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Original Article

Stem Cells and Other Emerging Agents as Innovative "Drugs" in Neurodegenerative Diseases: Benefits and Limitations

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Abstract

The brain has a limited process of repair/regeneration linked to the restricted and localized activity of *neuronal stem cells*. Consequently, it shows a reduced capacity to counteract the age-related loss of neural and glial cells and to repair the consequent injuries/lesions of nervous system. This progressively determines nervous dysfunction and onset/progression of neurodegenerative diseases, which represent a serious social (and economic) problem of our populations. Thus, the research of efficient treatments is encouraged. *Stem cell therapy* might represent a solution. Today, it, indeed, represents the object of intensive research with the hope of using it, in a near future, as effective therapy for these diseases and preventive treatment in susceptible individuals. Here, we report and discuss the data of the recent studies on this field, underling the obstacles and benefits. We also illustrate *alternative measures of intervention*, which represent another parallel aim for the care of neurodegenerative pathology-affected individuals. Thus, the road for delaying or retarding these diseases appears hard and long, but the advances might be different.

Keywords: brain, self-repair/regenerative process, neuronal stem cells, neurodegenerative pathologies, stem cell therapy, innovative intervention measures

Introduction: Stem Cells in the Central Nervous System

Today, the research community considers the concept that the nervous system lacks a system of repair and regeneration to be totally obsolete. The presence of stem cells in the brain is well recognized. They were discovered in 1965 by Altman and Das,[1] thanks to studies on adult rodent brains. From then to now, their presence has been confirmed by numerous groups of research. Stem cells reside in the brain of all mammals, humans included, and they are defined as *neural stem cells* (NSCs).[2–5]As any other adult stem cell (ADC), the NSCs constitute a heterogeneous mosaic of cells, which differ in their proliferation status, as well as in their responses to physiological inputs. Quiescent and active stem cells coexist in specific adult organs and systems, such as the brain.[6] In the case of the brain, the identity (or identities) of NSCs seems/seem to be different, and even if under debate. They prevalently stay *in vivo* in a quiescent status and essentially exhibit features of glial cells, as demonstrated by data obtained from genetic tracing, pharmacological ablation, morphological and immune-cytochemistry investigations.[7–9] Furthermore, NSCs show multipotency, self-renew and differentiation.[10] These peculiarities consent them of differentiating in various mature cellular types.[10,11] In addition, in adult mammalian brain, they reside in specialized niches, which have precise characteristics, as for other types of ADCs.[12] Thus, the fate of NSCs is constantly and finely regulated by changes in the microenvironment of niches.[13] These changes are provided by both intrinsic (*e.g.*, hormones, cytokines, neurotrophins, and growth factors) and extrinsic (*e.g.*, stress, aging, physical activity, environmental enrichment) factors.[13] Regarding the niches, some research groups have elegantly revised[14,15] the recent literature data and evidenced that, in the mammalian brain, adult neurogenesis mainly occurs in the subventricular zone (SVZ) of the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, leading to the formation, respectively, of new olfactory bulb interneurons and new granule cells. However, they also underlined the data obtained by other recent studies, which demonstrate the existence, in the adult mammalian brain, of a third noncanonical neurogenic area represented by the hypothalamus.[16–18] Furthermore, differences in the proliferative activity of these areas have been observed.[19]An extensive proliferative rate has been detected in the SVZ than SGZ in rodents.[20] A developmental study of the human SVZ also suggested that neurogenesis and neuronal migration extend

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into postnatal life, but it principally occurs in early childhood.[21] Surprisingly, during this window, a major migratory pathway of SVZ new neurons targets the prefrontal cortex, in addition to the olfactory bulb.[20,21] These results are consistent with previous evidence in the human brain that shows cortical neurogenesis only in perinatal period.[22] The latter data have been confirmed by negative results of many studies on cortical neuronal proliferation in adults, which employ the 14C as detector of DNA duplication. However, it cannot be excluded that these data are false negative, because of the limited sensitivity of the technique used.[23]

In the complex, these observations consent to affirm that NSCs are present in the nervous system for the entire life of an individual, but their repair/regenerative capacity results limited in adult. Consequently, it reveals inadequate to repair the injuries and lesions of nervous system, contributing to nervous dysfunction and onset of the neurodegenerative pathologies (NPs), including Huntington's disease (HD), multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS).[24,25] Furthermore, it hypothesizes that they can be a consequence of the deficiency of NSC pool in the affected brain regions, as summarized in the recent review from Zhao et al.[26]Accordingly, the research of efficient treatments, until now inexistent, is encouraged. However, the efforts in this field, currently performed by various research's groups, have led no effective solutions. In addition, the pathophysiology of these diseases still is unclear. Numerous gaps between the knowledge of precise cellular and molecular mechanisms of these diseases and the identification of disease pathways, to use as appropriate biomarkers and targets for efficient therapeutic treatments (*e.g.*, personalized therapies), remain to be solved.

In the light of these observations, the biomedical community is pursuing new ways in trying to counteract this imposing challenge. The recent discoveries and advanced knowledge in the field of stem cell biology, and their ability to provide a cue for counteracting several diseases, are leading numerous researchers to focus their attention on ''regenerative medicine'' as possible solution.[27] However, the lack of a consistent evidence in this arena has hampered the clinical application.[28] The same condition affects the research on endogenous NSCs, even if they can be isolated, expanded, and, notably, differentiated in many cell types of the brain.[29] This has led to investigate on other types of stem cells, including *mesenchymal stem cells (MSCs)*, *embryonic stem cells (ESCs)*, and *induced pluripotent stem cells (iPSCs)*[27] (Fig. 1). Despite the efforts until now executed, their clinical application still is not possible. In fact, the results achieved still are questionable. They have only permitted to demonstrate the absence of serious adverse events. In addition, the major number of these studies has been conducted in animal models, as preclinical studies, and other serious controversies and limitations also emerge, which certainly limit their importance and lead to have diverse concerns in their applications.[28] This is leading to test and develop alternative measures of intervention as more efficient and safe drugs for NPs.

Here, we report and discuss these aspect with emphasis. An overview of the current literature data on application of stem cells in the NPs, underling limitations and benefits until now obtained, is reported. Furthermore, we illustrate some alternative therapeutic approaches, including firstly metformin,[30] but also other emerging agents with potential therapeutic effects for NPs, ranging from melatonin hybrids[31] to natural antioxidants (*i.e.*, resveratrol, curcumin, and acetyl-L-carnitine), physical exercise,[32,33] and Mediterranean diet.[34]

Stem Cells as Therapeutic Agents for NPs

Recently, the clinical application of stem cells, as therapeutic agents in NPs, is acquiring a very consideration from

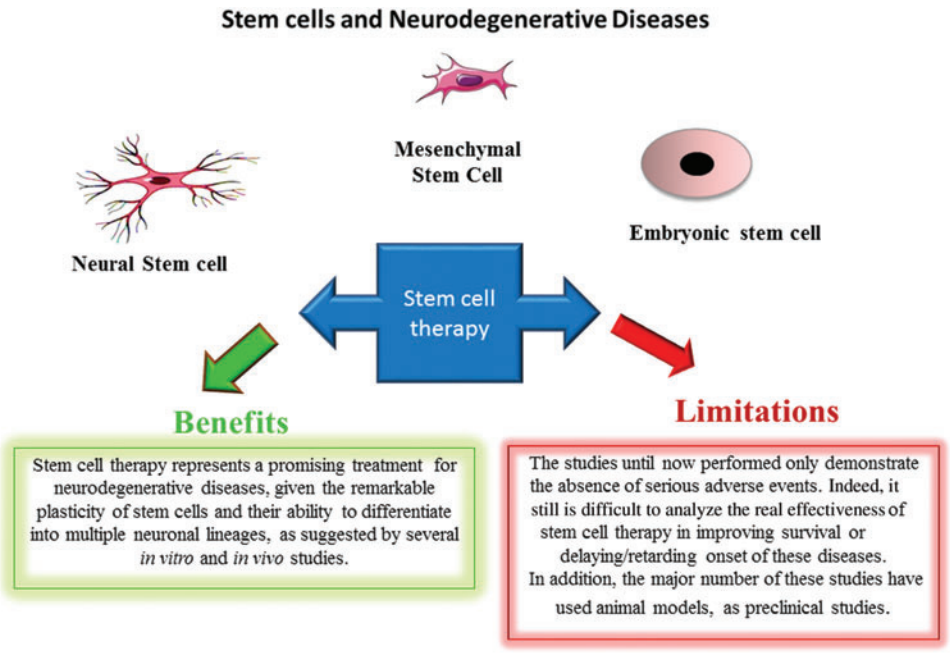

FIG. 1. Stem cell therapy and neurodegenerative disease. Advantages and limits of this treatment are reported. Color images available online at www.liebertpub.com/rej

the entire scientific community, since their incidence and prevalence in our populations are in continuous growth, as well as the number of disabling patients.[27–29] Thus, NPs represent a very social and economic problem. Stem cell therapy might, therefore, constitute a very solution for both delaying/retarding neurodegenerative process and the onset/progression of these diseases, and permitting to expand the survival of the affected patients. The hope also is of applying it in a near future as preventive treatment in susceptible individuals.

For facilitating the knowledge of the concepts about these topics reported and discussed in the subsequent paragraphs, a brief description on the classification and the features of stem cells, currently used in preclinical and clinical trials, is firstly reported and discussed.

Cell based therapy: stem cells as appropriate candidates and their features and limitations

Cell-based therapy is, today, became a real clinical application for some human pathologies, thanks to the use of stem cells and their progenitors.[6,28] Given their relevance, it is well to precise what it intends for *stem cells*.[6,28] Stem cells are defined as undifferentiated cells with the potential to renew themselves, and to differentiate into any other specialized cell of human body, and, therefore (potentially and theoretically), to create any tissues or organs. Under specific conditions, stem cells can, indeed, differentiate into diverse populations of mature and functionally specialized cellular types. To date, the following classes of stem cells are recognized: (a) *totipotent cells*, having the capacity to differentiate into embryonic and extra embryonic cell types, thereby generating the entire organisms, even if this capacity is limited to cells produced by the first few divisions after fertilization; (b) *pluripotent stem cell types* and (c) *adult multipotent/unipotent stem cells,* which can only differentiate into several closely related cell types.[6,28]

Some types of cells have been and are currently used in cell based therapy, including MSCs, ESCs, iPSCs and organ-specific stem cells, that is ASCs, such as NSCs.[6,28] Here, a brief description of these cells, which also are object of dissertation of this report, follows:

- ***ESCs:*** ESCs are derived from the inner cell mass of blastocysts (an early embryo). ESCs are pluripotent cells and give rise to all the derivatives of the primary germ layers, the ectoderm, endoderm, and mesoderm, and possess a high level of clonality, self-renewal, and pluripotency. ESCs can develop into each of the more than cell types of the adult body when stimulated (with the correct cues). A wide range of molecular biomarkers for their proper characterization and recognition (especially for humans) has been reported. Among these, membrane proteins (Stage Specific Embryonic Antigens-1, -3, -4, Cluster of Differentiation (CD) antigens 90, 117, and 133, integrins $\alpha5\beta1$, $\alpha v\beta5$, $\alpha6\beta1$, and $\alpha9\beta1$, Frizzled receptors, stem cell factor (or c-Kit Ligand), and others) represent the most important biomarkers, because they permit a direct ESC detection, without a preventive cell membrane lysis. Furthermore, several transcription factors, including Octamer-binding Protein 4 (Oct4), Sry-related High-mobility Group (HMG) Box-containing (Sox) family, Krupple-like Factor (Klf) family, Nanog, Reduced Expression 1 (Rex1 or Zfp-42), Undifferentiated Embryonic Cell Transcription Factor (UTF1), X-linked Zinc Finger Protein (ZFX), Taube Nuss (Tbn), Taube Nuss (Tbn), HMGA2, Nucleus Accumbens-1 (NAC1), Germ Cell Nuclear Factor (GCNF), Stat3, LEF1 and TCF, SALL Family, F-box 15 (FBXO15), ESC Associated *Transcript (ECAT) genes* have been also detected in stem cells and can be also used as key ESCs biomarkers. Other ESC potential biomarkers may be several signaling pathways, regulating ESC biology, including LIF-STAT3, Notch, BMP-SMAD, TGF-β/Activin/Nodal, IGF-IR, FGFR and Wnt-β-catenin intracellular pathways, but also enzymatic proteins (*i.e.*, *alkaline phosphatase* and *telomerase)* and other different molecules, such as lectin or short peptide. Since ESCs are able to differentiate into all cell types, they represent optimal potential candidates in cell-based therapies to track various human diseases, such as NPs.[6,28,35]
- ***MSCs:*** MSCs are multipotent stromal cells with a predominantly mesodermal origin, and they are one of the most translational attractive progenitor cell types, since these cells can be relatively easily isolated and expanded *in vitro* from individual patients. MSCs are obtained from BM, peripheral blood, adipose tissue, or other mesenchymal organs, and were originally defined by their plastic adherent properties and expression of specific cell surface biomarkers, such as CD105, CD90, and CD73. Currently, the global definition of MSCs is overly simplistic, since differences have been observed among MSC populations derived from different tissues, presenting an additional challenge to devising a universal definition. MSCs possess the capacity for self-renewal and the phenotypic potential to adopt a spectrum of different somatic cell types, including the osteoblast, adipocyte, endothelial, and chondrocyte lineages. Accordingly, MSCs may be able to generate nervous cells and consequently represent alternative therapeutic agents for NPs. In fact, several research's groups have focused their attention on MSCs. They have the capacity to enter the bloodstream and migrate to an injured site, where they can promote tissue regeneration because of their multilineage differentiation ability under certain environmental condition. Furthermore, MSCs are also characterized by low immunogenicity, because of both a reduced expression of the Major Histocompatibility Complex Class I (MHC I) molecules and the loss of MHC II antigens. These features make them a safe and promising tool in allogenic graft for the treatment of NPs. Several surface biomarkers have been used, until now, for the detection and purification of human circulating MSCs, including CD271, CD56, Stro-3, MSCA-1, and CD146. These biomarkers are, today, commonly used in many studies of NPs with encouraging results.[36]
- ***iPSCs:*** iPSCs[37] are not ADCs, but rather reprogrammed adult cells (*e.g.*, epithelial cells[38]) that give rise to pluripotent cells. For example, using genetic reprogramming with transcription factors (*i.e.*, Oct-3, -4, Sox2, c-Myc, and Klf4), pluripotent stem cells, which are equivalent to ESCs, may be obtained from human adult skin tissue. Like ESCs, iPSCs can also be differentiated into all three germ layers. These cells can be generated from human and mouse somatic cells and thereby overcome ethical and immunological issues such as those

identified for ESCs since both individual informed consent and patient-specific cells can be obtained. Nevertheless, iPSCs have an advantage over ESCs by avoiding ethical and immunological issues, significant safety concerns currently limit their clinical applicability. Accordingly, different methods and approaches may be used for iPSC generation. Among these, viral methods certainly are more efficient, but show the major risks because of the stochastic activation or inactivation of endogenous genes. Alternatively, nonintegrative methods (*e.g.*, mRNAs, or plasmids carrying the reprogramming factors) could lead to bypass this concern and consent future iPSC utilization in clinical practice.[39]

- ***NSCs:*** they are also studied as therapeutic NP agents.[28] They may be identified and characterized using a combination of molecules, including nestin, β-tubulin III, microtubule-associated protein 2 (MAP2), neuronal nuclei (NeuN), glial fibrillary acid protein (GFAP[28]), and other biomarkers (*e.g.*, lysophosphatidic acid receptor 1 (LPAR1[39]), and Prss56[40]) of more mature phenotypes.[41]

Testing the role of ESCs, MSCs, iPSCs, and ASCs as therapeutic agents, the enthusiasm of researchers has been reduced by diverse concerns related to the detection of various serious limitations.[35] In fact, diverse undesirable factors have been evidenced, including firstly the teratogenic and tumorigenic potential of these cells on the recipient organisms and the immune-reactivity, which consequently affect the safety of the treatments but also their reduced success related to the inadequate dose administrated, the unprecise phenotypic profile of cells used for the treatments, their biological age and senescent status, the inappropriate administration ways and methods used.[42] All these adverse factors have been reported by numerous published reports and recently summarized in an elegant manner by de Sá Silva et al.[42] This is leading some research's groups to establish standardized criteria and to point out some parameters related to the dose to administrate, phenotypes to use, methods, and administration ways, which must be observed for developing cell based therapies for human diseases, NPs included, in safety and with success. In 2015, Stoltz et al.[43] have suggested the following criteria:

- Can be found in abundant numbers.
- Can be harvested by a minimally invasive procedure with minimal morbidity.
- Can be differentiated along multiple cell lineage pathways in a controllable and reproducible manner.
- Can be safely and effectively transplanted to either an autologous or allogeneic host.
- Can be produced in accordance with current ''Good Manufacturing Practice'' guidelines.

Thus, ulterior studies are certainly needed to convert the research data in clinical applications, from preclinical to clinical studies.

The potential benefits of cell based therapy for the care and the research of NP diseases

The most acceptable opinion on the use of cell based therapy for NP diseases is that it might be advantageous for the following reasons:

1. Use of stem cells in the NP's investigations could firstly permit of identifying the real cellular and molecular mechanisms, and related pathways, involved in the complex pathophysiology of these diseases, until now unclear, which might be used as targets for the development of potential personalized therapies, at moment inexistent.
2. Cell based therapy might become the best therapeutic approach for these diseases, using doses, phenotypes, ways, or methods of administration, established by well-accepted and fixed criteria, parameters and guidelines. For example, the local expansion of endogenous NSCs in the mammalian nervous system might be very advantageous in the treatment of PD, administrating the transforming growth factor α (TGF-α), as reported in Parkinson's model of Fallon's study.[44] As an alternative way, stem cells or the different committed progenitors can also be directly transplanted in the injured areas of nervous system. In this case, the potential beneficial effects might be different: providing a trophic support to host cells, such as neurons; slowing the degenerative process; inducing a process of remyelination; producing neurotransmitters.[45] A typical example might be the transplantation of stem cells as treatment of the most severe NP of motor neurons (MN), the ALS. This treatment might be advantageous in alleviating the disease's symptoms thanks to the capacity of stem cells to differentiate in astrocytes and myoblasts, representing the two cell types extremely involved in the pathogenesis of this disease. The increase of both astrocytes and myoblasts might have beneficial effects, because these cells produce growth factors supporting the MN, including glial cell-derived neurotrophic factor (GDNF). In addition, these cells reduce the excitotoxic injury, releasing the glutamate-aspartate transporter (GLAST), brain-derived neurotrophic factor (BDNF), hepatocyte growth factor, neuronal growth factor, neurotrophin-3, and cardiotrophin-1 (produced by muscles).[45] Among stem cells, NSCs *in vitro* can differentiate into MN in the presence of chemical cues, such as retinoic acid or Sonic Hedgehog Homolog (Shh).[46,47] Regarding the mentioned beneficial effects, the most important consists in providing a trophic support, releasing growth factors recognized by several receptors with different affinity or neurotrophins. The beneficial role of all these factors is only shown when they work in concert (see Box 1, reporting the biological effects of different factors or neurotrophins). However, several limitations are attributed to neurotrophic factors: all neurotrophins could be toxic in areas without lesions; their half-life and bio-viability always are not sufficient and, therefore, they are not able to cross the highly selective permeability of blood–brain barrier. For overcoming all these difficulties, viral vectors or exosomes might be used as specific drivers of neurotrophins into the injured areas.[48,49]
3. Stem cells biomarkers could consent to monitor the effectiveness of the therapy into recipient organisms

BOX 1. THE BIOLOGICAL EFFECTS OF DIFFERENT FACTORS (OR NEUROTROPHINS) RELEASED BY NEURAL STEM CELLS

Several factors, or neurotrophins, derived from neural stem cells could determine host cell survival, induce cellular differentiation and modulate synaptic plasticity.[24] Among these factors, it includes: glial cell-derived neurotrophic factor (GDNF), produced by glial cells of central and peripheral nervous system, and able to influence the action of astrocytes and motor neurons, to defend them from death and to avoid the loss of choline acetyltransferase; neurotrophin-3 able to decrease the death of motor neurons being retrogradely caught by motor neuron's axon; Leukemia inhibitory factor with the capacity to induce myoblast proliferation, stimulate reinnervation, and consequently prevent muscle atrophy BDNF with the capacity to induce survival of motor neurons counteracting their death induced from nitric oxide or glutamate neurotoxicity; hepatocyte growth factor showing a similar action to GDNF; vascular endothelial growth factor, essential in the processes of repair and generation of new blood vessels and neuroprotection.[24]

Molecules and Environmental Factors as Emerging Therapeutic Agents for NPs: An Alternative Measure to the Undesirable Biological Effects of Cell-Based Therapy

As above mentioned, many limitations and reduced results emerge using the cell-based therapy for the human diseases, such as NPs (*see below for the details in the various NPs*).[35] This has led several researchers to put their efforts in establishing the role of several molecules in the treatment of NPs. This interest for a new field, but totally parallel, origins from the current evidence on NSC destiny (proliferation, differentiation, maintaining the undifferentiated state, *etc.*), which is completely dependent by the metabolic activity.[13,15] A sophisticated balance among diverse intrinsic metabolic pathways controls the fate of NSCs. These pathways may be induced or inhibited by nutrient signals.[50] Like to the other stem cells and progenitors, NSCs are characterized by a specific metabolic status and a specific energy expenditure, maintained by this fine balance, which regulates their NSCs' differentiation or entry in quiescence.[13,15] Different signals (both intrinsic and extrinsic) contribute to the cellular decisions.[13,15] Among the intrinsic signals,[13,15] the levels of oxygen in the niches, the bioavailability of calorie, the presence of insulin, insulin-like growth factor (IGF), the glycemia levels, and the levels of TGF-β[51] and BMPR-IA[52] pathways related to aging status of NSCs and the other cells of the niches, are of crucial relevance. All these cues influence adult neurogenesis, the formation of new neurons, and their death (Fig. 2). Among the signals inducing proliferation, *insulin and IGF1* are the most important. Their receptors have been shown especially in areas characterized by adult neurogenesis, even if they can only act together with another growth factors. These chemical signals are also produced locally, but what effectively induces this local synthesis is still unknown. Some hypotheses focus their attention on the role of nutrient availability, such as glucose and amino acids. Because of the elevated levels of glucose and insulin, these signals can be responsible of a premature exhaustion of NSCs. In this background, the depletion of IGF1 and deficits of insulin-like peptides can delay the onset of NPs.[13,15]

Furthermore, it has been evidenced that the status of transition of NSCs from quiescent cells to committed progenitors is characterized by the modifications ranging from the aerobic glycolytic metabolism to an energetic state based essentially on mitochondrial oxidative phosphorylation. This is regulated by *the oxygen tension*[53] in neural niches (usually very low: 1%–6%), which is essential to maintain the stem cell quiescence or to induce their differentiation. A hypoxic condition (2%–5% of oxygen tension in the niches) induces the expression of Hypoxia-inducible factor 1 (HIF1), which reduces apoptosis, maintains a state of quiescence, and prevents the differentiation of stem cells by inhibiting oxidative phosphorylation and favoring glycolysis. A premature exhaustion of stem cell pool (both neural and hematopoietic) has been shown in presence of HIF1 deficits.[53]

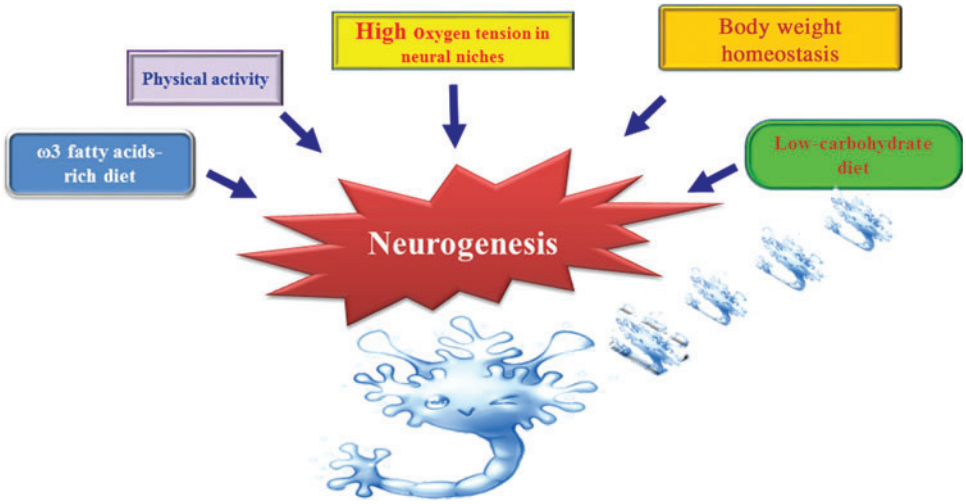

FIG. 2. Neurogenesis and inducing factors. Some factors of intrinsic or extrinsic nature, and related to behavioral choices or lifestyle have been demonstrated to modulate the neurogenesis process.[24] Color images available online at www.liebertpub.com/rej

The transitional status from stem cells to more committed progenitors is also regulated by *changes in lipid and protein metabolism*. A decreased fatty acid oxidation (FAO) and an increased ribosome formation and protein synthesis are shown in more differentiated cells. The fatty acid synthase (Fasn) enzyme is significantly expressed in NSCs and progenitors. Alterations in FAO and changes in lipid metabolism can generally promote neuropsychiatric disorders, such as autism and AD.[54]

Another intrinsic factor, related to behavioral choices or lifestyle, certainly is the *body weight homeostasis*,[55] which is the most important signal in regulating neuronal metabolism, as supported by data obtained from a study conducted in obese and diabetic mice.[55] A decreased neurogenesis and an excessive reduction of new-born neurons in hypothalamus have been observed in obese and diabetic mice, while mice in *calorie restriction* status showed increased hypothalamic neurogenesis and a major expression of BDNF.[55]

Among extrinsic factors, or exogenous behavioral factors, *the physical activity*, known to influence the synaptic plasticity, stimulates hippocampal neurogenesis and promotes all hippocampal-dependent abilities, such as memory, learning, and spatial memory.[32,33]

Based on the above stressed observations, some considerations can be evidenced. They are the following: (a) firstly, it is possible to evidence that nutrients and calorie are essential to support adult neurogenesis and the intrinsic functions of NSCs; (b) extreme conditions related to an excessive diet or metabolic disorders can increase the age-related brain decline.[13,15] These considerations suggest that *in future, the optimal therapeutic prospect to apply as preventive measure would possibly consider not only the possible pharmacologic approaches but also (or particularly) dietetic approaches to delay/retard the onset of NPs*.[15] In this context, it has been demonstrated that there is a connection between the endocannabinoid system,[56] the immune system and metabolism of omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In rats treated with EPA, it has been shown a significant proliferation of NSCs through the modulation of endo-cannabinoid pathways, consequently leading to the formation of 2-Arachidonoyl glycerol, the activation of p-38 mitogen-activated protein kinase and the action of interleukin-1β (IL-1β).[56] The use of antagonists of cannabinoid receptors attenuated these effects. The same result has been obtained in IL-1β-deficient mice.[56] On the other hand, DHA administration determined a less marked effect compared to EPA action.[56]

Interestingly, it has been also demonstrated that the metformin, normally used as diabetes drug, may also represent a therapeutic agent for NPs, as supported by many published reports.[30] Markowicz-Piasecka et al.[30] have recently summarized the recent literature data on this topic. Firstly, they have underlined the results of several clinical studies. The results obtained precisely suggest that the long-term use of metformin in diabetic patients contributes to better cognitive function, compared to participants using other antidiabetic drugs. The exact mechanisms used by metformin in inducing these advantageous effects are not fully clear. However, the researchers propose that the activation in human NSCs of Tumor Protein 73 (TAp73), AMP-activated protein kinase Protein Kinase C and *CREB-binding protein* pathway might be responsible for the neuroprotective activity of the metformin[30] (Fig. 3A). Furthermore, it has been also observed that the metformin markedly reduces the Beta-secretase 1 (BACE1) protein expression and its activity in cell culture models and *in vivo*, thereby reducing BACE1 cleavage products and the production of Aβ (β-amyloid).[30] This leads to suppose that metformin might be an optimal drug for example for AD patients. In this contest, other recent evidence demonstrates that metformin reduces the activity of acetylcholinesterase, responsible for the degradation of acetylcholine (Ach), a neurotransmitter involved in the process of learning and memory. Furthermore, many *in vivo* and *in vitro* studies have shown that metformin also has anti-inflammatory and antioxidative properties, ameliorating oxidative damage significantly associated with the NP pathogenesis.[30]

Of note also are the emerging studies about the beneficial effects of the melatonin hybrids (Fig. 3B).[31] They center their research on fact that aging is strictly associated with onset of many NPs. In addition, the investigations, addressed on identifying antiaging agents, have recently demonstrated the key role of melatonin as antiaging agent, and particularly a potential drug for NPs.[31] Accordingly, melatonin is an indoleamine produced mainly in the pineal gland. It mediates diverse pleiotropic actions, preventing several processes involved in neurodegeneration, including neuroinflammation, oxidative stress, excitotoxicity and/or apoptosis. However, melatonin shows a natural decline with advancing age, strongly contributing to the NP development.[31] Thus, the researchers are developing and testing melatonin hybrids resulting from the juxtaposition of tacrine, berberine, tamoxifen, curcumin, *N*,*N*-dibenzyl(*N*-methyl)amine, as potential therapeutic agents for the treatment of NPs. Recently, the Ramos's group[31] has summarized their effects and emphasized their advantageous actions as NP drugs.

It also appears promising the action of several natural molecules as NP drugs, having antioxidant beneficial effects, as largely emphasized by Mancuso et al.[57] Among these, resveratrol, curcumin and acetyl-L-carnitine (Fig. 3B).[57] Recently, Caruso's group is also proposing that the green olives Nocellara del Belice also have antioxidant effects[58] and might represent natural therapeutic agents for age-related diseases (Fig. 3B), NPs included.

Furthermore, the diet is another emerging risk factor for NPs, as well as for many human age-related diseases. Beneficial effects are deriving from investigations on the Mediterranean diet (Fig. 3B),[34] as potential solution. Thirteen meta-analyses of observational studies and 16 meta-analyses of randomized trials investigated the association between the adherence to the Mediterranean diet and 37 different health outcomes, for a total population of over than 12,800,000 subjects, as summarized in 2017 by Dinu et al.[34]Analyzing all data, they concluded that a robust evidence, supported by a p-value <0.001, a large simple size, and not a considerable heterogeneity among the studies, for a greater adherence to the Mediterranean diet and a reduced risk of mortality for cardiovascular diseases, coronary heart disease, myocardial infarction, over all cancer incidence, NPs and diabetes, is observed.[34]

Thus, all these agents and molecules would seem to be promising candidates as therapeutic or preventive NP approaches. Recent evidence shows no undesirable factors after their administration or observation. However, further

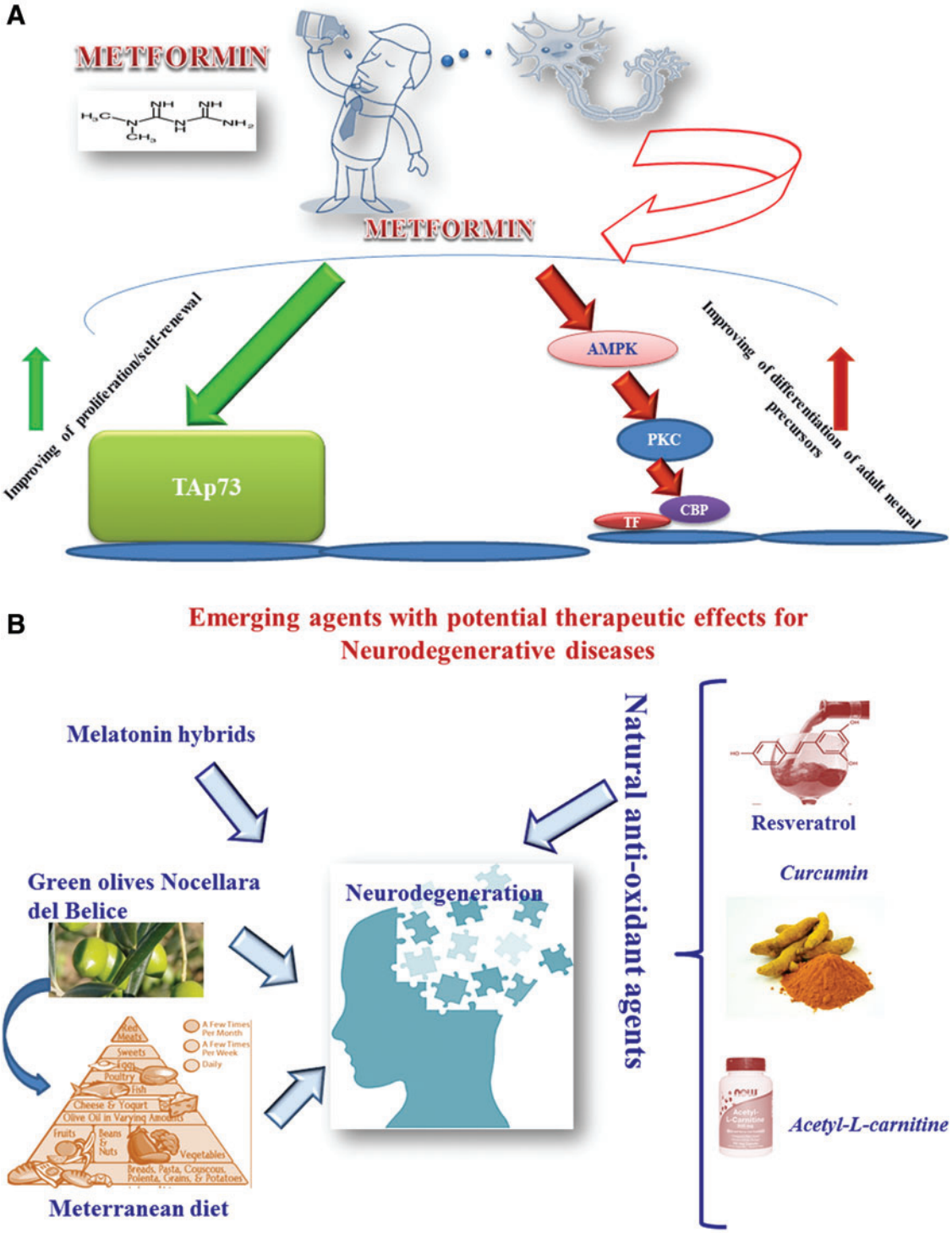

FIG. 3. **(A)** Metformin as promising drug of neuroprotection. Metformin induces proliferation/self-renewal and differentiation of adult neural precursors through two diverse molecular pathways. The first process is obtained through Tumor Protein 73 (TAp73), while the second is the result of activation of the AMP-activated protein kinase (AMPK), Protein Kinase C (PKC), and *CREB-binding protein* (CBP) pathway. In turn, their activation determines activation of different transcriptional factors (TF), and consequently the expression of several genes involved in differentiation process.[30] **(B)** Emerging agents with potential therapeutic effects for neurodegenerative diseases. Recent studies evidence the potential therapeutic effects of emerging agents, ranging from melatonin hybrids,[31] natural antioxidant molecules to the green olive of Nocellara del Belice[58] and the notable Mediterranean diet.[34] Color images available online at www.liebertpub.com/rej

drug development studies are necessary to achieve such successes.

An Overview on Literature Data About Stem Cell Therapy in NP Diseases: Advantages and Limitations

The *neurodegenerative diseases*—NPs—including HD, MS, AD, PD and ALS, represent a group of illnesses, all characterized by the following features: decline in neuronal function, brain atrophy, and often, abnormal deposition of proteins. HD, MS, AD, PD and ALS occur in diverse regions of the brain and show different etiology, but they show common cellular and molecular mechanisms.[59] As above described, they, today, constitute a very challenge in our countries, given the growing number of affected individuals. Nevertheless, at moment an effective therapy for NPs does not exist.[59] This seems linked to some difficulties that the researchers have in its experimentation, including firstly the identification of the exact cause of neurodegeneration, as for instance a unique signaling pathway capable to modify the NP onset or progression. In addition, the early diagnosis of the major number of these pathologies is difficult for the absence of efficient biomarkers and the progression often implies secondary clinical complications, such as systemic chronic inflammation, requiring changes in the treatments.[59]

Despite the results until now obtained and the related limitations, as above stressed, cell-based therapy remains the basis for the development of effective therapeutic strategies for a wide spectrum of NPs.[35] Here, we report a summary of experimental and preclinical studies previously published involving stem cell therapies for HD, MS, AD, PD and ALS, underling that stable and solid progresses in stem cell research in both basic and preclinical settings certainly are imperative for developing effective therapies. Our dissertation initiates, reviewing the data on HD disease, since it represents one of the first NP pathologies, in which cell-based therapy has been proposed.[60] Of follows, we describe the data on MS, where NSC transplantation had the first success in animals. This will facilitate us the description of the data on other pathologies.

HD as keystone in the investigations of stem cell therapy in NPs

HD is an autosomal dominant neurodegenerative disorder, clinically characterized by a progressive cognitive impairment, abnormalities of movement, and neuropsychiatric symptoms.[60] Its onset usually occurs during the fourth or fifth decade of life, and the disease symptoms and signs progress with aging, with a mean survival of 15–20 years.[60] Today, no effective therapy exists for HD. However, the stem cell biology has provided a way for experimenting therapeutic strategies, replacing the loss of neural cells with the transplantation of stem/progenitor cells.[60] Successful application of stem cell-based therapy in animal models of HD with functional recovery has been reported. Precisely, the earliest transplantation study in animal model of HD has been performed in 1983,[61] in which fetal rat striatal tissue fragments were transplanted into the kainic acid-lesioned striatum, and behavioral improvement was reported. Subsequently, numerous striatal (or other tissues) graft experiments have been performed in preclinical settings. Moreover, the first clinical study of cellular transplantation in HD patients has been conducted as a pilot study in Mexico in 1990.[62] The early trials have been, while, conducted in Cuba, Czechoslovakia, United Kingdom and California.[60] They provided data about implantation protocols and reported that the procedure showed no major complications.[63–66] In addition, several reports evidenced that the fetal striatal transplants can improve the cognitive symptoms associated with HD.[60] Thus, subsequent clinical trials used fetal striatal tissues from spontaneously aborted fetuses or elective abortions.[60] However, there have been ethical and social and logistical issues associated with the use of human fetal tissues for brain transplantation. Consequently, an alternative source of tissue for brain transplantation has been needed. Accordingly, other studies used striatal or systematic transplantation of human NSCs, and showed behavioral as well as anatomical recovery in a rodent model of HD.[60] However, the underlying mechanism of functional improvement induced by stem cell transplantation in the HD model is largely unknown.[60] Another research group tried autologous adult bone marrow MSC transplantation in HD rats. HD rats receiving bone marrow stem cells showed an improved behavioral function, but only a small number of cells expressed neural phenotype, suggesting that the release of the growth factors by the grafted cells allowed the host surviving cells to survive and function more efficiently and to facilitate other compensatory responses.[60] Other studies also used bone marrow MSCs in HD. However, autologous MSC transplantation in the human clinical setting has evidenced a problem that stem cells themselves also carry the mutant huntingtin gene.[60] Thus, autologous mesenchymal stem cell transplantation in HD patients seems not to be a definite modality for curing the disease. Further studies of bone marrow MSC cells for cell therapy in HD patients are necessary.

Furthermore, other studies used the systemic transplantation of NSCs,[60] which is probably the least invasive method of cell administration. However, other limitations have been evidenced.[60]

Thus, there still are many obstacles to resolve for the clinical application of stem cell therapy in HD: (a) it is still undefined what kind of stem/progenitor cells would be an ideal source for cellular grafts, and (b) it needs to be better understood by what mechanism transplantation of stem/progenitor cells leads to an enhanced functional recovery.

Stem cell therapy in MS disease

MS is the most common neurological disease of young adults in Western countries, and second only to trauma as one of the most debilitating.[67] The prevalence of MS has been last reported at 2.3 million people worldwide.[67] Patients experience a variety of clinical symptoms, including alterations in sensation, loss of balance, disturbances of vision or speech, extreme fatigue, muscle weakness, or paralysis.[68] Equally as significant are depression and cognitive impairment, which have only recently been appreciated for their prevalence and impact on quality of life. In each MS-affected individual, the presentation and course of disease may differ and is largely unpredictable.[68]

Today, it is in increasing the opinion to consider MS as chronic inflammatory and neurodegenerative disorder limited to the central nervous system (CNS).[68] The disease is

characterized by microvascular changes, extensive immune infiltration, demyelination, and axonal damage and cell loss.[69] Furthermore, irreversible axonal loss and neurodegeneration are considered the major alterations correlated to the chronic disability in MS.[68,69] MS presents in 80% of patients as a relapsing–remitting multiple sclerosis course with alternating clinical attacks associated with inflammatory activity, and periods of stability with complete or partial recovery.[68] This typically transitions into secondary progressive multiple sclerosis (SPMS) with progressive deterioration and disability secondary to neurodegeneration.[68] Approximately 15% of patients present with a primary progressive multiple sclerosis course, with increases in disability thought to result from degenerative processes occurring earlier in the disease.[68] Progressive MS, either the primary or secondary form, is associated with a more predictable and constant clinical deterioration, at a rate that is independent of previous disease.[68] Progressive MS typically presents later, occurring between the ages of 30 and 50 years, and remains poorly responsive to current treatment modalities. SPMS is thought to be present once a threshold of irreversible neurological symptoms is reached, and when the functional capacity to compensate has been exhausted.[70]

Stem cells have uncovered a new perspective as therapeutic tools in MS. In 2016, Meamar et al.[71] reported an overview of the studies on cell-based therapies in MS and underlined their clinical status. Accordingly, they underlined that in preclinical studies the optimal candidates are represented by MSCs and hematopoietic stem cells (HSCs) than NSCs.[71] However, NSCs have the unique feature of beneficial effects with remyelination, and this make them an attractive for further studies in clinical stages to see whether they show this benefit in practice, particularly in the progressive stages of MS.[71]

Furthermore, they evidenced[71] that there are several studies involving autologous therapies based on the recovery of mobilized bone marrow cells, including MSCs and HSCs on the treatment of MS. In summary, the major number of the trials are in phase 2 (to examine safety and tolerability of the stem cell treatment), and they had patients with median Expanded Disability Status Score (EDSS)\with follow-up median between 3 and 26 months.[71] All these trials provide the evidence of safety and effectiveness of MSCs. In fact, deaths or other adverse events have been not evidenced during the study's courses. To support of this evidence, Hou et al.[72] in a case report showed that repeated injections of bone marrow-derived MSCs followed by frequent injections of umbilical cord MSCs (both intravenously) improve one point on patient‘s EDSS score and diminish many magnetic resonance imaging lesions. However, in another case report, Alderazi et al.[73] described an MS patient, who received repeated intrathecal doses of allogenic CD 34+ MSCs derived from umbilical cord blood, as well as infusions of autologous adipose-derived stem cells obtained by liposuction. They observed severe meningo-encephalomyelitis in the patient, probably due to stem cell transplantation.[73]

Regarding the clinical trials based on autologous HS transplantation (AHSCT) in the treatment of MS, they underlined that many studies are conducted in small phase 1 (to determine toxicity and major side effects of the treatment) or 2, with SPMS participants who had a mean EDSS score baseline between 3 and 9.5 and a median follow-up between 5 months and 15 years.[74] Furthermore, some adverse events have been observed, including breakdown in task performance, bacterial infections, or sepsis.[74] However, fever is the most frequent adverse event reported.[74] It is also shown that AHSCT could result in significant improvement of patient's quality of life.[75]

In the complex, the data reported demonstrate that the transplantation of stem cells from either cell source could be a safe and effective therapy for MS. However, since up to now there is no controlled studies (randomized or nonrandomized) comparing stem cell therapy, finding a consistent answer regarding the safety and efficacy of this type of therapy for MS patients needs future comprehensive research with large group of patients.

Stem cell therapy in ALS pathology

A set of therapeutic strategies with stem cells is under clinical investigation for facilitating the treatment of NPs, such as ALS disease. ALS is a fatal NP characterized by the degeneration of both upper and lower MN in both brain and spinal cord.[76,77] It is frequently sporadic and characterized by an incidence, which varies between 1.2 and 4.0 per 100,000 individuals per year. Furthermore, ALS predominantly occurs in males.[77,78] Death occurs between 2 and 4 years after onset due to respiratory insufficiency.[76] Moreover, ALS is a complex disease associated with numerous pathologic mechanisms, including oxidative stress, mitochondrial dysfunction, axonal damage, microglial activation, inflammation, excitotoxicity, and protein aggregation.[79–82] Currently, diagnostic measures principally based on clinical examination and electrophysiological measurements,[83,84] result, in the major number of cases, inadequate for an early diagnosis, where potential therapies would likely be most effective. The clinical heterogeneity of ALS complicates the identification of the exact cause of the disease for the development of effective therapies. However, the drug riluzole may extend patient's survival by a few months.[82] In addition, multidisciplinary care, enteral nutrition and noninvasive ventilation can additionally extend patient survival.[82]

A hope in the development of effective therapeutic approaches might derive from the use of stem cells. Stem cell therapy might be a promising treatment for ALS, given the remarkable plasticity of stem cells and their ability to differentiate into multiple neuronal lineages.[85] In addition, stem cells can be used as important models for molecular pathway studies, drug screening, and cell therapy studies.

In 2016, the group of Casulari[84] summarized the existent literature data for analyzing the efficacy of stem cell therapy in clinical and preclinical studies. To this aim, stem cell therapy and survival studies in animal models and patients with ALS, published between March 2009 and March 2015, have been included.[84] Thus, a total of 714 studies have been considered. Among these, the researchers preferentially selected preclinical *in vivo* and retrospective clinical studies, since the number of clinical studies still is insufficient to assess their effectiveness, and it only demonstrates the absence of serious adverse events.[84]

The performed meta-analysis confirmed the efficacy of stem cell therapy in improving survival in preclinical trials, where a mean difference of 9.79 days (95% confidence interval: 4.45–15.14) in lifespan favored stem cell therapy.[84]

In addition, the Casulari group study[84] evidenced other very interesting aspects. Firstly, literature data demonstrate that various sources of stem cells can be used, including bone marrow cells, NSCs, MSCs, astrocyte precursor cells and pluripotent cells.[84] However, two stem cell types are prevalently used for disease modeling: ESCs and iPSCs.[86] Moreover, the study of Casulari group[84] underlined that the preclinical trials tend to use relatively young mutant $SOD1^{G93A}$ mice in homogeneous groups and in a controlled environment, in which the animals showed a similar clinical condition. Animal models are very useful for mimicking human diseases. However, they have some limitations: (a) they show different disease characteristics and a diverse progression; (b) they show different responses in trials[87]; (c) the sample size and sex of the animals often vary between studies. According to this evidence, there are, indeed, true concerns in translating preclinical studies into effective human treatments. In addition, in preclinical studies, (d) SOD1 animal models represent the familial ALS form more than sporadic ALS form, which is more common. In addition, ALS can be defined as a syndrome in which the pathophysiological mechanisms are poorly understood.[88] Accordingly, it is possible that familial and sporadic ALSs differ in some fundamental mechanisms that consequently influence in a diverse manner the effectiveness of treatments. Furthermore, the group of Casulari[84] discussed on therapeutic action of ESCs. Recent studies based on the use of ESCs have shown that the cell therapy, essentially applied for both inducing rejuvenation of MN and limiting their loss, results unable to impede the neurodegenerative process.[89,90] The potential reason seems linked to the complexity of mechanisms involved in ALS pathophysiology. Accordingly, it has been recently evidenced that the MN death is evocated not only by neuronal mechanisms, but also by the toxic environment provided by glial cells.[89,90] In agreement with this recent discovery, the literature data limited only to consider one gene, SOD1, appear inappropriate.[89,90] Indeed, the disease likely involves multiple pathways and genes, such as C90ORF72, TDP-43, FUS and cytoplasmic aggregates, suggesting an underlying convergence of cellular processes.[89,90]

Interesting results using stem cells from the bone marrow (HSCs or MSCs) have been, while, described by the Casulari study.[84] According to data reported in Vercelli and coworkers study,[90] MSCs can migrate to the spinal cord of mice, where they have neuroprotective actions (preventing the activation of microglia and the process of tissue gliosis and improving the count of MN, which explains the positive results observed in the animal studies and the trend observed in human studies).

Of note also are the data obtained by use of stem cells derived from the olfactory epithelium cells (OECs).[91] OECs are characterized to continue to multiply during the postnatal period, and are multipotent. They also serve as conductive connections between the central and peripheral nervous systems.[92] Accordingly, a clinical trial of 35 patients conducted in 2008, found that olfactory cell transplantation may slow disease progression.[93] OEC transplantation for ALS has been performed in China with positive effects in spinal cord injury studies, such as axonal regeneration, remyelination and functional improvements.[93] Although a large Chinese study reports that OECs may offer a benefit to cases, other reports criticize the observed outcomes and do not support the clinical translation of this therapeutic approach.[93]

Regarding iPSCs, studies, included in the Casulari's meta-analysis,[84] have shown that there are many similarities between iPSCs and ESCs.[94] This similarity suggests that iPSCs could potentially be used as patient-specific ESCs, consequently preventing rejection and eliminating any ethical issues. However, the iPSC recent studies in humans with ALS show many differences, such as the number of patients, cell type, delivery method and outcome measurement strategies. Although these studies show a low quality because of biases, they are important, because they contribute in increasing our current understanding on safety and feasibility of stem cell therapies for ALS.[94]

Moreover, in the major number of the studies, the group of Casulari[84] evidenced that the cell therapy procedure was uncontrolled and performed in patients with a very advanced stage of disease. The disease onset was variable and frequently prolonged at 2.32 ± 1.1 years. Accordingly, the major numbers of the authors agree that the treatment must be early performed in the disease course.[86] However, the goal of the major number of the studies essentially was to evaluate the presence or not of adverse events and consequently the tolerability to the treatments.

Considering all these limitations, the research community has recently introduced some guidelines[94] for reducing the number of false positives in preclinical studies, and therefore to prevent unnecessary clinical trials, which have been performed for evaluating various drugs. These recommendations include the following points: (1) rigorously assessing an animal's physical and biochemical characteristics with respect to human disease; (2) characterizing disease symptoms and the occurrence of death, and being alert to unexpected variations; and (3) creating a mathematical model to address questions about the experimental design, such as the number of mice that must be included in a study. To reduce concerns about animal research, Perrin[86] suggested to consider diverse factors, such as exclusion of irrelevant animals, balancing for gender, the use of siblings in the same treatment group, and inclusion in the study of genes that induce noninherited disease.

Thus, ALS is a rare heterogeneous disease, which still is poorly understood in terms of its pathophysiology. Moreover, from a clinical point of view, ALS is difficult to manage. As reported above, preclinical studies of stem cell therapy show great efficacy. However, more prospective and controlled studies are needed to establish the effectiveness of clinical studies in improving survival. Nonetheless, the most effective cell type to be used in transplantation must be determined, and it should be the one that shows better potential for neurogenesis and not only neuroprotective mechanisms.

PD and Stem Cell Therapy

PD is a dopaminergic neuron degeneration disease, which includes many risk factors.[95,96] Oxidative stress, genetic alterations, traumatic events in the brain, aging, mitochondrial harm by chemical agents, have been significantly associated with PD onset, even if its real etiology still is not completely clear.[96] It supposes that a heterogenic set of causes can contribute to its onset, with a crucial interplay between genetic and environmental factors.[96] Among genetic factors, mutations in 28 genes have been detected,

including mutations in the gene encoding the α-synuclein protein,[97] the main constituent found in insoluble aggregates inside the cells, called Lewy bodies.[98,99] Normally, α-synulclein's function is to regulate the sensitive stability between dopamine stored in the synaptic vesicles and cytoplasmic dopamine. As result of this alteration, there is a reduced output of vesicles available to fuse themselves with axonal membrane and release dopamine in the extracellular space. Therefore, this determines the dopaminergic cell death.[100] Recently in the brain of PD patients, it has been pointed out that α-synulcein underwent oxidative modifications on methionine residues. The protein contains four methionines, at Met1, Met5, Met116 and Met127, which can be modified by oxidation processes, with the result of a reduction of protein's hydrophobicity and a consequent increase of its polarity, leading to α-synuclein oligomer formations.[101–103]

Another crucial aspect on PD pathophysiology was evidenced in 1982, when in California, some drug-addicted boys developed a permanent Parkinsonism, using opiates contaminated by a neurotoxic substance, 1-methyl1-4phenyl1-1,2,3-tetrahydropiridine (MPTP), as quoted in Zhao et al. study.[95] Precisely, they began to suffer from motor dysfunctions as the result of the power of MPTP to cross the blood–brain barrier and destroy selectively dopaminergic neurons. This suggested that chemical disorders in the brain play a great role in the genesis of PD.[95]

Furthermore, a link between the disease and aging has been also identified. Aging subverts cellular homeostasis depleting the antioxidants reserves. Because endogenous protection systems are knock-out, ROS accumulate in the cell and may become responsible of the cell death or of an extended damage able to irreparably undermine the cellular functions.[96] Oxidative damage also plays the major role in the PD onset. In fact, when chemical agents or genetic disorders or other factors lower the antioxidant content, nucleic acid stability is exposed to a harm, because the balance between ROS and antioxidants hangs in favor of ROS. The exposure to excess ROS constitutes a widespread damaging mechanism, because of their trend to lead to covalent oxidative modifications. The harm is borne by components of macromolecules, undermining structures and affecting functions. ROS directly provoke damages in DNA structure, or they can induce modifications on the enzymatic systems involved in control replication, or proteins and lipids. Furthermore, when some nucleotide sequences have been amended, it is likely that the phenotypical expression of a gene, such as α-synuclein, doesn't happen properly and it may result in the increased formation of α-synucleinoligomer.[96]

Another interesting aspect of PD is that pharmacological PD approaches currently in use have only palliative action. Precisely, L-DOPA injections, administrations of dopamine receptor agonists and deep brain stimulation in subthalamic nucleus and globus pallidus are only able to alleviate symptoms for a very short period and side effects (such as on/off) appear very soon.[96] As consequence, the research of more accurate treatments able to delay or retard both PD onset and progression is very necessary. Accordingly, the use of stem cells might be favorable and aim to replace the dopaminergic neurons in pars compacta of substantia nigra.[104] To this aim, different stem cell lineages can be used, as above reported. In addition, in the specific case, cell replacement treatment might be considered successful only if the function of substantia nigra achieves the 30% of normal level. Moreover, other criteria must be respected, including the large bioavailability of the source of stem cells (*e.g.*, bone marrow for MSCs), the survival of transplanted cells, their differentiation into dopaminergic neurons and their secretion of dopamine, the behavioral recovery of the patient and the absence of a process of tumorigenesis. Each specific stem cell lineage has its advantages and limits, as above evidenced.[104] Here, these aspects are of follows illustrated.

ESCs in PD

Selected in an inner mass of a developing blastocyst, this cellular lineage can be used to give rise to: dopaminergic neurons *in vitro*; induced endogenous neurogenesis; NSC activation *in vivo*. ESCs have the capacity to differentiate into dopaminergic (DA) neurons *in vitro* in the presence of neurogenic stimulators, such as acid retinoic, Shh, FGF, EGF, BMP and GDNF.[105] The transplantation of these ESC-derived DA neurons in an PD animal model has shown beneficial effects with a behavioral recovery.[104] These cells achieve a dopaminergic phenotype and expressed synaptic markers. Transplantation of h-ESCs has displayed the same beneficial effects.[106] However, the very limited source of these cells and ethical problems are the most important disadvantages of this treatment. For this reason, there are not approved clinical trial with h-ESCs in PD patients. In addition, another limit in the use of these cells consists in their capacity to cross blood–brain barrier and, therefore, to release different trophic factors in the CNS.[104]

MSCs in PD

The rationality of the use of MSCs in the treatment of PD is due to different aspects. Firstly, MSCs produce growth factors, cytokines, extracellular matrix proteins and neuroregulatory molecules, all supporting neural regeneration.[104] Thus, MSCs induce local angiogenesis, have antiapoptotic effects (preventing the loss of dopaminergic neurons), and induce local neurogenesis and neuronal migration.[107] This is possible thanks to the activation of local glial cells that release essential factors acting on NSCs. After the transplantation of MSCs *in vivo*, it has been shown a larger release of EGF by glial cells and a more significant expression of EGFR in the SVZ.[107] It is important to focus on the capacity of MSCs to induce both angiogenesis and neurogenesis: two linked processes. MSCs are also able to have immunomodulation and anti-inflammatory effects. This is essential in patients affected by PD, because they show increased levels of proinflammatory molecules, such as Tumor necrosis factor, IL-1β and IFN-γ.[108] In addition, MSCs can transdifferentiate into neural and glial cells, which can be transplanted *in vivo.* However, these cells are small and achieve the correct morphological aspect with more difficulty. The huge advantage of the treatment based on MSCs than the other cells is that they are extremely available.[106] Indeed, they can be isolated and expanded from BM, peripheral blood, adipose tissue and umbilical cord. Clinical trials based on autologous transplantation of MSCs have already been approved.[104]

NSCs in PD

This cellular lineage is involved both in therapy and pathogenesis of this NP. Patients affected by PD show few

NSCs in affected regions of brain.[104] Their therapeutic potentiality is due to the capacity to differentiate in specific neural and glial types *in vitro*, and to restore nigrostriatal pathways and DA concentration *in vivo*.[104] This aim might be reached using autologous NSCs, autologous stem cell-derived NSCs and induced NSCs. The obstacle to this approach consists in the difficulty of obtaining neural tissue and insufficient number of adult NSCs.[104]

In this context, it is notable the study from Fallon et al.,[44] even if dated. They demonstrated as use of these cells may represent an optimal therapeutic approach.[44] Precisely, their results are crucial because they showed that NSCs are capable to induce proliferation and differentiation in response to exogenously administered growth factors.[44]

In the complex, the literature data show the use of several stem cell therapies, but the effects are not definitive, as above illustrated.

Therapy with stem cells in AD

AD is characterized by neurodegeneration, amyloid plaques, that settle around the cells and close to the membranes, hampering the connections between the cell and the microenvironment.[109] The cause of neuronal death is unknown, except for those cases (from 1% to 5%) in which the histopathological changes are related to abnormal genetic alterations. Mutated allelic forms have been found in the genes that encode for Presenilin 1 (PSEN1), Presenilin 2 (PSEN2) and Amyloid Precursor Protein (APP). APP is a type I transmembrane protein composed of 39–43 amino acids and, normally, it is cleaved by α, β or γ secretases.[110] When APP is sequentially cleaved by secretase enzymes, soluble Aβ 40, or insoluble Aβ 42 fragments can be generated. In physiological conditions, Aβ40 represents more than 90% of Aβ fragments while Aβ 42 is more prevalent among AD patients and aggregates[111,112] into a pleated sheet structure, making amyloid insoluble. β-Amyloid acts to activate complement, initiates reactive changes in microglia, and stimulates the release of chemokines and cytokines. The products include the membrane attack complex, oxygen free radicals, and excess glutamate.[111,112]

Furthermore, in the AD genesis, neurofibrillary tangles (NFTs) also play a concurrent role. These are aggregates containing a modified form of protein tau. In fact, when tau protein is hyperphosphorylated, it loses its function, becomes inactive, and falls leading to the formation of NFTs inside cell.[112]

Thus, pathogenesis of AD appears complex and insidious and currently no effective treatments have been developed. Consequently, stem cell therapy in AD might certainly be of help.[113] However, its success might be complex and show few favorable results, since the neurodegeneration is extended to large cerebral areas, including simultaneously amygdala, hippocampus, cortical areas and the basal forebrain cholinergic system.[113] Given heterogeneity of the affected areas, the stem cell lineages, which might be used, would be treated in an appropriate manner for predifferentiating into different types of cells, to replace the lost neurons in the various areas.[113] Furthermore, the reduction of the Ach levels in the AD brain might require an increase of cells, using stem cell-derived basal forebrain cholinergic neurons. To date, this remains only a hope-full hypothesis, even if an increasing number of data evidences the biological effects of ESCs, iPSCs, NSCs, and tissue-derived stem cells in AD.[113] The transplantation of ECSs in AD rat models seems to improve memory and cognition impairment, although these treatments show as complication the teratoma formation.[113] The same results have been obtained using iPSCs. In the specific case of h-iPSC application, the studies performed have also permitted detecting the molecular AD mechanisms.[114] iPCSs could also constitute a serious platform for studying the cellular responses induced by drug therapies.[114]

In addition, other published reported demonstrated that the transplantation of MSCs derived from BM, activating endogenous microglia, resulted to be able to remove plaque depositions in the hippocampal regions. Thus, an improved condition about memory and cognition functions was evidenced.[113] Therapeutic potential of MSCs has been also observed in other experimental studies conducted in APP transgenic mice. Precisely, the injection of human amniotic membrane-derived mesenchymal stem cells determined an improvement in mice learning ability and memory, a decline of oxidative stress with increased levels of glutathione, a significant reduction of amyloid plaque depositions and lipid peroxidation products.[114]

Regarding the NSC studies in AD, it has been shown their ability to home in brain of rat AD models and to differentiate into final cells, such as neurons, astrocytes and oligodendrocytes.[113] The transplantation of NCS cells results in a partial cognition recovery, which is suggestive of an increased number of cholinergic neurons in animal models.[113] This advantageous effect seems to be achieved through different ways. The observed neurogenesis might be not only the consequence of an increased NCSs differentiation, but also the result of NSC-induced modifications in microenvironment conditions stimulated by release of their neurotrophic factors.[113] Of note also are the studies performed in AD models. Among these, those performed by Blurton-Jones et al.[115] are very interesting. They transplanted murine NSCs in the hippocampus of 3xTg-AD mice (mice containing three mutations associated with familial AD) and in non-Tg mice. Control groups were characterized by the administration of a simple vehicle without NSCs. The 3xTg-AD mice, 18-month old, showed typical lesions of advanced AD, the loss of synapses and neurons, evidence of gliosis process and the clinical symptoms of memory and cognitive deficits. 3xTg-AD mice transfused with NSCs showed significant improvements in cognitive and memory abilities. In addition, after NSC transplantation, hippocampal synaptic density changed. This confirmed that typical AD memory and learning disability is not due directly to Aβ plaques and tau oligomers, but to the reduced synaptic density caused by the toxic effects of these histological alterations.[115] Densitometric analysis showed an increased synaptic density in the NSC-injected 3xTg-AD mice, but not in the vehicle-injected mice.[115]

Although NSCs in 3xTg-AD mice could differentiate into several mature cytotypes, the real beneficial effect of NSCs is caused by trophic support, as above mentioned.[115] The improvement of synaptic density seems to be the consequence of a significant expression of BDNF, especially in the hippocampal region.[116–118] In addition, there was not any cognitive improvements in 3xTg-AD mice receiving NSCs transduced with shRNA and expressing a 78% reduction in BDNF secretion.[116–118]

Another cell therapeutic strategy could aim at using a combination of NSCs and γ-aminobutyric acid (GABA) interneuron precursors, since patients with AD are characterized by altered GABAergic system. Cortical GABAergic interneurons have been found during embryogenesis in the medial ganglionic eminence (MGE). Ulterior positive results might be observed using GABAergic inhibitory neuron precursors from MGE because of their plasticity. It has been indeed demonstrated their ability to migrate and to differentiate into mature cells, until the structural interaction into the hippocampal circuitries.[94]

Thus, the literature data report the study of several stem cell therapies in AD, but with inconclusive results, as above described. The key reason might derive from the fact that the major number of physiopathological AD mechanisms are not completely understood.

Conclusions and Perspectives

Stem cell therapy might represent a promising treatment for NPs, given the remarkable plasticity of stem cells and their ability to differentiate into multiple neuronal lineages (Fig. 1). In addition, stem cells might also have other applications in this context. Indeed, they might represent important models useful for performing molecular pathway studies and drug screening. Notably, their valence is also leading to effectuate some clinical trials. However, until now it is not possible to evaluate the effectiveness of stem cell therapy in improving survival or in delaying/retarding the onset of these diseases, because the number of both preclinical trials and clinical studies is still insufficient. They consent only to demonstrate the absence of serious adverse events, as above mentioned. Despite these limitations, they evidence an important aspect, which might be of help in future investigations, their heterogeneity in the design, methodologies, clinical status of cases, and sample size. Thus, the studies until now performed appear of an unsatisfactory quality.

Another critical aspect, which is possible to evidence, regards the preclinical trials. Their major number is based on use of mutant mice, precisely the relatively young mutant $SOD1^{G93A}$ mice divided in homogeneous groups, and maintained in a controlled environment, in which the animals show a similar clinical condition. This certainly does not reflect the real environmental conditions of humans. Thus, animal models are very useful for mimicking human diseases, but they show several limitations. Among these, they have a distinct disease progression and show diverse responses in trials with drugs.[85,87] Moreover, their sample size and gender are not identical among studies. Another limitation is linked to the translation of the data obtained with preclinical animal studies into effective human treatments.[85,87] In preclinical studies, for example the SOD1 animal models represent the typical model of NP familial forms, respect to sporadic forms (*e.g.*, ALS studies as above mentioned). In addition, these diseases can generally be defined as a syndromic, in which the pathophysiological mechanisms still are poorly understood.[88] Consequently, it is not possible to discriminate the fundamental mechanisms involved in the effectiveness of treatments. In addition, their valence seems to be reduced. An example is given by recent studies with ESCs. They have shown that the use of cell therapy to substitute MN is not sufficient to impede the neurodegenerative process, as evidenced in ALS studies above reported. In addition, it has been underlined that the trophic support of stem cell therapy might be of major relevance than the replacement of cells. Consequently, ulterior studies are necessary.

Furthermore, various sources of stem cells can be used, as above described. Among these, certainly the ESCs and iPSCs

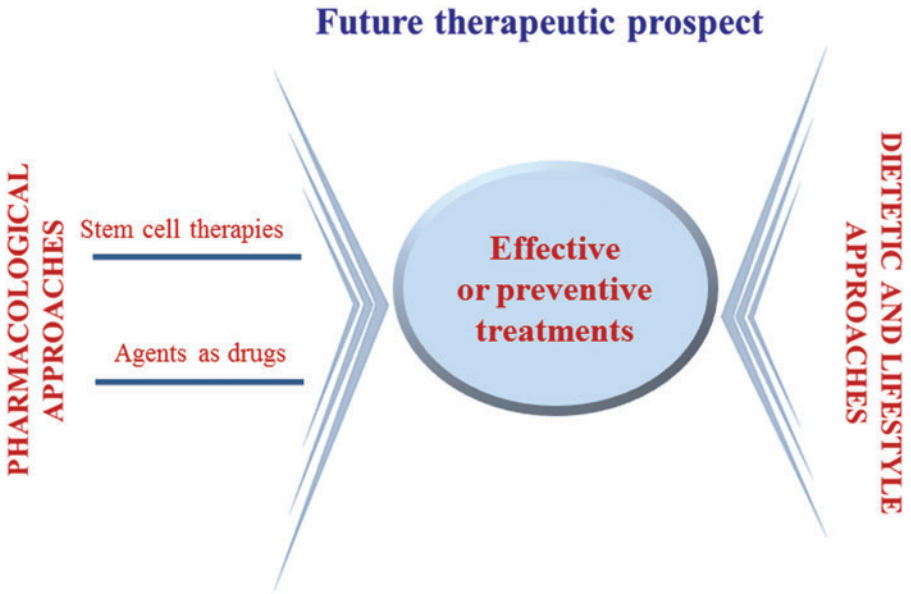

FIG. 4. Future therapeutic prospect. In a near future, a new therapeutic prospect must be considered, which must contemplate not only the pharmacological approaches but also the dietetic and lifestyle approaches, which can facilitate or modulate the effectiveness of drugs or measures of intervention used as effective or preventive treatments. Color images available online at www.liebertpub.com/rej

represent the best candidates. However, ethical problems particularly limit the use of ESCs, which constitute the multipotent cells ''par excellence.'' Regarding the iPSCs, their development has led to remarkable changes in stem cell science. This technology has been able to obtain pluripotent stem cells directly from a patient's adult cells. These cells are usually induced to form embryonic bodies and subsequently form NPCs,[86] which holds new promise for the treatment of NPs. However, studies have shown that there are many similarities between iPSCs and ESCs, such as telomere renewal during cell reprogramming into iPSCs and telomere shortening upon differentiation into somatic cells.[93] This analogy suggests that iPSCs could potentially be used as patient-specific ESCs, consequently preventing rejection and eliminating any ethical issues.

Another alternative candidate might be represented by MSCs. They have several sources as adipose tissue, umbilical cord, placenta and embryonic tissues. They show several potentialities. For example, the group of Vercelli et al.[90] demonstrated that mesenchymal cells can migrate to the spinal cord of mice. Here, they have neuroprotective actions, such as preventing the activation of microglia and the process of tissue gliosis and improving the count of MN, which result in the positive results observed in all animal studies, and explain the trend observed in human studies. Thus, these promising results lead to suggest their use as optimal candidates for the treatment of NPs.

In the complex, the use of stem cells in preclinical studies show great efficacy. Certainly, more prospective and controlled studies are needed to establish the effectiveness of clinical studies in improving survival. Furthermore, the most effective cell type to use in transplantation must be determined, and it might be the one that shows better potential for neurogenesis and not only neuroprotective mechanisms. In addition, criteria, parameters and guidelines related to the dose to administrate, phenotypes to use, methods and administration ways, must be observed for developing cell-based therapies for human diseases, NPs included, in safety and with success.

Since the clinical application of stem cell therapy still appears to be a myth and not a fact, alternative measures or intervention strategies might be used for delaying or retarding the neurogenerative process and the related diseases. Some examples in this report have been described and discussed, from the physical activity[32,33] and Mediterranean diet[34] to use of metformin,[30] melatonin hybrids,[31] natural antioxidant agents,[57] which show multiple protective biological actions (Fig. 3A, B). Metformin and melatonin's studies[30,31] particularly suggest the necessity of synthesizing new molecules, which can interact with multiple targets with the aim to improve the balance of efficacy and the safety compared to the use of a single drug. In this context, the diet type has been also shown to modulate the effectiveness of the drugs used. This also leads to reflect in assuming new ways of interventions in management of these pathologies, which contrast the old methodology of research based on a unique discipline. Today, the translation medicine might be the way of success for counteracting these diseases. As above reported, in near future a therapeutic prospect must consider not only the pharmacological approaches but also the dietetic and lifestyle approaches, which can facilitate or modulate the effectiveness of drugs or measures of intervention used as effective or preventive treatments (Fig. 4).

Authors' Contribution

C.R.B. was involved in conception and design of the study. C.R.B., M.N., and G.S. (two university students of Medicine School) were involved in drafting the article. F.C contributed to making some figures. C.R.B. was involved in the study's supervision and revision. All authors participated in the study, and they read and approved the final article.

Author Disclosure Statement

No competing financial interests exist.

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