et al., [127]
38	Preclinical	MSCs	IV	When MSCs were transplanted into the hippocampus of a 10-month-old transgenic mouse model of AD for 10, 20, or 40 days, NEP expression was increased in the mice brains. Moreover, $A\beta_{42}$ plaques in the hippocampus and other regions were decreased by active migration of MSCs toward Aβ deposits. This suggests that MSC-derived sICAM-1 decreases Aβ plaques by inducing neprilysin expression in microglia through the sICAM-1/LFA-1 signaling pathway.	Kim et al., [128]
39	Preclinical	NSCs	IC	(1)H-MRS displayed IC metabolite changes before and after NSC transplantation in AD mice models and had an applicable value in evaluating the therapeutic effect of NSCs on AD.	Chen et al., [129]

(*Continued*)

Table 1
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	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
40	Preclinical	ADSCs	IV & IC	Intravenously transplanted ADSCs passed through the BBB and migrated into the brain. The learning, memory and pathology in an AD mouse model (Tg2576 mice) greatly improved for at least 4 months after IV injection of the cells.	Kim et al., [53]
41	Preclinical	NSCs	IC	Authors showed that transplanted epidermal-NSCs survived and produced many neurons and a few glial cells, presenting glial fibrillary acidic protein. In addition, the total number of granule cells in hippocampus was higher in the AD model with transplanted cells as compared to the control group.	Esmaeilzade et al., [54]
42	Preclinical	Amniotic stem cells	IC	Amniotic stem cells transplantation significantly ameliorated spatial memory deficits in AD transgenic mice, as well as increased acetylcholine levels and the number of hippocampal cholinergic neurites.	Xue et al., [130]
43	Preclinical	NSCs	IC	Transplantation of F3. ChAT human NSCs fully recovered the learning and memory function of AF64A animals, and induced elevated levels of acetylcholine in cerebrospinal fluid. Transplanted F3.ChAT human NSCs were found to migrate to various brain regions including cerebral cortex, hippocampus, striatum and septum, and differentiated into neurons and astrocytes	Park et al., [131]
44	Preclinical	MSCs	IC	Aβ deposition, BACE1 levels, and tau hyperphosphorylation were dramatically reduced in MSC transplanted AβPP/PS1 mice. These effects were associated with reversal of disease-associated microglial neuroinflammation, as evidenced by decreased microglia-induced pro-inflammatory cytokines, elevated alternatively activated microglia, and increased anti-inflammatory cytokines.	Lee et al., [24]
45	Preclinical	MSCs	IC	Intraventricular transplantation of native and glucagon-like peptide-1 transfected MSC was shown to be effective. Decreased amyloid deposition or suppression of glial and microglial responses were observed.	Klinge et al., [132]
46	Preclinical	Amniotic epithelial cells	IC	Transplanted amniotic cells can survive for at least 8 weeks and migrate to the third ventricle without immune rejection. Not only they significantly improved the spatial memory of the AD transgenic mice, but they also increased acetylcholine concentration and the number of hippocampal cholinergic neurites.	Xue et al., [133]
47	Preclinical	MSCs	IC	Immunohistochemistry showed that GFP-MSCs distributed uniformly and the number of Alzheimer's senile plaques reduced after transplantation, leading to retard AD-like pathology and upregulation of DeltaNp73 expression in hippocampus of AβPP/PS1 transgenic mice.	Wen et al., [134]

(*Continued*)

Table 1
(*Continued*)

	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
48	Preclinical	MSCs	IC	Immortalized MSCs possessed better ability of proliferation and anti-senescence compared with primary MSCs, while maintained multilineage differentiation capacity. Neural-like cells derived from immortalized MSCs had similar expressions of neural-specific genes, protein expression patterns and resting membrane potential (RMP) compared with their counterparts derived from primary MSCs; they play same role as primary MSCs in improving learning ability and spatial memory in AD models.	Gong et al., [135]
49	Preclinical	MSCs	IC	MSCs reduced the hippocampal apoptosis. Moreover, acute markers of glial activation, oxidative stress and apoptosis levels were decreased in AD mouse brain. MSCs treated AD mice demonstrated cognitive rescue with restoration of learning/memory function.	Lee et al., [136]
50	Preclinical	NSCs	IC	Histological examinations showed that there was no obvious changes in Aβ deposition between tested groups; in addition, the NSCs differentiated and expressed neuronal nuclei-positive cells, and continuously expressed 5-bromodeoxyuridine-positive cells for six weeks.	He et al., [137]
51	Preclinical	MSCs	IC	MSCs attenuated Aβ-induced apoptotic cell death in primary cultured hippocampal neurons by activation of the cell survival signaling pathway. These anti-apoptotic effects of MSCs were also observed in an acutely-induced AD mice model produced by injecting Aβ intrahippocampally. In addition, MSCs diminished Aβ -induced oxidative stress and spatial memory impairment in the *in vivo* model.	Lee et al., [74]
52	Preclinical	MSCs	IC	MSCs into AβPP/PS1 mice significantly reduced Aβ deposition with decreased tau hyperphosphorylation and improved cognitive function. These effects were associated with restoration of defective microglial function, evidenced by increased Aβ-degrading factors, decreased inflammatory responses, and elevation of alternatively activated microglial markers.	Lee et al., [73]
53	Preclinical	NPCs	IC	NPCs transplantation significantly reduced microgliosis (by 38%), but not astrogliosis in peptide-injected hippocampus.	Ryu et al., [138]
54	Preclinical	NPCs	IC	Immunohistochemical analysis revealed that the majority (approximately 70%) of the NPCs retained neuronal phenotype and approximately 40% of them had a cholinergic cell phenotype following transplantation with no tumor formation, indicating that NPCs may be safe for transplantation. Behavioral assessment revealed a significant behavioral improvement in memory deficits following transplantation with NPCs.	Moghadam et al., [37]
55	Preclinical	NSCs	IC	The number of cholinergic neurons of the NSCs-transplanted group was significant higher than that of the glia-transplanted group in medial septum and vertical diagonal branch. The results indicate that transplanted NSCs can differentiate into cholinergic neurons, which may play an important role in the therapeutic effects of transplanted NSCs.	Xuan et al., [139]

(*Continued*)

Table 1
(*Continued*)

	Level of evidence	Cell Type	Cell Routing	Main findings	Reference
56	Preclinical	NSCs	IC	BDNF can enhance the treatment effects of NSCs transplanted into brain lesion model as shown by immunohistochemical detection of p75(NGFR) indicating that the number of cholinergic neurons of the had more significant improvement	Xuan et al., [140]
57	Preclinical	NSCs	IC	NSCs survived after transplantation, integrated into the host brain and enhanced cognitive performance.	Wu et al., [141]
58	Preclinical	NPCs	IC	The development of amyloidosis in the neurogenic regions attenuated the age-associated reduction of proliferation of NPCs, proving that their proliferation in culture did not depend on the age or presence of AβPP-transgene.	Kolecki et al., [142]
59	Preclinical	UCBCs	IV	UCBC infusions reduced cerebral vascular Aβ deposits in the Tg2576 AD mouse model. These effects were associated with suppression of the CD40-CD40L interaction, as evidenced by decreased circulating and brain soluble CD40L (sCD40L), elevated systemic immunoglobulin M (IgM) levels, attenuated CD40L-induced inflammatory responses, and reduced surface expression of CD40 on microglia.	Nikolic et al., [143]
60	Preclinical	MSCs	IC	Results showed that MSCS survived, migrated and expressed NGF as well as differentiated into ChAT-positive neurons with a significant improvement in learning and memory in AD rats.	Li et al., [144]
61	Preclinical	NSCs	IC	NSCs transplanted into the brain after neuronal ablation survived, migrated, differentiated and, most significantly, improved memory.	Yamasaki et al., [145]
62	Preclinical	NSCs	IV	After transplantation at different time points, NSCs were diffusely distributed in the brain and around the blood vessels in the first 48 h, then migrated gradually towards the hippocampus and cortex until 4 weeks later.	Zhan et al., [146]
63	Preclinical	NSCs	IC	Not only NSCs survived well after transplantation into hippocampus of AD rats, but also they possessed the capacity of continuous proliferation and migrated along the hippocampus.	Yang et al., [147]
64	Preclinical	NSCs	IC	Neurospheres transplanted into the mouse cortex survived and produced many ChAT-positive neurons and a few serotonin-positive neurons in and around the grafts, thus decreasing significantly the working memory error in the mice.	Wang et al., [148]
65	Preclinical	NSCs	IC	The behavioral amelioration of AD model rat was obtained by transplanting single NSCs and BDNF-gene-modified NSCs. The effect of the NSCs group modified with BDNF gene proved to be better than NSCs alone.	Zhao et al., [149]

Aβ, amyloid-β peptide; AβPP amyloid-β protein precursor; AchE, acetylcholinesterase; ADSCs, adipose tissue derived stem cells; AD, Alzheimer's disease; BACE1, beta-secretase 1; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; BMMNCs, bone marrow mononuclear cells; ChAT, choline acetyltransferase; DAPI, 4',6-diamidino-2-phenylindole; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adaptor molecule 1; IC, intracerebral; IL, interleukin; IN,intranasal; iPSCs, induced pluripotent stem cells; IV, intravenous; LFA-1, lymphocyte function-associated antigen-1; MRS, magnetic resonance spectroscopy; MSCs, mesenchymal stem cells; NGF, nerve growth factor; NPCs, neuronal progenitor cells; NSCs, neuronal stem cells; sICAM-1, soluble intracellular adhesion molecule-1; SVZ, subventricular zone; PGE2, Prostaglandin E2; PS1, presenilin 1; SYN, synaptophysin; TLR, Toll-like receptor; TNF, tumor necrosis factor; UCBCs, umbilical cord blood cells; VEGF, vascular endothelial growth factor.

Table 2
Clinical trials performed for testing stem cells therapy in Alzheimer's disease

	Study	Type of cell / Drug	Design/type	Classification	Estimated enrollment/ Follow-up period	Outcomes
1	NCT02600130	Longeveron mesenchymal stem cells	Randomized/ Interventional Double blind	Safety demonstration (Phase I)	30 subjects Follow up: primary outcome: first 30 days. Secondary outcome: 2, 6, 13, 26, 39 & 52 post infusion	Not yet recruiting
2	NCT02054208	Human umbilical cord blood derived mesenchymal Stem cells	Randomized/ Interventional Double blind	Safety and Exploratory Efficacy Versus Placebo (Phase I and II)	42 subjects Follow up: 24 weeks after the first dose	Recruiting
3	NCT00927108	Adult neuronal Progenitor stem cell culture	Interventional/ Open label	Exploratory Efficacy (Phase II)	10 subjects Follow up: not provided	Not yet recruiting
4	NCT01547689	Human Umbilical cord derived MSCs	Interventional/ Open label	Safety and Efficiency (Phase I and II)	30 subjects Follow up: 10 weeks post-administration and year	Not yet recruiting
5	NCT01297218	Human Umbilical cord derived MSCs	Interventional/ Open label	Safety and Efficiency (Phase I)	9 subjects Follow up: 12 weeks post-administration	Completed with no published results yet
6	NCT02513706	Human Umbilical cord derived MSCs	Interventional/ Double Blind	Safety and Exploratory Efficacy (Phase I and II)	40 subjects Follow up: 10 weeks post-administration	Not yet recruiting
7	NCT01696591	Human Umbilical cord derived MSCs	Observational/ Case Control/ Prospective	Safety and Exploratory Efficacy (Phase I and II)	14 subjects Follow up: 24 months postop	Recruitment status is unknown
8	NCT02672306	Human Umbilical cord derived MSCs	Randomized/ Interventional Double blind	Safety and Exploratory Efficacy (Phase I and II)	40 subjects Follow up: 10 weeks post-administration	Not yet recruiting
9	NCT00391833	Panax Ginseng	Non-Randomized/ Interventional Open label	Safety and Exploratory Efficacy (Phase I and II)	Not provided	Completed with no published results yet
10	NCT01765803	Mellaril	Interventional/ Open label	Feasibility Study (Phase 0)	6 subjects Follow up: 24 hours after treatment	Terminated with no published results yet
11	NCT00874783	Induced pluripotent stem cells development from donated somatic cells	Observational/ Case Control/ Prospective		120 subjects Follow up: not provided	Not yet recruiting
12	NCT02646982	Candesartan	Randomized/ Interventional Double blind	Exploratory Efficacy (Phase II)	72 subjects Follow up: 12 months	Not yet recruiting
13	NCT01617577	Filgrastim (G-CSF)	Randomized/ Interventional Double blind	Safety and Exploratory Efficacy (Phase I and II)	8 subjects Follow up: 2 weeks and 4 weeks	Results: Granulocyte colony stimulating factor decreased brain amyloid burden and reverses cognitive impairment in Alzheimer's mice (Sanchez-Ramos et al., 2009) [150].

MSCs, mesenchymal Stem cells, G-CSF, granulocyte colony stimulating factor.

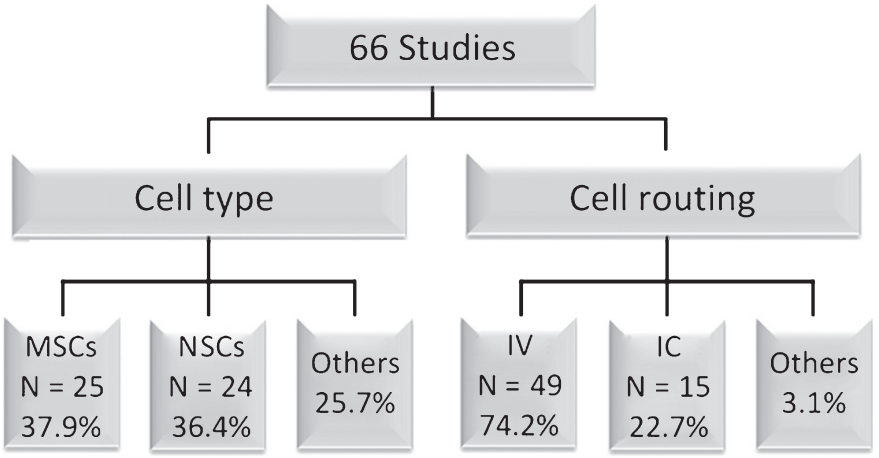

Fig. 2. Flow chart summarizing the main findings of this systematic review. In 66 studies, MSCs (mesenchymal stem cells) and NSCs (neuronal stem cells), proven to be the most commonly used cell types among all stem cell lines, whereas the IV (intravenous) together with IC (intracerebral) techniques are the most preferred routes for cell delivery.

In 2015, Kanamaru and colleagues proposed the use of cells other than MSCs that usually takes long culture time for preparation. Therefore, they used bone marrow mononuclear cells (BMMNCs), which do not need such culture time, containing MSCs together with other forms of cells [28]. Still, issues with these cells lines unwanted proliferation or differentiation, and the need for irradiation or sometimes other toxic preconditioning to achieve proper engraftment [29]. Nevertheless, there are concerns regarding severe side effects resulting from MSCs use, such as immunogenicity and potential tumourgenicity [30, 31].

Neuronal stem cells. Notwithstanding the remarkable results obtained with the use of MSCs, research involving neurodegenerative diseases have pointed their efforts to NSCs. Indeed, NSCs transplantation became one of the main lines used for AD research (Fig. 2). The robust migratory capacity of NSCs provided a compelling approach to deliver their therapeutic effect to the brain. The subventricular zone of the lateral ventricles is considered the largest source of NSCs in the adult mammalian brain [32]. NSCs have proved in multiple settings to be safe and effective when transplanted into the hippocampus, improving cognitive functions, synaptic connectivity and neuronal survival in animal models of AD [33]. Genetically modified NSCs expressing the proteolytic enzyme neprilysin that underlies the endogenous degradation of Aβ, led to marked and significant reduction in the pathology of AβPP transgenic mice. A major concern, particularly in the study done by Spencer and colleagues, was the long-term side effects associated with the vector assisted delivery method (lentivirus, adenovirus, or herpes simplex virus) they used to deliver the neprilysin to the target areas. It was also unclear the reason of the adenovirus delivered neprilysin localization at the presynaptic sites, whereas the lentivirus localization was in the cell soma and the endoplasmic reticulum [34–36]. Transplantation of neuronal precursor cells (NPCs), derived from mouse embryonic stem cells (ESCs), was shown to promote behavioral recovery in a nbM-lesioned rats AD model following commitment to a cholinergic cell phenotype [37]. Unlike other previous studies, it clearly showed an increase in the number of cholinergic neurons after treatment. Still, the question whether NPCs is only a cholinergic survival factor or also contributes to differentiation of cholinergic neurons is yet to be elucidated.

In an *in vitro* induction study, the combined effect of both nerve growth factor and brain derived neurotropic factor (BDNF) on NSCs differentiation led to increased neurogenesis rate, which may be interesting for future clinical applications, especially for dosage optimization [38]. In addition, NSCs showed significant neuroprotective effect against AD inflammation by suppressing glial and toll-like receptor-4 (TLR4) mediated inflammatory pathway activation, leading to down-regulation of the proinflammatory mediators [interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha (TNFα)] in AβPP/PS1 mouse model, thus ameliorating the cognitive deficits, but with no change in Aβ concentration [39]. It is worth noticing the amelioration of cognitive deficits without difference in Aβ concentration, but associated with the

attenuation of the inflammatory response, which warrants further investigation.

Other hypothesis regarding NSCs mechanism of action lies on their ability to enhance mitochondrial biogenesis via increasing the number of mitochondria, and mitochondrial proteins (dynamin related protein 1, mitochondrial fission 1 protein, optic atrophy 1) expression in AβPP/PS1 mice models, thus rescuing the cognitive outcome [40]. These results are of pivotal relevance as mitochondrial bioenergetic deficits usually precede AD pathogenesis, thus adding functional mitochondria through NSC transplantation may be a promising therapeutic strategy. Kim and colleagues showed that NSCs transplantation at the early stages of the disease improved the learning and memory ability and reduced Aβ plaque load and tau hyperphosphorylation in Tg2576 mice. In addition, NSCs transplantation decreased microglial activation [41]. However, it should be mentioned that in advanced stages of the disease, NSCs failed to restore behavioral/cognitive deficits, which correlates well with previous results in older stages of the disease.

As an attempt to identify a suitable line for clinical application, a study used human neural stem cell population (HuCNS-SCs) transplantation in animal models of neurodegeneration. In 3xTg-AD mice, HuCNS-SCs proved to ameliorate context and place dependent learning (tested using the novel subject recognition test). In addition, HuCNS-SCs improved spatial learning and memory impairments (tested with the Morris water maze test, MWM) with proper cell survival and engraftment detected by immunofluorescence and confocal microscopy. The intriguing observation was the improvement in MWM probe trial and novel subject recognition test performance, with no effect on MWM acquisition, suggesting specificity of the processes involved in memory formation and consolidation. Moreover, cognitive improvements were not associated with altered Aβ or tau pathology. Rather, human NSC transplantation seems to improve cognition by enhancing endogenous synaptogenesis [42].

It is important to highlight that despite NSCs and NPCs appear to overlap to a certain degree in terms of gene expression, they can be clearly distinguished based on differences in notch signaling and functional attributes. NPCs collectively describe a mixed population of both NSCs and neural progenitor cells. Neural progenitor cells are generally capable of limited transient self-renewal with generation of one type of cell, in contrast to the highly plastic NSCs that can maintain unlimited lifetime differentiation along the three known cell lineages (multipotent) [43].

Human umbilical cord blood cells (HUCBCs). HUCBCs transplantation has been therapeutically beneficial in many neurodegenerative disorders [44]. Despite acknowledged as clinical waste, HUCBCs provide an alternative source for plenty of MSCs [45]. They have been suggested to modulate the peripheral inflammatory processes, which in turn affect inflammation in the brain parenchyma, and the mobilization of adult stem cells from the bone marrow, leading to extension of the lifespan in AβPP mice [46, 47]. Multiple low doses of HUCBCs were shown to improve cognitive impairment, reduce Aβ-associated neuropathology, and improve the motor skills in long term treated AD mice [48]. Despite the usage of multiple doses to detect the optimum one in those studies, the problem of dose optimization is yet to be solved.

Adipose tissue derived stem cells (ADSCs). ADSCs appeared as a new attractive source for stem cells in regenerative therapy as they are readily abundant, easily accessible, with a detected lower senescence ratio compared to bone marrow-MSCs [49]. In addition, they are known for their ability to differentiate into mesenchymal and non-mesenchymal lineages [50]. One study proposed ADSCs as a safe stem cell source for intravenous (IV) injections, owing to the absence of immune rejections, ethical problems or tumorigenesis. [51]. Long term follow-up (up to 26 months post injection) to determine their potential tumorigenicity in nude mice showed ADSCs to be safe even with high doses.

In AD, intracerebral (IC) transplanted or intravenous (IV) administered ADSCs showed dramatic improvement of impaired memory and neuropathology in AβPP/PS1 mice models, with huge reduction of Aβ peptide deposition with microglial activation, thus suggesting that they have a high therapeutic potential for AD [17, 52]. It was very interesting to show that in cases of IV ADSC delivery, the labeled fluorescence magnetic nanoparticles for *in vivo* live tracking were demonstrated in all brain regions except the olfactory bulb in Tg2576 model [53]. This study highlights once more the importance of the early detection and intervention in AD, as the early onset injected cells produced major changes in the disease progress and pathology, while late onset treatment was minimally effective.

Hair follicle stem cells = Epidermal neural crest derived stem cells (Epi-NCSCs). A very interesting study assessed the ability of Epi-NCSCs derived from hair follicles, which are the multipotent remnants of

ESCs, for neuronal differentiation for the first time. By direct stereotactic transplantation of the cells into the hippocampus of $A\beta_{40}$ induced AD rat model, Esmaeilzade and colleagues showed that Epi-NCSC can differentiate into neurons *in vivo*, offering them the potential for treating neurological diseases with significant advantages over other NSCs sources, as they are readily accessible, with no risks of obtaining NSCs from the brain, and provide a renewable population for autologous transplantation [54]. Yet, further studies are needed to determine the fate of the newly differentiated neurons.

Induced pluripotent stem cells (iPSCs). Since their discovery in 2006 by Yamanka and Takahashi [55], a huge interest focused on induced pluripotent stem cells (iPSCs) for various reasons, such as their high capacity for scaling up and the potential ability to get autologous cells, thereby reducing or eliminating the need for immune-suppression. Recently, xeno-free clinical grade iPSCs have been generated, moving this approach several steps closer to clinical reality [56]. It is worth mentioning that iPSCs exhibit molecular and functional characteristic similar to those of the ESCs, but unlike ESCs, iPSCs raise no major ethical concerns regarding their source. Moreover, they provide autologous cells for cell-based therapies [57]. In spite of the huge potential of iPSCs to reach therapies in neurodegenerative disease, trials on iPSCs are still limited to the basic laboratory boundaries [58, 59].

In summary, while progress has been made last years, the best cell line to be used is still a point of argument. Noteworthy, MSCs and NSCs together accounted for almost 75% of the cases (37.9% and 36.4% respectively). An important question arising from the complex pathological nature of AD is: *when to inject the cells?* Early injection proved to be more beneficial in protecting and/or reversing the pathology, unlike the late stages management that is not as promising so far.

Cell routing

The best route through which stem cells can reach the target brain tissues for neurogenesis is still a matter of debate despite numerous efforts in elucidating it. It is noteworthy that over 70% of the studies used the IV route (Fig. 2).

It is yet intuitive to assume that to deliver cells to a particular organ, direct implantation techniques should be the best. The safety and tolerability of stereotactic administration was proven by a phase-one clinical study for gene delivery in AD patients [60]. A recent phase-one clinical trial used the same reported successful stereotactic surgery to deliver MSCs intracerebrally, directly into the hippocampus and the precuneus, which was proven to be feasible, safe, and well tolerated [61]. Despite lacking a control group in both studies and the small study sample (10 and 9, respectively), they both proved interventional safety, thus opening the gate for future more controlled wide scale evidence-based clinical trials.

Choosing a cell delivery route for clinical translation is a huge challenge, since the human brain is considerably larger than the mouse brain (the most used animal model for AD). Peripheral delivery of cells and proteins appears to be less effective than intracerebral injections. For instance, a study found that IV delivery route could reduce plasma Aβ, but failed to clear Aβ plaques within the brain [62].

Kanamaru and colleagues rationally debated the need for other less invasive ways of cell delivery, particularly in humans [28]. Based on the theory of blood-brain barrier disruption and leakage in AD patients [63, 64], lots of researchers have constructed their preclinical experiments using the IV route for cell delivery, as an easier and less invasive method. Although Lee and colleagues in 2008 postulated that NSCs are best delivered IV than IC, they also mentioned that in case of MSCs IV injection, most of the injected cells were found in the splenic marginal zone, rather than homing in the brain tissues [65]. This was also consistent with the results of IV injection of cord blood cells that were found all over the body of a murine model [66]. Moreover, Yang and colleagues first reported that the IV injection of BMMNCs is not inferior to the intra-arterial route. Interestingly, Kanamaru and colleagues also reported the inability to detect the IV injected cells in brain tissues after 3 months of transplantation [28]. Such reports illustrate the unpredictable fate of the IV route, and the minimal percentage of localization of the injected cells centrally (in the brain), despite its apparent efficacy, which render this technique debatable.

Direct intra-arterial stem cells transplantation has been used mainly for stroke and ischemic diseases to induce functional recovery in the acute therapeutic window [67, 68]. In 6-hydroxydopamine (6-OHDA)-induced animal model of Parkinson's disease, MSCs were directly infused in the carotid artery. Systemically injected cells could not efficiently cross the blood-brain barrier to home in the nigrostriatal region unless another agent is given with them facilitating their permeability [69]. Such technique is not employed so far for stem cells delivery in AD.

However, preclinical trials used this route in AD models to deliver Monodisperse Iron Oxide Nanoparticles (MIONs) targeting the cerebrovascular amyloid deposits that are found in approximately 90% of patients with AD [70]. The same research group applied it for the delivery of antibody fragments for immunotherapy [71]. Still, this technique is far from optimization for clinical practice.

Other less commonly utilized routes were suggested, even without a clear or convincing justification for their use. For instance, it was tried a direct intra-cardiac injection in a trial to prove the systemic effectiveness as an alternate pathway for cells delivery [20].

A recent study postulated the intranasal (IN) route for cell delivery. In this study, the authors showed that after 7 days of IN application of MSCs, cells were predominantly distributed within the olfactory bulb, hippocampus, cortex, and cerebellum in two different animal models, a Thy1-h[A30P] αS model for Parkinsonism and an AβPP/PS1 model of AD [72]. To achieve functional improvement using such technique, dosage, number of injections and time of treatment should be further investigated for optimization.

Cell interactions

Stem cells' mechanism of action

Several studies have shown that stem cells trigger anti-inflammatory reactions that activate microglia [73, 74]. The microglia was shown to increase the expression of Aβ degrading enzymes such as insulysin and neprilysin, thus helping in improving AD manifestations [75]. One study postulated that a stem cell-induced neurotrophic factor, namely BDNF, is responsible for the improvement in cognition in 3xTg-AD mice model [76]. It was very interesting in this study to show that the NSCs *per se* did not induce any direct cognitive beneficial effects via alteration of Aβ or tau pathology, as hypothesized previously in literature. In contrast, NSCs induced elevation in the hippocampal levels of BDNF, leading to increased synaptic density and restoring hippocampal-dependent cognition.

In multiple studies, stem cells were shown to upregulate the anti-inflammatory cytokine IL-10 and to downregulate pro-inflammatory cytokines (TNFα, IL-1, IL-6, IL-8, IL-12) [77–79], in addition to increase expression of vascular endothelial growth factor that is known to have neuroprotective and neurogenic roles [80].

A more recent explanation to stem cells' effects is the role of the extracellular vesicles as key components of their paracrine effect. Various types of stem cells release 'secretomes'. Studies have shown that they deliver bio-active cargo to the neighboring diseased or injured cells, modifying the cellular receptors function, inducing anti-inflammatory, immune modulation, angiogenesis, neurogenesis and synaptogenesis [81–84]. Moreover, recent reports linked them as mediators to intercellular communication in cancer [85], which warrants more elucidation on their mechanism of action.

In spite of the numerous trials along the past decade to unravel the actual mechanism by which stem cells induce their effect, to date, it is still largely unknown and mostly hypothetical. Furthermore, despite their promise, secretomes clinical application still faces several challenges, including their isolation and production techniques (time consuming ultracentrifugation is the only available technique so far) and heterogeneity.

In vivo stem cell tracking

One of the biggest challenges that face all sorts of stem cells therapies is the lack of reliable *in vivo* imaging methods to evaluate the biological interactions of transplanted cells [86].

Nanomedicine and nanoparticles-based delivery systems for stem cells are very interesting. Superparamagnetic iron oxide has been used for decades to track stem cells *in vivo* after *in vitro* labeling. Nanotechnologies revolutionized the high resolution of *in vivo* imaging for cell tracking [87]. Magnetic resonance imaging (MRI), positron emission tomography (PET), or single-photon emission computed tomography are used to visualize the labeled stem cells *in vivo*. MRI imaging has the advantage of high spatial resolution and anatomical information but its major drawback is the limited sensitivity [88–90]. PET scans are highly sensitive but have low spatial resolution, poor in anatomical delineation, with notable short half-life of the radioisotopes [91, 92]. Fluorescence imaging is another noninvasive imaging method for *in vivo* tracking. It is also highly sensitive with good resolution at the subcellular level, but suffers from limited penetration power even with the prolonged release dye techniques [93, 94]. Near-infrared fluorescence imaging (NIRFI) using NIR fluorophores improved penetration depth and provided more specific signals, but still has a major drawback of photobleaching [95, 96]. Several multimodal imaging techniques have been developed

to overcome the limitations of each single method [97–99].

Notwithstanding the advances achieved in nanotechnology field, still proper methods of *in vivo* cell tracking and reporting are far from optimization for reliable use in human subjects.

AD modeling

Preclinical trials use small animal models (mice or rats). Mice models, the most commonly used (72.7%), focused on the overexpression of familial AD associated mutant genes, particularly amyloid precursor protein, presenilin-1, and presenilin-2 [100]. Still, it was shown that the expected outcomes reported by the previous preclinical trials are not consistent with the clinical studies. This was attributed to fundamental discrepancies including the anatomical, pathophysiological and micro-environmental differences in addition to the divergent reporting mechanisms used in humans compared with animals [101, 102].

This problem in particular gave momentum to the uprising iPSCs modeling technologies. Israel and colleagues described the initial iPSCs based *in vitro* AD model in 2012 focusing on replicating already known familial and sporadic aspects of the disease [103]. Genomic editing enabled the creation of patient specific (isogenic) cell lines. 'Alzheimer's in a dish model' developed by Choi and colleagues marked a cornerstone progress in AD modeling [104, 105]. Based on the rational of the 3D model, Zhang and colleagues developed a 3D-iPSCs derived neuronal model for AD [106]. The importance of 3D-iPSCs model technology stems from their ability to differentiate into multiple neurologic cell types. As these derived cells carry all the information packed in the genome of the patient from where they are derived, they allow disease testing, drug screening and possible treatments modalities, in addition to predicting the possible patient's interaction with treatments. So far, most iPSC-AD modelling trials have constructed either embryoid body/neurosphere or small molecule-based neuronal differentiation known for glutamatergic cortical forebrain neuron generation. In terms of gene and neuronal marker expression, these cortical neuron cultures comprise a mixture of different cell types of variable maturity levels, such as neurons, astrocytes, oligodendrocytes, and other brain-derived cells. As these diverse cells carry the genomes of patients with AD, most closely resembling early human fetal neurons, expressing AD phenotypes such as increased $A\beta_{42}$ production and tau-phosphorylation changes, they are very important for *in vitro* study models. Very interestingly, compounds that inhibit gamma-secretase activity were effective at reducing Aβ production in AD iPSC-derived neuronal cultures, a concept used in drug screening studies. This will hopefully lead to rational selection of the most successful pharmacological strategy for preventing early AD changes [107].

This new stem cells frontier will enable scientists to genuinely study human-based AD pathology and its response to genetic and pharmacologic manipulation, for more effective future clinical applications.

CONCLUSION AND FUTURE PERSPECTIVES

Cell based therapies offer promising results, but it is clear that they still need more research to optimize lots of hanging problems around them. On one hand, the potential of regeneration of brain tissue using stem cells is sure. On the other hand, we still do not know the best way to deliver these cells to the brain. The complexity of neurodegenerative diseases results in difficulty in optimizing a single direct cell based therapy to reverse all the effects, particularly AD, in which there is no uniform disease progression with multiple levels of pathological changes incorporated along different time points (early, intermediate, and late courses). This is one of the major hurdles that face a potential cell based therapy to such complex disease process. NSCs, claimed to be the best and most effective type for AD management, need proper optimization regarding doses and mode of delivery and whether a concomitant treatment should be administered or not.

In addition, we learned that stem cells are effective, but we are still incapable of clearly identifying their mechanistic effects as well as detect them *in vivo* after transplantation, which is in our opinion the ultimate hurdle facing their clinical applications. It is paramount to be able to trace your treatment, to detect its dosage level, its toxicity and cellular interactions, not just administer it and applauding an outcome that we are not sure of its cellular level biochemical processes yet.

In iPSCs based therapy, the first landmark clinical trial on humans was launched in 2014 to treat macular degeneration [108, 109]. Even though it started favorably with their first patient, an undefined genomic mutation occurred with the iPSCs of the second patient, which forced them last year to

officially suspend the trial till further notice. This brings us back to the point of the real comprehensive understanding of the cellular level based interactions of such cells.

The complex nature of the human brain and its interconnected circuit of neurons make it unlikely for an individual stem cell type or other current management modality to be fully effective in AD treatment single-handedly. Most likely, a future cure will be based on a multimodal approach at different intervals, which will hold the collective benefits of optimized stem cell technology, probably via their medically promising secretomes, in addition to the advances in neurogenesis induction.

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