Biochemical Pharmacology 211 (2023) 115522

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

# Alzheimer's disease: Insights and new prospects in disease pathophysiology, biomarkers and disease-modifying drugs

Ana R. Monteiro [a,b], Daniel J. Barbosa [c,d], Fernando Remião [a,b], Renata Silva [a,b,*]

[a] UCIBIO - Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, Porto University, 4050-313 Porto, Portugal
[b] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[c] TOXRUN - Toxicology Research Unit, Department of Sciences, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[d] Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal

ARTICLE INFO

*Keywords:*
Alzheimer's disease
Amyloid-beta
Tau hyperphosphorylation
Disease biomarkers
Disease-modifying drugs

ABSTRACT

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases that affect millions of people worldwide, with both prevalence and incidence increasing with age. It is characterized by cognitive decline associated, specifically, with degeneration of cholinergic neurons. The problem of this disease is even more fundamental as the available therapies remain fairly limited and mainly focused on symptoms' relief. Although the aetiology of the disease remains elusive, two main pathological hallmarks are described: i) presence of neurofibrillary tangles formed by unfolded protein aggregates (hyperphosphorylated Tau protein) and ii) presence of extracellular aggregates of amyloid-beta peptide. Given the complexity surrounding the pathogenesis of the disease, several potential targets have been highlighted and interrelated upon its progression, such as oxidative stress and the accumulation of metal ions. Thus, advances have been made on the development of innovative multitarget therapeutical compounds to delay the disease progression and restore cell function.

This review focuses the ongoing research on new insights and emerging disease-modifying drugs for AD treatment. Furthermore, classical and novel potential biomarkers for early diagnosis of the disease, and their role in assisting on the improvement of targeted therapies will also be approached.

## 1. Genetics and epidemiology of Alzheimer's disease: An overview

Alzheimer's disease (AD) is a neurodegenerative disorder that mostly affects the elderly population worldwide, and which is characterized by a progressive decline in cognitive function [1,2]. It represents the most predominant form of dementia estimating that, by 2050, it will affect approximately more than 100 million people worldwide [3,4]. Thus, AD is categorized by the World Health Organization as a disease of public health priority [3,4]. A study related to the epidemiology of dementia and AD in Portugal, reported that both incidence and prevalence increase with age, doubling approximately every 5 years [5]. From the number of patients over 60 years-old diagnosed with dementia, and knowing that 50 to 70 % of the cases are due to AD, they estimated that, in 2013, there were between 80,000 and 112,000 people diagnosed with AD in Portugal [5]. Indeed, ageing is the most striking risk factor underlying this disease, as indicated by the increased prevalence in the aged population (65 to 85 years old) [2,6]. However, the causes of AD are far from being fully known.

Clinically, AD is characterized by a slowly progressive loss of memory and cognitive impairments [2,6]. The decline in patients is mostly due to the aberrant accumulation of toxic protein fragments in the nervous system, namely amyloid-beta (Aβ) deposition within senile plaques, outside of neurons, and intracellular accumulation of microtube-associated Tau protein. Aβ is pointed to contribute to cell death by interfering with neuron-neuron communication at synapses,

*Abbreviations:* AD, Alzheimer's disease; Aβ, amyloid-beta; APP, amyloid precursor protein; ATP, adenosine triphosphate; BACE-1, beta-site APP cleaving enzyme 1; CNS, Central Nervous System; CSF, cerebrospinal fluid; CYP450, cytochrome P450; GPX4, glutathione peroxidase 4; GSH, reduced glutathione; GSK-3, glycogen synthase kinase-3; MCI, mild cognitive impairment; NADPH, nicotinamide adenine dinucleotide phosphate; NMDA, *N*-methyl-*D*-aspartate; Nrf2, Nuclear factor erythroid 2-related factor 2; PET, Positron Emission Tomography; PP2A, protein phosphatases 2A; ROS, reactive oxygen species; siRNA, small interfering RNA.

* Corresponding author at: UCIBIO/REQUIMTE-Laboratory of Toxicology, Biological Sciences Department, Faculty of Pharmacy of University of Porto, Rua Jorge Viterbo Ferreira n° 228, 4050-313 Porto, Portugal.
*E-mail address:* rsilva@ff.up.pt (R. Silva).

https://doi.org/10.1016/j.bcp.2023.115522
Received 23 February 2023; Received in revised form 21 March 2023; Accepted 22 March 2023
Available online 28 March 2023

while neurofibrillary tangles of Tau restrict the passage of essential nutrients and other compounds inside neurons [1,2,6]. These two predominant changes associated with AD will be explored in further detail in chapter **3. Pathophysiology of Alzheimer's disease**. In addition to these causes, abnormalities in lipid-carrier protein apolipoprotein E, especially associated to the ε4 allele, and in the abundance of the presynaptic protein α-synuclein are also associated. While apolipoprotein E4 plays a role in the accumulation of Tau and Aβ peptides, α-synuclein can self-aggregate into large inclusions inside the neurons, known as Lewis bodies [6].

There are two forms of AD, described as late-onset (AD with the age greater than 65 years old) and early-onset (AD in ages between 30 and 65 years old). Genetically, AD can be divided into familial or sporadic cases [7]. The familial cases, in which individuals inherit a mutation, are largely early-onset, affecting a minor number of patients. These cases are characterized by inherited autosomal mutations in genes coding for proteins associated with Aβ aggregates, such as amyloid precursor protein (APP) and presenilin-1 or 2, both present in the catalytic center of the enzyme γ-secretase. The *APP* gene is constituted by 18 exons, which can give rise to 10 different isoforms, with APP 695 being the most predominant in the central nervous system (CNS) [7]. The enzymes involved in the cleavage of APP are α-secretase, β-secretase (identified as BACE-1, beta-site APP cleaving enzyme 1) and γ-secretase [7,8]. There are several mutations in the *APP* gene, primarily located in the exons 16 and 17, that give rise to an increase or change in the production of Aβ, since these mutations can occur very close to the cleavage site of either α-secretase or γ-secretase [7]. Most mutations that occur in the genes coding for presenilin-1 or 2 result in an increased production of the toxic Aβ fragments [7]. Sequencing analysis identified mutations in *APP*, *presenilin*-1 and *presenilin*-2 genes in 170 families with early-onset AD and in 18 sporadic cases (17 presented a *presenilin*-1 mutation and 1 an *APP* duplication; total of 129 sporadic cases analysed) with onset under age 51 [9]. In 10 of the 18 sporadic cases, the mutation was not identified in direct relatives, indicating a "*de novo*" mutation. This study supports a connection between familial cases of AD and mutations in these 3 genes, and indicated that gene mutations can also account for nonfamilial cases of AD with onset at an early age [9].

Sporadic AD, with no familial history described, is the most prevalent form of the disease, where environmental factors, such as exposure to pesticides, metals and industrial surfactants, as well as anomalies in apolipoprotein E4, constitute important risk factors [2,6,10]. Indeed, the *apolipoprotein E* gene is the predominant genetic risk factor linked to late-onset AD, being responsible for more than 99 % of the cases [11]. The apolipoprotein E is present in the brain, mostly produced by astrocytes, playing a key role in cholesterol homeostasis [7,11]. There are three described polymorphic forms of the *apolipoprotein E* gene, namely ε2, ε3 and ε4, where the polymorphic form ε4 is associated with an increased risk of AD [7,11]. Although the mechanism involving apolipoprotein E in the pathogenesis of the disease is not yet fully elucidated, it has been shown to influence Aβ seeding and aggregation, as well as its clearance [11,12]. The apolipoprotein E4 is able to bind to Aβ, forming a complex that can be detected in senile plaques [7,11]. Besides, apolipoprotein E is involved in the conversion of Aβ monomers (non-toxic fragments) into toxic oligomers and fibrils [7,11]. Regarding Aβ clearance, which involves mechanisms like proteolytic degradation and cellular clearance, apolipoprotein E is engaged in Aβ clearance in an isoform-dependent path (apolipoprotein E2 > apolipoprotein E3 > apolipoprotein E4) [12]. In Aβ clearance, astrocytes are responsible for its intracellular uptake and degradation. Microglia migration and phagocytosis activation also play a key role in Aβ clearance [11,12]. Structural differences in the three apolipoprotein isoforms may influence their functionality, causing changes in the distribution of cholesterol and other lipids and, consequently, affecting neuronal plasticity [7,11].

In 2018, Ferretti and colleagues noticed a heterogeneity of the disease among AD patients, in both manifestation and progression [4]. Accordingly, after studying the sex-related differences in neuronal anatomy and function, they verified that sex might also constitute a relevant risk factor in the variability of AD. Thus, a recognition of these differences can be a breakthrough in more personalized treatments [4]. In fact, in general, AD affects more women than men, as revealed by the analysis of prevalence by sex, and some female-related risk factors contribute to this phenomenon. Indeed, some evidence link conditions as pregnancy and menopause to AD [1,4]. Therefore, the key points in this study highlighted that i) women have some protection relative to men in the symptomatic phase of AD before dementia. Nonetheless, later they exhibit higher cognitive decline and higher rates of brain atrophy; ii) evaluation of the levels of Aβ showed no differences between genders; iii) a gender-specific susceptibility to the effects of apolipoprotein E4 was detected, which revealed that the risk of developing AD is higher in women carrying the mutation than in men [4]. In line with a gender difference in the risk of AD development, Ferris and colleagues, in 2009, also verified that rivastigmine was able to delay the disease progression from mild cognitive impairment to AD only in women [4,13]. This makes clear the importance of considering the emerging evidence of gender differences to improve treatment efficacy [4].

AD is supposed to begin years before perceptible symptoms appear [1]. In fact, an higher accumulation of Aβ was detected in the brain of symptomatic individuals from families carrying autosomal dominant mutations in *presenilin*-1, *presenilin*-2 and *APP* genes than in non-carriers, which started 22 years before the onset of dementia. Most importantly, impairments in glucose metabolism started 18 years before expected symptomatology, and brain atrophy began 13 years before symptoms appear [14]. This supports the idea that the initial stages of the disease are characterized by molecular and cellular alterations that are not clinically detected.

Currently, epigenetics has also been applied to AD [8,15]. Both onset and progression of the disease involve several factors such as ageing, genetic mutations, metabolic impairments and exposure to environmental factors, which can induce epigenetic changes or AD-like pathogenesis at an early age [8]. The epigenetic modifications include DNA methylation and hydroxymethylation, impairments in chromatin structure caused by histone modifications and RNA-mediated pathways from miRNA (noncoding RNAs) [8,15,16]. These epigenetic mechanisms play an important role in the development of memory processing and maintenance, both in physiological and pathological conditions [8,16]. The methylation of cytosine residues is associated with gene silencing. In the cytosine-guanine regions of the DNA, the DNA methyltransferases (DNMT) are responsible for arbitrating this process, where S-adenosyl-L-methionine (SAM) acts as a donor of methyl groups. Four types of DNMT are identified, with DNMT1 being the major methylase responsible for the maintenance of the methylation in somatic cells [16]. Some studies linked DNA methylation with a deterioration in neuronal plasticity [8,16]. In fact, several processes are regulated by this mechanism, such as silencing and imprinting germline-specific genes and long-term memory formation [16]. Thus, since DNA methylation decreases with age, it might represent a potential biomarker for the disease [8,16]. In fact, a less pronounced immunoreactivity of 5-methylcytosine was detected in cortical neurons of *postmortem* brain samples of AD patients, when compared to neurons of non-disease controls at a similar age. In addition, the low levels of 5-methylcytosine inversely correlated with markers of neurofibrillary tangles in the same neurons [16,17]. This supports an altered epigenetic regulation in AD.

In addition, DNA hydroxymethylation is also described as an epigenetic modification, as a result of the oxidation of methylated cytosines residues [18,19]. This hydroxylation of 5-methylcytosine is involved in both transcriptional activation and suppression, and can be mediated by two processes: by oxidative stress or by the specific oxidant enzyme, ten-eleven-translocation-1 (TET1) [16,18]. Similarly to DNA methylation, differences in DNA hydroxymethylation correlate with AD. Some studies have reported a 20 % reduction in the immunoreactivity of 5-hydroxymethylcytosine in the hippocampus of AD patients, when compared to

**Table 1**
Different stages of Alzheimer's disease and their main signs and symptoms.

| Stage | Characteristics/symptoms | References |
|---|---|---|
| **Preclinical AD** | - measurable biomarkers and detectable changes in the brain, CSF and blood;<br>- absence of symptoms such as memory loss; | [1,25,26,28,29] |
| **MCI due to AD** | - measurable biomarkers and changes in the brain related to AD pathology;- moderate cognitive decline, mainly felt in the execution of small daily tasks<br>(*e.g.* paying the bills or preparing a meal), making evident the need for a longer period of time to execute them and lower efficiency in the process; | |
| **Dementia** | - measurable biomarkers and changes in the brain related to AD pathology;<br>- outstanding memory loss;<br>- behavioral and personality changes;<br>- severe impairments in realizing daily tasks; | |

AD - Alzheimer's disease; CSF - cerebrospinal fluid; MCI - mild cognitive impairment

control patients [18,19]. By contrast, other studies have reported increased levels of 5-hydroxymethylcytosine in the hippocampus of AD patients [20]. Therefore, despite the growing interest in this thematic, further studies are needed to accurately establish how DNA methylation/hydroxymethylation changes with AD. [18].

## 2. Clinical manifestations of Alzheimer's disease

Over the past decades, several studies highlighted that the severity of AD is intimately linked to a progressive interference with the cholinergic system, essential not only in memory and learning mechanisms, but also in neuronal plasticity. Therefore, this progressive loss of cholinergic neurons contributes to the age-associated memory loss and impaired cognitive abilities that characterize the disease, accompanied by other brain alterations, such as atrophy, resulting from cell death and inflammation [21–23].

The brain is able, at an early stage of the disease and through compensatory mechanisms, to enable patients to maintain normal cognitive functions that rely on adequate cholinergic neurotransmission. As the disease progresses, the decline in cognitive functions becomes more noticeable, with the spreading of plaques and neurofibrillary tangles to other parts of the brain. As a result, and as the cognitive damage becomes more significant, memory loss, confusion, behavioral changes accompanied by depression, personality change, and apathy start to become evident. Eventually, basic functions, such as swallowing, are also affected as a result of outstanding nerve cell damage [1,24].

Currently, 3 stages of the disease are described, namely preclinical AD, Mild Cognitive Impairment (MCI) due to AD and dementia [1]. The symptoms and signs of the disease vary according to the stage, with a positive correlation with the damage inflected to the neuronal cells.

The disease begins years before the onset of symptoms, as suggested by the presence of disease biomarkers in the cerebrospinal fluid (CSF) or amyloid positron emission tomography (PET) imaging. Thus, the preclinical AD stage is, by definition, assumed when biomarkers are present in individuals with normal cognitive functions and are theoretically referred to from the first onset of pathological brain damage until the appearance of the first clinical symptom. This definition includes presymptomatic individuals (once carriers of an autosomal dominant mutation are fated to develop full clinical AD) and asymptomatic at risk state (encompassing an absence of clinical signs of AD, accompanied by the presence of at least one biomarker of the disease) [25].

In MCI due to AD, individuals become symptomatic, but not yet demented, being accepted as a syndrome described by clinical, cognitive and functional criteria. Although they are able to maintain independence with minimal assistance, individuals diagnosed with MCI experience impairments in the performance of several cognitive domains, including memory, language, attention and visuospatial skills [26,27].

In dementia, the final and more severe stage of the disease, the symptoms emphasize the degree of neuronal damage and the extension to different parts of the brain [28,29]. Table 1 gathers the main characteristics and symptoms of the 3 different stages of AD.

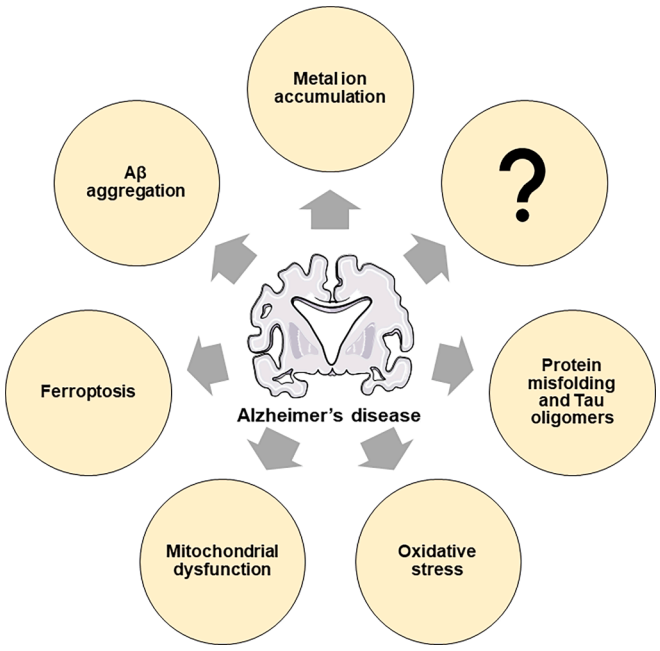


**Fig. 1. Main pathophysiological mechanisms involved in Alzheimer's disease**. Despite advances made in the last decades, the disease's true aetiology is still far from being fully known. To the well-established pathological hallmarks of AD (Aβ aggregates and hyperphosphorylated Tau), other possible causes, interrelated upon disease progression, are nowadays emerging as putative targets for disease treatment.

## 3. Pathophysiology of Alzheimer's disease

Despite the increasing knowledge on the molecular, biochemical and cellular mechanisms of the disease, the true aetiology and pathogenesis of AD remain unknown. This makes difficult the development of novel effective disease-modifying drugs [30]. The main pathological hallmarks (Fig. 1) remain the excessive accumulation of Aβ plaques, the formation of neurofibrillary tangles and neuronal loss. Despite the genetic predisposition, increased evidence have linked gliosis, inflammation, impairments in the balance between production and removal of reactive oxygen species (ROS), mitochondrial dysfunction and excessive accumulation of metal ions to the pathogenesis of AD disease (Fig. 1) [8,31–33]. The following subchapters focus on the most relevant pathological pathways linked to the disease, as well as putative drug targets for AD treatment.

### 3.1. Amyloid-β cascade

The amyloid cascade hypothesis highlights that Aβ, the main proteolytic fragment of APP cleavage, is the key factor in AD pathogenesis. APP, present in the somatodendritic and in the axonal compartments of neurons, is a single-pass type I membrane protein, described by a single transmembrane domain with a large extracellular domain and a short cytoplasmic tail [34,35].

The cleavage of APP within its extracellular domain can occur by two distinct mechanisms: the nonamyloidogenic and amyloidogenic pathways (Fig. 2). Firstly, α-secretase or β-secretase are responsible for cleaving APP in the extracellular domain, forming soluble ectodomains and membrane-tethered C-terminal fragments. Afterwards, in both

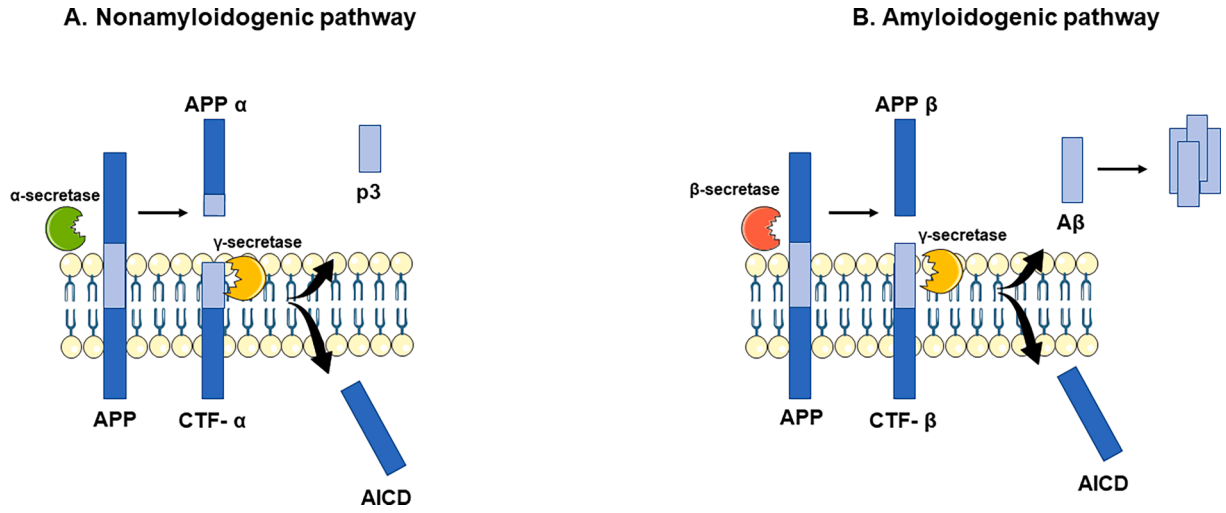


**Fig. 2. The nonamyloidogenic (A) and amyloidogenic (B) pathways involved in Alzheimer's Disease.** (A) In the nonamyloidogenic pathway, α-secretase cleavage produces the soluble ectodomain APPα and the C-terminal fragment α (CTF-α). Then, the cleavage of the CTF-α by γ-secretase produces the non-amyloidogenic fragment p3. (B) In the amyloidogenic pathway, β-secretase (BACE-1) produces the soluble ectodomain APPβ and the C-terminal fragment β (CTF-β). The cleavage of the CTF-β by γ-secretase generates Aβ peptides, which, due to their ability to aggregate, gives rise to the characteristic amyloid plaques of the disease. In both pathways, γ-secretase is also responsible for producing identical cytosolic polypeptides termed APP intracellular domain (AICD).

pathways, γ-secretase is responsible for the transmembrane cleavage of the membrane-tethered C-terminal fragments formed and for generating identical cytosolic polypeptides termed APP intracellular domain (AICD) [36,37].

In the nonamyloidogenic pathway, APP processing involves α-secretase followed by γ-secretase activity. First, α-secretase cleavage produces the soluble ectodomain APPα and the C-terminal fragment α. Then, the cleavage of the C-terminal fragment α by γ-secretase results in the production of an APP fragment named p3, classified as a non-amyloidogenic peptide, lacking pathological effects (Fig. 2). On the other hand, the amyloidogenic pathway involves BACE-1 (β-secretase) followed by γ-secretase processing. The cleavage of the APP by β-secretase produces the soluble ectodomain APPβ and the C-terminal fragment β. Subsequently, the cleavage of the C-terminal fragment β by γ-secretase at multiple sites generates Aβ peptides (Fig. 2) [34–36]. The Aβ peptides induce neurotoxicity due to their ability to self-aggregate. Indeed, Aβ monomers are prone to oligomerize, aggregating into protofibrils and fibrils, whose toxicity is explained by their capability of inducing oxidative stress, mitochondrial dysfunction, alterations in membrane permeability, inflammation, synaptic dysfunction and excitotoxicity. Furthermore, Aβ also interferes with channels, membrane proteins and receptors, impairing cell signaling [34,38].

### 3.2. Tau hyperphosphorylation

The formation of neurofibrillary tangles that contains aggregates of Tau protein, found embedded within neurons, represents the second most predominant histopathological hallmark of AD. The phosphoprotein Tau belongs to the family of microtubule-associated proteins, and is found predominantly in axons and, in less extension, in somatodendritic compartments and glial cells [34,39]. Tau is responsible for the stabilization and assemble of microtubules, and promotes axonal transport and dendrite structure. However, its biological activity is regulated by post-translational modifications, including phosphorylation at multiple sites. An abnormal Tau phosphorylation decreases its affinity for microtubules and makes the protein more prone to aggregate. Therefore, Tau hyperphosphorylation leads to a loss of its inherent functions, culminating in downsets in microtube assembly, axonal trafficking and dendritic structure, loss of synapses, neuronal death and, eventually, dementia [34,40]. Like Aβ, prefibrillar aggregates are responsible for the Tau-mediated neurotoxicity. Phosphorylated Tau aggregates to form oligomers that later mature into paired helical and straight filaments [34,39].

With several factors linked to Tau-mediated toxicity, including disturbance of calcium levels and oxidative stress, two main causes remain the most associated to the abnormal hyperphosphorylation of this protein: i) Tau conformational change(s) in AD-affected brain, making it a more favorable substrate for phosphorylation rather than for dephosphorylation and ii) imbalance between the activities of protein kinases and protein phosphatases 1 and 2A (PP1 and PP2A, respectively), whose joint balance activities are responsible for the regulation of its normal phosphorylation state. Dephosphorylation catalyzed by PP1 and PP2A is responsible to revert abnormal Tau to its normal-like state [39,40]. Tau is phosphorylated in more than 30 serine/threonine residues in AD by several different kinases like glycogen synthase kinase-3 (GSK-3), cyclin-dependent protein kinase-5 (cdk5), protein kinase A (PKA) and calcium and calmodulin-dependent protein kinase-II (CaMKII) [40–42]. In AD, the broad specificity serine-threonine kinase GSK3 isoform β (GSK-3β) is a crucial kinase contributing to the hyperphosphorylation of Tau. Thus, like BACE-1, it has been considered an attractive therapeutic target for stopping/delaying disease progression [39,43,44].

### 3.3. Oxidative stress

Oxidative stress is described as an imbalance between the production and removal of oxidants due to an ineffective action of the antioxidant defenses. Thus, the accumulation of high levels of reactive species derived from oxygen and nitrogen is favored. The major consequence of this imbalance is the oxidative modification of lipids (lipid peroxidation), proteins and nucleic acids [45,46]. Generated from endogenous sources, like molecular oxygen ($O_2$) metabolism and cellular signaling, and exogenous stimulus, reactive species are necessary, however, for several biological functions, such as receptor-mediated signaling pathways and cellular homeostasis throughout the regulation of apoptosis. Nitrogen-containing oxidants include nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO-$) and nitrogen dioxide ($NO_2$), while ROS include superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide ($H_2O_2$), hydroperoxyl radical ($^{\bullet}O_2H$), singlet oxygen ($^1O_2$), peroxide ($O_2^{2-}$) and hydroxide ion ($OH-$) [34,45]. Additionally, nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), while transferring electrons from NADPH to oxygen, generates high amounts of superoxide

anion radicals, representing, therefore, an important source of intracellular ROS [34].

However, oxidative stress can contribute to disease progression and to the exacerbation of its symptoms throughout direct and indirect mechanisms. Directly, reactive species can affect cellular functions, which can lead to cell death. Indirectly, while acting as a second messenger, $H_2O_2$ can cause dysfunction in redox signaling pathways, affecting normal biological functions through the modification of proteins and mitochondrial function, and also can promote inflammation, apoptosis or meddling with autophagy. In AD, oxidative stress is linked to neurodegeneration over four major aspects: i) Aβ production and accumulation; ii) microglia activation; iii) dysregulation of redox-active metal ions and iv) mitochondrial dysfunction [34,46,47].

Regarding Aβ production, several studies enlightened that oxidative stress contributes to an increased formation of senile plaques since it contributes to decreasing α-secretase activity, while enhancing both β- by γ-secretase activities [48,49]. Moreover, oligomeric accumulation also enhances oxidative stress, as it has been described that Aβ is able to induce a concentration-dependent accumulation of ROS and to increase its generation by directly activating NADPH oxidase [50–53]. Indeed, some studies highlighted a significant accumulation of oxidative stress markers in *postmortem* AD brains, including indicators of damage to lipids, proteins and nucleic acids, impaired antioxidant defense levels and accumulation of redox-active metals. Similarly, redox proteomics analysis has indicated oxidative damage of enzymes involved in energy metabolism, of proteins linked to neurotransmission and to mitochondrial function and the damage of proteasomal components in AD [49,54–56].

As a normal biological response of the CNS to the accumulation of Aβ and hyperphosphorylated Tau, activated glial cells mark another histopathological sign of the disease, with the presence of neuroinflammation in regions of AD-affected brains. In spite of being considered a neuroprotective mechanism, chronic neuroinflammation triggers neurotoxicity, which leads to neurodegeneration [34,49,52]. As resident immune cells of the CNS, microglia are considered the first line of defense of the CNS, quickly responding to inflammatory stimuli, thus playing a key role in tissue maintenance, injury response and phagocytosis. In response to Aβ accumulation, microglia cells are activated to eliminate the toxic stimuli. Consequently, deleterious effects in AD may occur due to the inflammatory factors that are released, mediating neuronal damage [57–60]. Oxidant species can also stimulate proinflammatory genes' transcription in both microglia and astrocytes. The activated microglial cells produce both ROS and proinflammatory cytokines, such as interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α), thus initiating the inflammatory response in AD brains. Furthermore, IL-1, IL-6 and TNF-α favor Aβ production and accumulation [49,52,60]. Inflammatory mediators secreted by activated microglia may also induce reactive astrocytosis, mediating a secondary inflammatory response. Astrocytes communicate with neurons, protecting and supporting them mainly in the maintenance of neurotransmitters, regulation of blood flow and formation and maturation of synapses [57,61]. Nonetheless, in AD, reactive astrocytes play a key role in neuroinflammation and in the production of ROS and RNS, which can promote, or even exacerbate, neuropathology and neurodegeneration. Several studies highlighted the role of reactive astrocytes in the clearance of Aβ plaques. However, when the clearance capacity of the formed plaques is reached, or when morphological changes occur, the release of Aβ from astrocytes mediates an aggravation of Aβ pathology [62–64]. Therefore, astrocytes are emerging as putative therapeutic targets in the treatment of neurodegenerative diseases, as reviewed elsewhere [65–68]. Both reactive astrocytes and microglia activated by oxidative stress can induce synaptic loss, increase Tau pathology through the upregulation of kinases responsible for the hyperphosphorylation of Tau, contribute to Aβ production and accumulation and produce proinflammatory cytokines involved in neuronal death via apoptosis [52,60,69,70].

The concentration of metal ions is well regulated across the blood–brain barrier (BBB) as they play a pivotal role in several biological mechanisms, including cell metabolism, signal transmission, and catalysis and stabilization of proteins. The most abundant redox-active metals are iron (Fe) and copper (Cu) [32,34,71]. In the human body, Cu can be found in its labile or protein-bound (*e.g.* ceruloplasmin and cytochrome *C* oxidase) forms. Being a transition metal, Cu (I/II) is an essential cofactor involved in enzymatic reactions that include $O_2$ biochemistry. Cu provides neuroprotection to neurons and glia cells, as it participates in homeostasis of neurotransmitters and neuropeptides and is part of the antioxidant enzyme superoxide dismutase. Nonetheless, an imbalance in Cu (I/II) levels can induce ROS overproduction, leading to oxidative stress, by two different mechanisms: through the formation of hydroxyl radicals (via Fenton reaction) and through the depletion of reduced glutathione (GSH), a remarkably important cellular antioxidant and substrate for several enzymes involved in ROS neutralization. Under high levels of Cu, GSH suppresses Cu catalytic activity by directly chelating it. By keeping Cu in a stable oxidation state, the participation of Cu in redox reactions, such as the Fenton reaction, may be prevented [34,71]. Similarly, Fe (II/III) is involved in several biological actions that generate ROS. Fe is an essential metal ion for heme and non-heme enzymes, playing a key role in several biological functions, such as $O_2$ metabolism and transport, gene regulation and regulation of cell growth and differentiation. Given its ability for electron transfer, Fe is a cofactor for enzymes with essential roles in metabolic processes, including enzymes involved in both ROS formation (*e.g.* NADPH oxidases, nitric oxide synthases and cytochrome P450 (CYP450)) and scavenging (*e.g.* catalase and peroxidases). The levels of Fe are tightly regulated to assure their adequate intracellular labile amount. However, its release from the protein ferritin or iron-sulfur [Fe-S] cluster-containing enzymes through the action of superoxide anion radicals, with consequent enzyme inactivation, contributes to its intracellular accumulation. This favors the Fe-dependent generation of ROS by direct (via Fenton and Haber–Weiss reactions, producing highly reactive hydroxyl radicals) and indirect mechanisms [72–74]. In addition, dysregulation of metal ions has been linked to AD, with Aβ-mediated oxidative stress involving redox-active metals, particularly Cu. Several studies indicated that metals form high-affinity complexes with Aβ, modifying its aggregation, as well as contributing to the formation of redox-cycling reactions with the generation of ROS involved in the neurotoxicity of Aβ. Although further studies are needed to deep elucidate the precise mechanism of metal-Aβ neurotoxicity, iron chelating therapy has demonstrated a potential applicability in the treatment of AD [34,54,71].

Despite being a major source of intracellular reactive species, paradoxically mitochondria are organelles highly sensitive to oxidative stress. Proteinopathy (amyloidopathy and tauopathy), characteristics of the disease, induce the production of ROS that can trigger cellular death pathways. Studies in isolated mitochondria, cultured cells, and *postmortem* AD-affected brains highlighted mitochondrial damage in response to proteinopathy, such as impairments in mitochondrial bioenergetics, alterations in fusion/fission and impaired mitophagy [49,75,76]. Mitochondria possesses an outer and inner membrane, surrounding its matrix. In their inner membrane, through the oxidative phosphorylation mechanism that happens in respiratory chain complexes, the transmembrane potential produced is used by the ATP-synthase enzyme complex to synthesize adenosine triphosphate (ATP). Mitochondrial dysfunction has been linked to AD, including morphological alterations and reduction in mitochondria number, impairment of organelle bioenergetics, reduced ATP levels, mitochondrial membrane depolarization, increased ROS production and variations in mitochondrial biogenesis and dynamics [49,77]. Therefore, mitochondria have emerged as a potential target in disease therapeutics, with pharmacological approaches showing beneficial effects by increasing ATP levels, reducing ROS accumulation and restoring the mitochondrial membrane potential [77,78].

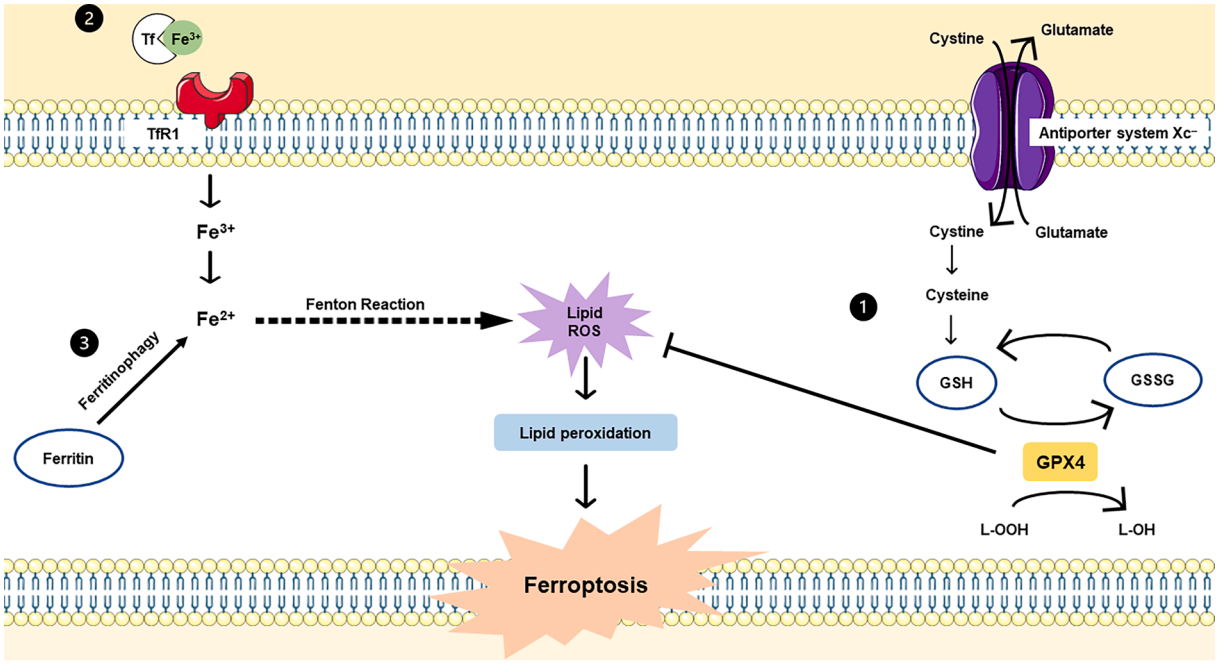


**Fig. 3. Key mechanisms involved in Ferroptosis.** The ferroptotic cell death is mediated by a redox imbalance between iron and antioxidant defenses [specially glutathione (GSH)-dependent glutathione peroxidase 4 (GPX4) pathway], consequently affecting membrane phospholipids. (1) The regulation of the GSH-dependent GPX4 pathway may be affected by an impairment in the antiporter system $Xc^-$ or in the rate-limiting activity of enzymes implicated in the biosynthesis of GSH. Inactivation of GPX4 consequent to GSH depletion, limits GPX4-mediated detoxification of cytotoxic lipid hydroperoxides (L-OOH), which favors the accumulation of free radicals and toxic lipid products, such as peroxides, causing an extensive lipid peroxidation. (2) Likewise, $Fe^{3+}$ is delivered into the cells [bound to transferrin (Tf)] through transferrin receptor 1 (TfR1). Once within the cells, $Fe^{3+}$ is reduced to $Fe^{2+}$, and the excess labile iron pool stored in ferritin. (3) Ferritinopaghy is a process responsible to the degradation of ferritin, leading to a release of cytoplasmic iron. Free labile $Fe^{2+}$ within the cells enhances the formation of toxic lipid ROS mostly via the Fenton reaction, thus contributing to the peroxidation of plasma membrane phospholipids. GSSG - Glutathione disulfide. L-OOH - lipid hydroperoxides. L-OH - Lipid alcohols.

**Table 2**
Morphological and biochemical features of ferroptosis.

| Morphological characteristics | Biochemical characteristics | References |
|---|---|---|
| - intact nuclei and unaggregated chromatin;<br>- plasma membrane remains unruptured, but suffers from loss of selective permeability due to lipid peroxidation;<br>- mitochondrial shrunk, maintaining, however, a good inner membrane density, accompanied by outer membrane rupture; | - elevated levels of Fe (II);<br>- ROS accumulation;<br>- increased levels of lipid hydroperoxides;<br>- GSH depletion;<br>- inhibition of the antiporter system $Xc^-$, with the consequent reduction in cystine uptake; | [73,80–82] |

Fe – iron; ROS – reactive oxygen species; GSH – reduced glutathione

### 3.4. Ferroptosis

As the most abundant redox-active metal in the human body, Fe accumulation in the brain is one hallmark for neurodegenerative diseases. Fe accumulation in AD is responsible: (i) for the generation of ROS, which, as mentioned, induces several cellular damages; (ii) for increasing Aβ aggregation and oligomerization, contributing to Aβ toxicity and (iii) for participating in the hyperphosphorylation and consequent aggregation of Tau protein. All these processes mainly involve Fe in Fenton chemistry [34,73]. Recently, Fe activity has been implicated in a new form of programmed cell death named ferroptosis. Ferroptosis is an iron-dependent form of cell death that is initiated by oxidative distresses, which can be mostly initiated by the inactivation of the antioxidant GSH-dependent system, culminating in the accumulation of toxic lipid-ROS (Fig. 3). Ferroptosis morphological and biochemical features differ from apoptosis, necrosis and autophagy [73,79,80]. The main characteristics are gathered in Table 2.

Currently, three key hallmarks are responsible for the ferroptotic cell death: (i) labile redox-active Fe and iron-dependent peroxidation enzymes, as lipoxygenases and CYP450, responsible for catalyzing the peroxidation of polyunsaturated fatty acids, generating lipid peroxyl radicals; (ii) presence of phospholipids with polyunsaturated fatty acids tails, once ferroptosis is driven by their peroxidation; and (iii) failure in the clearance of lipid-ROS, contributing to lipid oxidative stress due to ineffective action of the GSH – glutathione peroxidase 4 (GPX4) system. GPX4 uses GSH to reduce toxic lipid hydroperoxides present in cell membranes [73,83–85]. Indeed, high amounts of labile Fe (II), promote, through Fenton reaction, the non-enzymatic lipid peroxidation, accompanied by excessive ROS formation, thus triggering ferroptotic cell death. Moreover, the synthesis of the tripeptide GSH, with a pivotal role in controlling the activity of GPX4, is essential to protect the cells from this programmed cell death. The antiporter system $Xc^-$, which promotes glutamate and cystine exchange, contributes to the biosynthesis of GSH by providing optimal intracellular levels of cystine, which is intracellularly converted into cysteine, an amino acid used for GSH biosynthesis. Therefore, the inhibition of this antiporter system contributes to GSH depletion and to the consequent inactivation of GPX4. As a response, the cells became more sensitive to ferroptosis due to an increase in lipid-ROS production [80,84,85].

Ferroptosis can be inhibited by iron chelators and antioxidants, thus underlining ferroptosis as an iron- and lipid-ROS-dependent cell death. Moreover, several studies point out that cellular mechanisms involved in the storage and uptake of Fe are essential for ferroptosis control. Indeed, increased Fe uptake, decreased storage or even dysfunction in its regulatory pathways or transcription factors may contribute to its overwhelming contribution to ferroptosis. During this process, stress-mediated catabolic autophagy can be triggered, leading to lysosomal

**Table 3**
Classical and novel potential biomarkers in Alzheimer's disease.

| Biomarkers | Main features | References |
|---|---|---|
| **Aβ** | - reduced Aβ CSF levels, as a result of its aggregation in brain parenchyma and formation of senile plaques (around 50 % of normal levels of concentration of the $Aβ_{42}$);<br>- amyloid PET remains the most used biomarker in clinical trials, with 3 PET tracers approved for Aβ imaging in cognitive impairment evaluation;<br>- to increase diagnosis precision, currently the ratio of peptide $Aβ_{42}$ to soluble $Aβ_{40}$ aggregation concentration is used, which correlates approximately 100 % with amyloid PET; | [95–111] |
| **Total and phosphorylated Tau** | - The CSF concentrations of both total and phosphorylated Tau are considered valuable criteria for AD, reflecting the pathophysiology across neurogenerative dementia;<br>- enhanced concentrations of phosphorylated Tau in CSF corroborate the phosphorylation state of the protein and consequent formation of neurofibrillary tangles;<br>- higher concentrations of total Tau in CSF indicate the release of Tau from degenerating neurons, thus highlighting the intensity of neuronal degeneration;<br>- currently, the lack of selectivity and efficacy of Tau tracers due to decomposition limits the visualization, mapping and quantification of Tau pathology;<br>- despite the recognized proteolytic degradation of Tau in the blood, which limits its half-life and detection, recent studies underline advances in plasma biomarkers, namely P-Tau181 and P-Tau217. Indeed, studies highlight that plasma P-Tau181 correlates positively with both Tau CSF concentration and amyloid PET; | |
| **Volumetric magnetic resonance imaging (MRI)** | - considered a biomarker for AD-neurodegeneration, used in the diagnosis of dementia patients;<br>- weighted imaging tool that assesses morphological changes in the whole brain and medial temporal volumes;<br>- measures include brain atrophy, gray matter atrophy, regional atrophy and surface-based cortical thickness; | |
| **Neurofilament light (NfL)** | - a biomarker of neuroaxonal damage and degeneration that can be measured in both CSF and plasma/serum;<br>- NfL is a neuronal cytoplasmic protein expressed in myelinated axons. Its levels increase in CSF and blood correspondingly to the degree of axonal damage;- in familial AD (mutation carriers) , impaired levels of NfL appear years before the clinical onset; | |
| **Fluorodeoxyglucose PET** | - a radiolabeled sugar (glucose) molecule used for AD diagnosis via synaptic dysfunction;<br>- technique used to evaluate the presence of lesions with high glycolytic metabolism;<br>- hypometabolism reflects a combination of synaptic and metabolic dysfunction, as well as neuronal cell loss;<br>- detects abnormalities at early stages of the disease; | |
| **PET tracers targeting synaptic vesicle protein 2A (SV2A)** | - biomarker of synaptic loss;<br>- PET imaging allows to quantify the density of brain SV2A;<br>- in AD patients, studies revealed a decreased SV2A density in the hippocampus; | |
| **Dendritic protein neurogranin** | - neurogranin is a protein of the dendritic spines responsible for receiving and integrate information, thus considered a biomarker for synaptic dysfunction and degeneration;<br>- CSF neurogranin concentration increases in AD patients, correlating with cognitive deterioration over time; | |
| **Inflammation/ astroglial activation** | - CSF levels of chitinase-3-like protein 1 (or YKL-40), a glycoprotein expressed in both astrocytes and microglia, and triggering receptor expressed on myeloid cells-2 (TREM2) are increased in patients diagnosed with AD, correlating positively with CSF levels of Tau and Aβ;- glial fibrillary acidic protein (GFAP) , a marker of astrocyte activation subsequent to injury or stress in CNS, is indicated as a CSF biomarker;- activated microglia express translocator protein (TSPO) on the outer membrane of mitochondria. 80 % of MCI-positive due to AD patients revealed active inflammation and higher concentrations of TSPO ligand retention in the brain;<br>- other inflammation markers in CSF and blood are being used for treatment monitoring proposes only due to their lack of specificity for AD (*e.g.* pro- and anti-inflammatory cytokines and chemokines); | |
| **microRNA (miRNA)** | - recent studies pointed to reduced miRNA-125b concentrations in serum of AD patients, as compared to non– inflammatory controls, thus indicating its potential as a biomarker of the disease; | |

Aβ – amyloid-beta; CNS – Central Nervous System; CSF – cerebrospinal fluid; PET - Positron Emission Tomography; AD – Al zheimer's disease; MCI – mild cognitive impairment

degradation. Furthermore, reduced levels of cysteine activate autophagy, which degrades ferritin (responsible for the storage of excessive Fe), contributing to the increased labile Fe in the cytoplasm [83,85]. Ferroptosis-inducing stimuli can also contribute to the overload of labile Fe. The small molecule Erastin induces ferroptosis through the inactivation of antiporter system $Xc^-$. A study enlightens that the knockdown of the gene coding for ferroportin, a membrane protein responsible for the efflux of Fe from the cells, accelerated Erastin-induced cytotoxicity in human neuroblastoma SH-SY5Y cells. Additionally, evidence

reenforced that a decrease in Fe overload promoted by iron-chelating agents inhibited Erastin-induced cytotoxicity, while promoting additional stress with exogenous sources of Fe exacerbated the Erastin-induced cytotoxicity [86,87]. [Fe-S] clusters represent another source of labile Fe in ferroptosis, since they are highly vulnerable to peroxides formed as a consequence of oxidative stress [83,86–89]. Based on the fact that lipid peroxidation, reduction in GSH levels and Fe overload have been already found in the brains of AD patients, ferroptosis inhibition has been explored as a potential therapeutic strategy for delaying AD progression [80,90–94].

The key ferroptosis checkpoints are depicted in Fig. 3.

## 4. Biomarkers of Alzheimer's disease

Over the last decade, improvements in the diagnostic of AD have been made with advances aiming for an early detection and effective therapeutical intervention. Currently, the diagnostic of AD is primarily made by cognitive testing, neuroimaging and screening of distinct biomarkers [95,96]. Identifying the disease with reliability, ideally before the onset of symptoms, with minimal invasive and low-cost techniques drove the combination of CSF biomarkers and imaging to be investigated [95,97]. Indeed, the development of techniques to measure specially Aβ *in vivo*, which remains the main target of disease-modifying drug candidates in clinical trials, via biomarkers in CSF and amyloid PET imaging, helped to clarify the underlaying mechanisms at a molecular level. This can ultimately improve the development of target therapies by facilitating monitoring pharmacodynamics effects of novel drugs candidates [96].

The ideal biomarker, detectable or measured in biological fluids as blood, CSF or peripheral tissue, must comply with several characteristics including: (i) high sensitivity, high specificity, allowing to differentiate AD from other dementias, and high reliability; (ii) a positive predictive value; (iii) be involved in AD pathology; (iv) allow an early detection, enabling either providing patients with diagnostic and prognostic information about their disease or the optimization of treatment strategies and (v) be obtained by a non-invasive and un-expensive technique [97,98].

The biomarkers can be divided into classical and novel candidates for AD, leaning towards pathological features of the disease, such as neuronal atrophy, synaptic dysfunction (an early feature of the disease, directly involved in the characteristic decline in cognitive function), progressive accumulation of senile plaques and Tau hyperphosphorylation, innate immune response (since astrocytes and microglia activation became key players in neurodegenerative dementias) and metabolic dysfunction [96–98].

Table 3 presents the main classical and novel potential biomarkers for AD.

## 5. Current treatment and an overview of new disease-modifying therapies

Current treatment for AD involves design molecules targeting the basic biology of the disease, aiming to attenuate its progression by using symptomatic cognitive enhancers. The treatment includes the used of acetylcholinesterase inhibitors (donepezil, galantamine and rivastigmine) and of the antagonist of N-methyl-D-aspartate (NMDA) receptors memantine, seeking to increase the cholinergic neurotransmission and to activate the NMDA receptors, respectively [34,112].

The discovery of new disease-modifying drugs that may delay disease progression and prevent cell death represent an emergent research topic. Although all the efforts that have been employed so far, a large number of clinical trials with new molecules for the treatment of the disease have dramatically failed. Indeed, 99 % of new drugs developed between 2002 and 2014 showed no effect, with only one getting approved to manage the disease (memantine). Presently, the ongoing AD drug development involves disease-modifying strategies focused on either immunotherapies or small molecules for oral administration [32,113–115].

In the process of drug discovery, following the identification of a disease target (*e.g.* Aβ aggregation, hyperphosphorylation of Tau, neuroinflammation) libraries of compounds have been created for further screening. Normally libraries include molecules derived from different pharmacophores. Due to the complex nature of AD, the development of multitarget-directed ligands/multifunctional molecules has become an appealing strategy to target multiple pathways implicated in the progression of the disease [32,34,114]. This multifunctionality, aiming to increase drug efficacy by targeting several pathophysiological features of AD, includes mostly metal chelation and enzyme inhibition [32,34,114]. Tacrine was developed as an acetylcholinesterase inhibitor with potential for the treatment of AD, but was withdrawn from therapeutics due to serious cases of hepatotoxicity. Over the years, tacrine derivatives have been studied as potential multitarget compounds for AD treatment. They demonstrated a multitarget mode of action, simultaneously presenting an inhibitory activity against cholinesterases and also against Aβ aggregation, with several compounds being classified as BACE-1 inhibitors. Additionally, tacrine hybrids have also been developed as potential multifunctional compounds for AD treatment. Starting with neuroprotective and antioxidant features, tacrine has been used to design hybrids with ferulic acid, caffeic acid and Trolox, a potent antioxidant that protects liver from oxidative damage and shows neuroprotective effects by scavenging ROS and diminishing Aβ-mediated neurotoxicity [112,116]. Advances in the design of multitarget tacrine hybrids for targeting neuroinflammation and Tau hyperphosphorylation have also been made. In fact, tacrine-coumarin hybrids have been depicted as multifunctional compounds owing to coumarin the capability of preventing Aβ aggregation and inhibiting Tau hyperphosphorylation. On the same line, efforts have been made on the development of multitarget donepezil-related derivatives [112,116]. In 2018, Iraji and colleagues designed and screened novel triazine derivatives with different aryl hydrazone moieties. Some compounds showed a multitarget mode of action, with significant BACE-1 inhibitory activity, as well as antioxidant and metal-chelating properties (namely with ability to chelate $Fe^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Cu^{2+}$). Noteworthy, the most potent BACE-1 inhibitor lacked significant cytotoxicity towards neuronal cells [117].

Given the impact of ferroptosis in AD pathology, and to explore the iron-chelating capability of deferiprone (approved for the treatment of iron overload in thalassemia, and already in clinical trials for Parkinson's disease), Bortolami and colleagues, in 2020, developed derivatives of this compound as potential multifunctional cholinesterases inhibitors with potentiality to also simultaneously chelate metal ions associated with Aβ aggregates and involved in oxidative stress [118].

Recent advances in nanotechnology and nanomedicine have also been made to overcome the limited BBB permeation of some compounds and to improve their stability and bioavailability. In fact, curcumin-loaded nanoparticles are being tested to overcome the poor stability, bioavailability and quick oxidation of coumarin. Furthermore, nanoparticles have shown to be a good alternative to protect against some well-established effects of chelating therapy, including hepatotoxicity, low capability to across the BBB and neurotoxicity [119–122]. In addition to the nanoparticles loaded with Fe and Cu chelating agents, as well as their derivatives, Ceria nanoparticles have been used to protect mitochondria against ROS-mediated oxidative stress. Similarly, gold- and selenium-containing nanoparticles have been studied for their inhibitory effects on Aβ aggregation [119–122].

On the other hand, nanoparticles have been used in the diagnosis of AD. Ultra-small gadolinium-nanoparticles showed the ability to bind to Aβ peptide. In addition, when functionalized with polyisobutylene (PIB) or a nanobody (B10AP) they interact with Aβ plaques. Thus, this functionalization increases the targeting of nanoparticles to disease sites, powering its application as a multimodal imaging tool for early

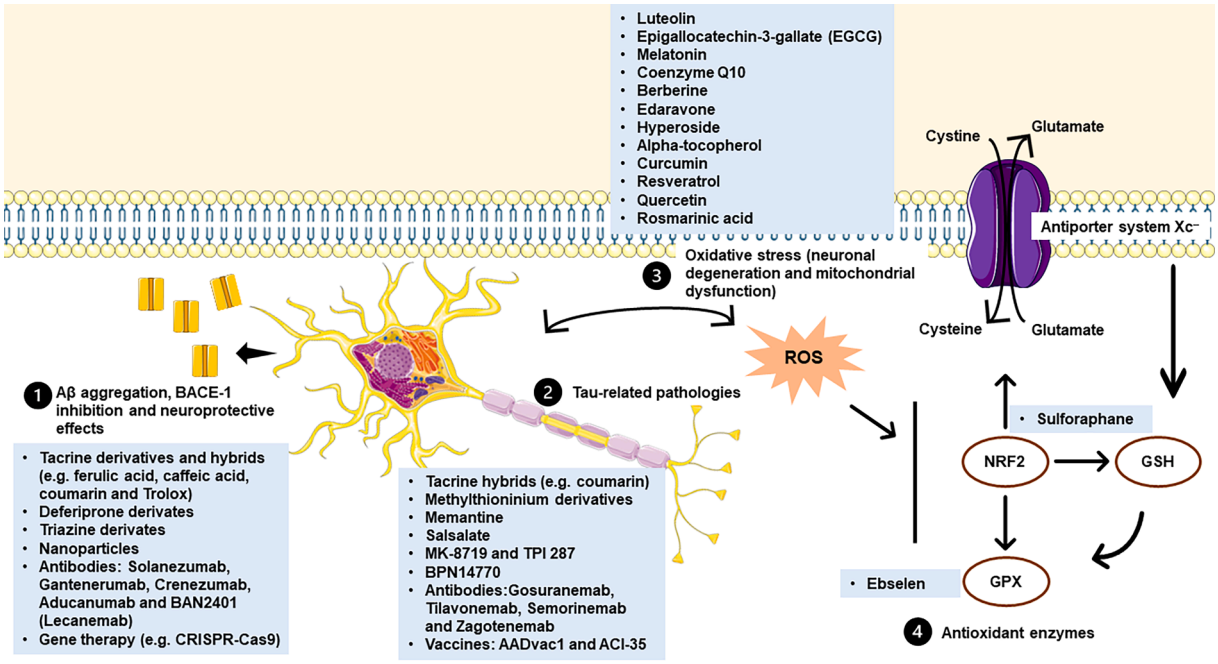


**Fig. 4. Emerging new disease-modifying and multifunctional drugs, screened or in ongoing clinical trials, towards the main hallmarks of Alzheimer's disease.** Distinct strategies targeting distinct hallmarks of the disease have been tested, including (1) Aβ aggregation or (2) Tau-related pathology. Considering the increased knowledge on the involvement of oxidative stress on the disease pathogenesis, (3) molecules with antioxidant properties and (4) activators of endogenous antioxidant systems have been explored. Aβ – amyloid-beta; BACE-1 – beta-site APP cleaving enzyme 1; GPX – glutathione peroxidase; GSH – reduced glutathione; Nrf2 – Nuclear factor erythroid 2-related factor 2; ROS – reactive oxygen species.

diagnosis of AD [119]. Gold, silver and magnetic nanoparticles are currently used for AD diagnosis in the design of biosensors (devices that incorporate a biochemical binding component with a signal conversion used in the evaluation of several AD biomarkers). Specifically, gold nanoparticles possess high chemical stability and conductivity, suitable catalytic activity, wide surface area, biological compatibility, and simplicity of functionality improving the immobilization of biological recognition elements such as DNA, antibodies, and enzymes. These features make them widely explored in the design of biosensors to validate AD biomarkers for diagnosis, mainly due to their ability to bind to Aβ peptide [119,123].

Tau-targeting small molecules have also been developed with several already reaching clinical trials in AD. They have the ability of modulating Tau function at different levels: i) expression; ii) aggregation propensy; iii) post-translational modifications; iv) degradation and v) microtubule stabilizing properties [124–126]. Studies performed in cell-based and animal models have highlighted that small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) can reduce Tau expression [124–126]. Likewise, methylthioninium derivatives have shown the ability to reduce Tau aggregation. Phosphatase enhancers (both memantine and sodium selenate induce PP2A activity) and kinase inhibitors (Tideglusib and lithium chloride act as inhibitors of GSK3-β) have been explored to modulate the post-translational modifications of Tau. However, Tideglusib was discontinued after failing efficacy in a phase II trial. Furthermore, the small molecule Salsalate inhibits the acetylation of Tau at Lys174, while MK-8719 inhibits the *O*-GlcNAcase enzyme, suppressing Tau deglycosylation. Davunetide or NAP and TPI 287 (abeotaxane) have been explored for their ability to act as microtubule-stabilizing agents [124–126]. Several oral BACE-1 inhibitors were also developed (*e.g.* atabecestat, lanabecestat, umibecestat, elenbecestat and verubecestat), although later discontinued after showing lack of efficiency, worsening of symptoms and toxicity in humans [127]. However, the inhibitor of phosphodiesterase-4, BPN14770, has shown promising results in improving memory [128].

Monoclonal or polyclonal antibodies directed to specific epitopes on the target molecule have also been increasingly tested, as they show reduced off-target effects when compared to small molecules [114,124]. Currently, five antibodies targeted to Aβ aggregates are in ongoing clinical trials – solanezumab, gantenerumab, crenezumab, aducanumab and BAN2401 (Lecanemab). They showed some effectiveness in reducing CSF biomarkers' levels and Aβ levels within the brain [127,129,130]. Similarlly, four anti-Tau antibodies – Gosuranemab, Tilavonemab, Semorinemab and Zagotenemab – are also currently in clinical trials [127]. An alternative strategy with anti-Tau vaccines also is in ongoing clinical trials [127]. AADvac1 has shown good immunogenicity, leading to considerable changes in blood and CSF biomarkers, thus highlighting its potential to reduce tauopathy. Another anti-Tau vaccine, ACI-35, revealed a promising efficacy in diminishing tauopathy, with an acceptable safety profile [124,127].

With oxidative stress increasingly linked to AD, antioxidant therapy has also been explored in the management of the disease, specially by minimizing neuronal degeneration and counteracting mitochondrial dysfunction. Some exogenous antioxidants currently screened for their potential applicability in AD include luteolin, epigallocatechin-3-gallate (EGCG), melatonin, coenzyme $Q_{10}$, berberine, edaravone and hyperoside [131,132]. Other natural antioxidants like alpha-tocopherol, curcumin, resveratrol, quercetin, and rosmarinic acid are also highlighted as potential therapeutical strategies to counteract oxidative damage and ROS production in AD [133]. Noteworthy, with some small molecules failing in providing a proper scavenging protection against oxidative stress, the rationale strategy has been shifted to target the activity of antioxidant enzymes to counteract oxidants-induced cell damage. In this class of compounds are included ebselen, a glutathione peroxidase mimic, and sulforaphane, an activator of the Nuclear factor erythroid 2-related factor 2 (Nrf2) [46]. Nrf2 transcription factor, when activated, regulates gene expression of several antioxidants/detoxifying enzymes like glutathione peroxidase, glutathione *S*-transferase, superoxide dismutase, catalase and some CYP450 oxidoreductases, thus allowing protection against oxidative stress that drives age-related pathologies, such as AD [134,135].

**Table 4**
Main advantages and limitations for the different therapeutic approaches in Alzheimer's disease.

| Therapy | Advantages | Disadvantages/ limitations | References |
|---|---|---|---|
| **Symptomatic cognitive enhancers** | | | |
| **Acetylcholinesterase inhibitors**:<br>- donepezil<br>- galantamine<br>- rivastigmine | - approved therapy;<br>- relief of symptoms;<br>- improve quality of life; | - management of symptoms for a short period of time;<br>- continuous decline of cognitive function;<br>- no impact in disease progression; | [34,113] |
| **Antagonist of N-methyl-D-aspartate receptor**:<br>- memantine | | | |
| **Disease-modifying drugs** | | | |
| **Multi-target small molecules:**<br>**1. Aβ aggregation, enzyme inhibition and neuroprotective effects**<br>- tacrine derivatives and hybrids<br>- deferiprone derivates<br>- triazine derivates<br>**2. Tau-related pathologies and neuroprotective effects**<br>- tacrine hybrids<br>- methylthioninium derivatives<br>- memantine<br>- salsalate<br>- MK-8719 and TPI 287<br>- BPN14770<br>**3. Oxidative stress (neuronal degeneration and mitochondrial dysfunction)**<br>- luteolin<br>- epigallocatechin-3-gallate<br>- melatonin<br>- coenzyme Q10<br>- berberine<br>- edaravone<br>- hyperoside<br>- alpha-tocopherol<br>- curcumin<br>- resveratrol<br>- quercetin<br>- rosmarinic acid | - multifunctional molecules with structural moieties capable of targeting several underlying causes of cognitive decline in AD;<br>- administration of a single multifunctional drug decreases potential drug-drug interactions and facilitates patient compliance and adherence to the treatment;<br>- targets include amyloid-beta cascade, Tau hyperphosphorylation, oxidative stress, metal ion accumulation, mitochondrial dysfunction and enzyme inhibition; | - limited target specificity;<br>- difficult in crossing the blood–brain barrier;<br>- poor pharmacokinetics parameters, limiting their efficacy;<br>- limited efficacy in clinical trials;<br>- high incidence of side effects; | [34,114,119,122,129,131,132,136–138,140,142,143,145,150–156] |
| **Antibodies:**<br>- solanezumab<br>- gantenerumab<br>- crenezumab<br>- aducanumab- BAN2401 (Lecanemab)<br>- gosuranemab<br>- tilavonemab<br>- semorinemab<br>- zagotenemab | - specificity to bind to a target epitope, neutralizing the pathogenic protein;<br>- lower toxicity when compared to other therapies;<br>- well-tolerated by patients; | - limited efficacy in clinical trials;<br>- risk of immunogenicity;<br>- invasive methods for administration;<br>- increased risk of side effects; | |
| **Nanoparticles** | - targeted drug delivery;<br>- the encapsulation process allows to reduce the amount of drug required to achieve therapeutic effects, thus minimizing possible side effects;<br>- improved drug bioavailability and effectiveness, since they can encapsulate drug molecules, protecting them from degradation;<br>- potential use as imaging agents for non-invasive disease progression monitoring, earlier detection and treatment of AD; | - some nanoparticles can present toxicity to cells and tissues, leading to unwanted side effects;<br>- impairment in the nanoparticles' clearance can lead to accumulation and potential toxicity;<br>- nanoparticle-based therapies can be expensive to develop and manufacture; | |
| **Vaccines:**<br>- AADvac1<br>- ACI-35 | - strategy for the treatment of AD through immunization;<br>- expected to improve patients' quality of life by delaying the onset of symptoms; | - require long-term follow-up and can have side effects;<br>- as a relatively new therapy, long-term effectiveness and safety remain unknown; | |

(*continued on next page*)

**Table 4** (*continued*)

| Symptomatic cognitive enhancers | | | |
|---|---|---|---|
| **Therapy** | **Advantages** | **Disadvantages/ limitations** | **References** |
| **Sigma-2 receptor antagonist**<br>- CT1812 (Elayta) | - potential to reduce the formation of amyloid-beta plaques and neuroinflammation;- improvement in cognitive function (animal studies) ; | - research still in early stages, therefore efficacy and safety remain fully undescribed; | |
| **Senolytic therapy:**<br>- dasatinib and quercetin | - improved cognitive function and reduced inflammation;<br>- by removing senescent cells, senolytic therapy may delay the progression of the disease; | - potential adverse side effects;<br>- further research is needed to establish the safety and effectiveness of the therapy in AD; | |
| **Cell therapy:**<br>- Lomecel-B | - prevents neuronal loss and reduces inflammation;<br>- improved cognitive function; | - ethical concerns and risk of immune-mediated rejection; | |
| **Gene therapy:**<br>- CRISPR-Cas9 | - allows to target specific genes that are associated with AD;<br>- minimized risk of off-target side effects; | - safety concerns regarding potential immune reactions or undesirable gene alterations;<br>- ethical concerns | |

Aβ – amyloid-beta; AD – Alzheimer's disease

The advent of gene therapies for multiple conditions has been extended to neurodegenerative diseases. In fact, several clinical trials are currently ongoing [136]. At this level, both viral [mostly based on adeno-associated viruses (AAVs)] and non-viral vectors (*e.g.* nanoparticles and liposomes) have shown outstanding results in directing the expression of therapeutic proteins, antibodies, Cas9/gRNA for gene editing, microRNAs and siRNA. In AD, studies in cellular and animals models with viral and non-viral vectors have shown the potential of CRISPR-Cas9-mediated genome editing to reverse mutations in *APP*, *PSEN2* and *Bace1*, revealing, therefore, a promising applicability in Aβ-related pathologies [136,137].

Fig. 4 schematizes the new disease-modifying and multitarget drugs for the main hallmarks of AD that have been screened or are currently in ongoing clinical trials.

Emerging as a putative target to manage AD progression is the sigma-2 receptor [implicated in cellular processes as autophagy, cholesterol synthesis, lipid membrane-bound protein trafficking, and receptor stabilization at the cell surface], with ongoing preclinical and clinical trials for the small molecule CT1812 (Elayta), a selective allosteric antagonist of the complex [138–140]. The mechanism of action of CT1812 includes downstream synaptotoxicity by the regulation of the affinity of the neurotoxic Aβ oligomers to bind to the receptor protein. The induction of this destabilization to the binding sites made by the small molecule modulates the release of the oligomers as well their consequent clearance from the brain [138,139].

With cellular senescence becoming increasingly relevant in the disease process of AD, senolytic therapies are also becoming an appealing therapeutic approach [141–143]. Indeed, being AD an age-dependent neurological disorder, the accumulation of senescent cells can occur as a consequence of their ineffective removal by the immune system in old age, of their increased resistance to apoptosis, or as a consequence of the combination of these two factors. Senescent cells are characterized by cell cycle arrest, apoptotic-resistance, and the production of catabolic factors known as the senescence-associated secretory phenotype (SASP). Therefore, the senolytic therapy proposes that apoptosis can be selectively initiated in senescent cells through the inhibition of the pro-survival mechanisms upregulated during senescence, thus contributing to the elimination of senescent neurons that could lead to cognitive dysfunction [142,144]. Evidences in *in vivo* (mouse model of tauopathy) and *in vitro* studies have successfully highlighted the role of dasatinib and quercetin in counteracting gliosis and tau-mediated neurodegeneration, driving the entry of this cocktail into two clinical trials to assess the potential of this senotherapy in AD: SToMP-AD and ALSENLITE [141,145,146]. Indeed, in a study performed by Musi and colleagues, using four AD transgenic mouse models of tau pathology, the major findings highlighted that neurofibrillary tangles display a senescence-like phenotype and that Tau transgenic mice, when treated with senolytic therapy, revealed improvements in brain structure, neuron loss and aberrant cerebral blood flow [147]. Based on these remarkable evidences in preclinical studies and complying with the first clinical trial of senolytic treatment, the SToMP-AD open-label pilot clinical trial was designed to individuals with early-stage dementia due to AD [145,148]. The aims of the study involved i) determination of the ability to cross the BBB and target engagement (treatment-related changes in CSF total and phosphorylated tau levels and Aβ levels); ii) establishment of the safety and tolerability of the treatment; iii) assessment of changes in cognition, functional status, and physical performance and iv) evaluation of changes, by neuroimaging, of biomarkers of brain structure (hippocampal atrophy) and function. This design acted as an initial proof-of-concept for a larger phase II clinical trial [145].

Furthermore, with several clinical trials already assessing their potential therapeutic role for other pathological conditions, and animal studies supporting the beneficial effects in inflammation and proteinopathy characteristic of AD, mesenchymal stem cells possess relevant therapeutical features to be explored in the treatment of the disease. Indeed, in a phase I clinical trial, the allogeneic medicinal signaling cell Lomecel-B proved its safety and potential applicability to act as a disease-modifying candidate for AD [with significant changes in preliminary biomarker results] [149,150]. Thus, cell therapy may constitute another remarkably approach for AD.

Table 4 summarizes the main advantages and disadvantages of the different therapeutic approaches approved and being exploited to the management of Alzheimer's disease.

## 6. Concluding remarks

AD is a neurodegenerative disease characterized by a progressive decline in the cognitive function, where the available therapies are mainly focused on symptoms relief, with no effect on disease progression. Despite the advances made in the last decades, the disease's true aetiology is still far from being fully known, as it presents a multifactorial nature. To the well-established pathological hallmarks (neurofibrillary tangles formed as a consequence of Tau hyperphosphorylation, and formation of aggregates of Aβ, due to an impairment in its

clearance), oxidative stress, neuroinflammation, metal ion dyshomeostasis and mitochondrial dysfunction also gained notable evidence as possible causes of the disease.

An early diagnosis of AD is as important as its treatment. One of the biggest challenges remains the establishment of reliable biomarkers, resorting to minimal invasive techniques. With several small molecules failing over the years in clinical trials, the rational strategy for developing new disease-modifying molecules has been changed from "single-target" to "multi-target", aiming at improving their effectiveness, while reducing side-effects. The emerging new disease-modifying therapies include small molecules or immunotherapies, with several already in preclinical or in clinical trials. In addition, nanomedicine, senolytic and gene therapy strategies have emerged as well. These approaches may constitute an undoubtedly powerful breakthrough in the treatment of patients diagnosed with AD.

## CRediT authorship contribution statement

**Ana R. Monteiro:** Conceptualization, Writing – original draft. **Daniel J. Barbosa:** Conceptualization, Writing – review & editing. **Fernando Remião:** Writing – review & editing, Supervision. **Renata Silva:** Conceptualization, Writing – review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work is supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

## References

[1] 2019 Alzheimer's disease facts and figures, Alzheimer's Dement 15:321–387.
[2] J.L. Haines, Alzheimer disease: Perspectives from epidemiology and genetics, J. Law Med. Ethics 46 (2018) 694–698.
[3] B. Duthey, Background Paper 6.11 Alzheimer Disease and other Dementias, Update on 2004. World Heal Organ 1–77, 2013.
[4] M.T. Ferretti, M.F. Iulita, E. Cavedo, et al., Sex differences in Alzheimer disease — The gateway to precision medicine, Nat. Rev. Neurol. 14 (2018) 457–469.
[5] I. Santana, F. Farinha, S. Freitas, V. Rodrigues, Á. Carvalho, Estimativa da prevalência da demência e da doença de Alzheimer em Portugal, Acta Med. Port. 7 (2015) 182–188.
[6] L. Mucke, Lots of people are forgetful. Are there any particular warning signs of Alzheimer's disease? Neuroscience 461 (2009) 895–897.
[7] I. Piaceri, B.S.S. Nacmias, Genetics of familial and sporadic Alzheimer's disease, Front Biosci 5 (2013) 167–177.
[8] S. Tiwari, A. Venkata, A. Kaushik, Y. Adriana, M. Nair, Alzheimer ' s Disease Diagnostics And Therapeutics Market. Int J Nanomedicine Jul 2019 (2019) 5541–5554.
[9] D. Wallon, A. Rovelet-lecrux, A. Richard, et al., APP, PSEN1, and PSEN2 mutations in early- onset Alzheimer disease : A genetic screening study of familial and sporadic cases, PLoS Med. 1 (2017) 1–16.
[10] L.G. Gunnarsson, L. Bodin, Occupational exposures and neurodegenerative diseases—a systematic literature review and meta-analyses, Int. J. Environ. Res. Public Health (2019), https://doi.org/10.3390/ijerph16030337.
[11] T.P.V. Huynh, A.A. Davis, J.D. Ulrich, D.M. Holtzman, Apolipoprotein E and Alzheimer's disease: The influence of apolipoprotein E on amyloid-β and other amyloidogenic proteins, J. Lipid Res. 58 (2017) 824–836.
[12] R. Najm, E.A. Jones, Y. Huang, Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease, Mol. Neurodegener. 14 (2019) 1–13.
[13] S. Ferris, R. Lane, N. Sfikas, B. Winblad, M. Farlow, H.H. Feldman, Effects of gender on response to treatment with rivastigmine in mild cognitive impairment: A post hoc statistical modeling approach, Gend. Med. 6 (2009) 345–355.
[14] B.A. Gordon, T.M. Blazey, Y. Su, et al., Spatial patterns of neuroimaging biomarker change in individuals from families with autosomal dominant Alzheimer's disease: a longitudinal study, Lancet Neurol. 17 (2018) 241–250.
[15] J.B.J. Kwok, Role of epigenetics in Alzheimer ' s and Parkinson ' s disease R eview. 1 (2010) 671–682.
[16] T.J. Qazi, Z. Quan, A. Mir, H. Qing, Epigenetics in Alzheimer's Disease: Perspective of DNA Methylation, Mol. Neurobiol. 55 (2018) 1026–1044.
[17] D. Mastroeni, A. Grover, E. Delvaux, C. Whiteside, P.D. Coleman, J. Rogers, Epigenetic changes in Alzheimer's disease: Decrements in DNA methylation, Neurobiol. Aging 31 (2010) 2025–2037.
[18] A.S. Yokoyama, J.C. Rutledge, V. Medici, DNA methylation alterations in Alzheimer's disease, Environ Epigenetics 3 (2017) 1–11.
[19] L. Chouliaras, D. Mastroeni, E. Delvaux, A. Grover, G. Kenis, P.R. Hof, H.W. M. Steinbusch, P.D. Coleman, B.P.F. Rutten, D.L.A. van den Hove, Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients, Neurobiol. Aging 34 (2013) 2091–2099.
[20] M.A. Bradley-Whitman, M.A. Lovell, Epigenetic changes in the progression of Alzheimer's disease, Mech. Ageing Dev. 134 (2013) 486–495.
[21] H. Hampel, M.M. Mesulam, A.C. Cuello, et al., The cholinergic system in the pathophysiology and treatment of Alzheimer's disease, Brain 141 (2018) 1917–1933.
[22] S. Verma, A. Kumar, T. Tripathi, A. Kumar, Muscarinic and nicotinic acetylcholine receptor agonists: current scenario in Alzheimer's disease therapy, J. Pharm. Pharmacol. 70 (2018) 985–993.
[23] M.E. Hasselmo, The role of acetylcholine in learning and memory, Curr. Opin. Neurobiol. 16 (2006) 710–715.
[24] P.J. Snyder, D. Ph, E. Giacobini, et al., Alzheimer's & Dementia : The Journal of the Alzheimer's Association Revisiting the cholinergic hypothesis in Alzheimer's disease : emerging evidence from translational and clinical research, J. Prev. Alzheimer's Dis. 6 (2020) 2–15.
[25] B. Dubois, H. Hampel, H.H. Feldman, et al., Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria, Alzheimer's Dement (2016), https://doi.org/10.1016/j.jalz.2016.02.002.
[26] M.S. Albert, S.T. DeKosky, D. Dickson, et al., The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease, Alzheimer's Dement 7 (2011) 270–279.
[27] B.R. Ott, R.N. Jones, L.A. Daiello, S.M. de la Monte, E.G. Stopa, C.E. Johanson, C. Denby, P. Grammas, Blood-cerebrospinal fluid barrier gradients in mild cognitive impairment and Alzheimer's disease: Relationship to inflammatory cytokines and chemokines, Front. Aging Neurosci. 10 (2018) 1–12.
[28] M. Kivipelto, F. Mangialasche, T. Ngandu, Lifestyle interventions to prevent cognitive impairment, dementia and Alzheimer disease, Nat. Rev. Neurol. 14 (2018) 653–666.
[29] F.S. Dafsari, F. Jessen, Depression—an underrecognized target for prevention of dementia in Alzheimer's disease, Transl. Psychiatry 10 (2020) 1–13.
[30] X. Du, X. Wang, M. Geng, Alzheimer's disease hypothesis and related therapies, Transl Neurodegener 7 (2018) 1–7.
[31] T. Guo, D. Zhang, Y. Zeng, T.Y. Huang, H. Xu, Y. Zhao, Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease, Mol. Neurodegener. 15 (2020) 1–37.
[32] M.M. Ibrahim, M.T. Gabr, Multitarget therapeutic strategies for Alzheimer's disease, Neural Regen. Res. 14 (2019) 437–440.
[33] P. Scheltens, B. De Strooper, M. Kivipelto, H. Holstege, G. Chételat, C. E. Teunissen, J. Cummings, W.M. van der Flier, Alzheimer's disease, Lancet 397 (2021) 1577–1590.
[34] M.G. Savelieff, G. Nam, J. Kang, H.J. Lee, M. Lee, M.H. Lim, Development of multifunctional molecules as potential therapeutic candidates for Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis in the last decade, Chem. Rev. 119 (2019) 1221–1322.
[35] U.C. Müller, T. Deller, M. Korte, Not just amyloid: Physiological functions of the amyloid precursor protein family, Nat. Rev. Neurosci. 18 (2017) 281–298.
[36] R.J. O'Brien, P.C. Wong, Amyloid precursor protein processing and alzheimer's disease, Annu. Rev. Neurosci. 34 (2011) 185–204.
[37] G. Thinakaran, E.H. Koo, Amyloid precursor protein trafficking, processing, and function, J. Biol. Chem. 283 (2008) 29615–29619.
[38] P. Carrillo-Mora, R. Luna, L. Colín-Barenque, Amyloid beta: Multiple mechanisms of toxicity and only some protective effects? Oxid. Med. Cell. Longev. (2014) https://doi.org/10.1155/2014/795375.
[39] N.M. Kanaan, D.S. Himmelstein, S.M. Ward, B. Combs, L.I. Binder, Tau Protein: Biology and Pathobiology, Second Edi. Mov Disord Genet Model Second Ed., 2015. https://doi.org/10.1016/B978-0-12-405195-9.00056-1.
[40] K. Iqbal, C. Del, A. Alonso, S. Chen, et al., Tau pathology in Alzheimer disease and other tauopathies, Biochim. Biophys. Acta – Mol. Basis Dis. 1739 (2005) 198–210.
[41] J.Z. Wang, I. Grundke-Iqbal, K. Iqbal, Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration, Eur. J. Neurosci. 25 (2007) 59–68.
[42] S. Mondragón-Rodríguez, G. Perry, J. Luna-Muñoz, M.C. Acevedo-Aquino, S. Williams, Phosphorylation of tau protein at sites Ser396-404 is one of the earliest events in Alzheimer's disease and Down syndrome, Neuropathol. Appl. Neurobiol. 40 (2014) 121–135.
[43] K. Yang, Z. Chen, J. Gao, W. Shi, L. Li, S. Jiang, H. Hu, Z. Liu, D. Xu, L. Wu, The Key Roles of GSK-3β in Regulating Mitochondrial Activity, Cell. Physiol. Biochem. 44 (2017) 1445–1459.
[44] D.C. Souder, R.M. Anderson, An expanding GSK3 network: implications for aging research, GeroScience 41 (2019) 369–382.
[45] F. Ito, Y. Sono, T. Ito, Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis,

and chronic inflammation, Antioxidants (2019), https://doi.org/10.3390/antiox8030072.
[46] H.J. Forman, H. Zhang, Targeting oxidative stress in disease: promise and limitations of antioxidant therapy, Nat. Rev. Drug Discov. 20 (2021) 689–709.
[47] M.L. Circu, T.Y. Aw, Reactive oxygen species, cellular redox systems, and apoptosis, Free Radic. Biol. Med. 48 (2010) 749–762.
[48] Y. Zhao, B. Zhao, Review Article Oxidative Stress and the Pathogenesis of Alzheimer ' s Disease. Oxid. Med. Cell. Longev. 2013, 2013.
[49] G. Ganguly, S. Chakrabarti, U. Chatterjee, L. Saso, Proteinopathy, oxidative stress and mitochondrial dysfunction: Cross talk in alzheimer's disease and parkinson's disease, Drug Des. Devel. Ther. 11 (2017) 797–810.
[50] D.A. Butterfield, D. Boyd-Kimball, Amyloid β-peptide(1–42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain, Brain Pathol. 14 (2004) 426–432.
[51] D. Boyd-Kimball, R. Sultana, H. Fai Poon, B.C. Lynn, F. Casamenti, G. Pepeu, J. B. Klein, D.A. Butterfield, Proteomic identification of proteins specifically oxidized by intracerebral injection of amyloid β-peptide (1–42) into rat brain: Implications for Alzheimer's disease, Neuroscience 132 (2005) 313–324.
[52] Z. Cai, B. Zhao, A. Ratka, Oxidative stress and β-amyloid protein in Alzheimer's disease, NeuroMol. Med. 13 (2011) 223–250.
[53] P.B. Shelat, M. Chalimoniuk, J.H. Wang, J.B. Strosznajder, J.C. Lee, A.Y. Sun, A. Simonyi, G.Y. Sun, Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons, J. Neurochem. 106 (2008) 45–55.
[54] X. Huang, R.D. Moir, R.E. Tanzi, A.I. Bush, J.T. Rogers, Redox-active metals, oxidative stress, and Alzheimer's disease pathology, Ann. N. Y. Acad. Sci. 1012 (2004) 153–163.
[55] D.A. Butterfield, F. Di Domenico, A.M. Swomley, E. Head, M. Perluigi, Redox proteomics analysis to decipher the neurobiology of Alzheimer-like neurodegeneration: Overlaps in Down's syndrome and Alzheimer's disease brain, Biochem. J 463 (2014) 177–189.
[56] D.A. Butterfield, A.M. Swomley, R. Sultana, Amyloid β-Peptide (1–42)-induced oxidative stress in alzheimer disease: Importance in disease pathogenesis and progression, Antioxidants Redox Signal 19 (2013) 823–835.
[57] C. Rinaldi, L. Donato, S. Alibrandi, C. Scimone, R. D'angelo, A. Sidoti, Oxidative stress and the neurovascular unit, Life 11 (2021) 1–17.
[58] M. Colonna, O. Butovsky, Microglia function in the central nervous system during health and neurodegeneration, Annu. Rev. Immunol. 35 (2017) 441–468.
[59] D.V. Hansen, J.E. Hanson, M. Sheng, 311. Microglia in Alzheimer ' s disease, J. Cell Biol. 2014 (2014) 1–14.
[60] H.S. Kwon, S.H. Koh, Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes, Transl Neurodegener 9 (2020) 1–12.
[61] C.A. Juźwik, S. Drake, Y. Zhang, N. Paradis-Isler, A. Sylvester, A. Amar-Zifkin, C. Douglas, B. Morquette, C.S. Moore, A.E. Fournier, microRNA dysregulation in neurodegenerative diseases: A systematic review. Prog Neurobiol 182 (2019) 101664.
[62] C. Acioglu, L. Li, S. Elkabes, Contribution of astrocytes to neuropathology of neurodegenerative diseases, Brain Res. 1758 (2021), 147291.
[63] C. Escartin, E. Galea, A. Lakatos, et al., Reactive astrocyte nomenclature, definitions, and future directions, Nat. Neurosci. 24 (2021) 312–325.
[64] S. Sarkar, S.C. Biswas, Astrocyte subtype-specific approach to Alzheimer's disease treatment, Neurochem. Int. 145 (2021), 104956.
[65] A.M. Arranz, B. De Strooper, The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications, Lancet Neurol. 18 (2019) 406–414.
[66] P. Hong, X. Zhang, S. Gao, P. Wang, Role of monocarboxylate transporter 4 in Alzheimer disease, Neurotoxicology 76 (2020) 191–199.
[67] B. Liu, A.G. Teschemacher, S. Kasparov, Neuroprotective potential of astroglia, J. Neurosci. Res. 95 (2017) 2126–2139.
[68] C. Veyrat-Durebex, P. Corcia, E. Piver, et al., Disruption of TCA Cycle and Glutamate Metabolism Identified by Metabolomics in an In Vitro Model of Amyotrophic Lateral Sclerosis, Mol. Neurobiol. 53 (2016) 6910–6924.
[69] B.G. Perez-Nievas, A. Serrano-Pozo, Deciphering the astrocyte reaction in Alzheimer's disease, Front. Aging Neurosci. 10 (2018) 1–23.
[70] K.A. Walker, B.N. Ficek, R. Westbrook, Understanding the Role of Systemic Inflammation in Alzheimer's Disease, ACS Chem. Nerosci. 10 (2019) 3340–3342.
[71] K. Jomova, S. Baros, M. Valko, Redox active metal-induced oxidative stress in biological systems, Transit. Met. Chem. 37 (2012) 127–134.
[72] E. Gammella, P. Buratti, G. Cairo, S. Recalcati, The transferrin receptor: The cellular iron gate, Metallomics 9 (2017) 1367–1375.
[73] T. Nakamura, I. Naguro, H. Ichijo, Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases, Biochim Biophys Acta - Gen Subj 1863 (2019) 1398–1409.
[74] M. Wessling-Resnick, Crossing the iron gate: Why and how transferrin receptors mediate viral entry, Annu. Rev. Nutr. 38 (2018) 431–458.
[75] X. Wang, B. Su, H.G. Lee, X. Li, G. Perry, M.A. Smith, X. Zhu, Impaired balance of mitochondrial fission and fusion in Alzheimer's disease, J. Neurosci. 29 (2009) 9090–9103.
[76] L. Devi, B.M. Prabhu, D.F. Galati, N.G. Avadhani, H.K. Anandatheerthavarada, Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction, J. Neurosci. 26 (2006) 9057–9068.
[77] G. Cenini, W. Voos, Mitochondria as potential targets in Alzheimer disease therapy: An update, Front. Pharmacol. 10 (2019) 1–20.
[78] C. Sharma, S.R. Kim, Linking oxidative stress and proteinopathy in alzheimer's disease, Antioxidants 10 (2021) 1–21.
[79] J.Y. Cao, S.J. Dixon, Mechanisms of ferroptosis, Cell. Mol. Life Sci. 73 (2016) 2195–2209.
[80] C. Han, Y. Liu, R. Dai, N. Ismail, W. Su, B. Li, Ferroptosis and Its Potential Role in Human Diseases, Front. Pharmacol. 11 (2020) 1–19.
[81] J.P.F. Angeli, R. Shah, D.A. Pratt, M. Conrad, Ferroptosis Inhibition: Mechanisms and Opportunities, Trends Pharmacol. Sci. 38 (2017) 489–498.
[82] Y. Mou, J. Wang, J. Wu, D. He, C. Zhang, C. Duan, B. Li, Ferroptosis, a new form of cell death: Opportunities and challenges in cancer, J. Hematol. Oncol. 12 (2019) 1–16.
[83] B.R. Stockwell, X. Jiang, The Chemistry and Biology of Ferroptosis, Cell Chem. Biol. 27 (2020) 365–375.
[84] Y. Yu, Y. Yan, F. Niu, et al., Ferroptosis: a cell death connecting oxidative stress, inflammation and cardiovascular diseases, Cell Death Discov (2021), https://doi.org/10.1038/s41420-021-00579-w.
[85] J. Li, F. Cao, H. Yin liang, Z jian. Huang, Z tao Lin, N. Mao, B. Sun, G. Wang, Ferroptosis: past, present and future. Cell Death Dis., 2020 https://doi.org/10.1038/s41419-020-2298-2.
[86] Y. Li, X. Zeng, D. Lu, M. Yin, M. Shan, Y. Gao, Erastin induces ferroptosis via ferroportin-mediated iron accumulation in endometriosis, Hum. Reprod. 36 (2021) 951–964.
[87] N. Geng, B.J. Shi, S.L. Li, Z.Y. Zhong, Y.C. Li, W.L. Xua, H. Zhou, J.H. Cai, Knockdown of ferroportin accelerates erastin-induced ferroptosis in neuroblastoma cells, Eur. Rev. Med. Pharmacol. Sci. 22 (2021) 3826–3836.
[88] Y. Xie, W. Hou, X. Song, Y. Yu, J. Huang, X. Sun, R. Kang, D. Tang, Ferroptosis: Process and function, Cell Death Differ. 23 (2016) 369–379.
[89] S.J. Dixon, K.M. Lemberg, M.R. Lamprecht, et al., Ferroptosis: An iron-dependent form of nonapoptotic cell death, Cell 149 (2012) 1060–1072.
[90] M. Jakaria, A.A. Belaidi, A.I. Bush, S. Ayton, Ferroptosis as a mechanism of neurodegeneration in Alzheimer's disease, J. Neurochem. 159 (2021) 804–825.
[91] N. Kajarabille, G.O. Latunde-Dada, Programmed cell-death by ferroptosis: Antioxidants as mitigators, Int. J. Mol. Sci. (2019), https://doi.org/10.3390/ijms20194968.
[92] A. Ashraf, J. Jeandriens, H.G. Parkes, P.W. So, Iron dyshomeostasis, lipid peroxidation and perturbed expression of cystine/glutamate antiporter in Alzheimer's disease: Evidence of ferroptosis, Redox Biol. 32 (2020), 101494.
[93] M. Bulk, W.M. Abdelmoula, R.J.A. Nabuurs, et al., Postmortem MRI and histology demonstrate differential iron accumulation and cortical myelin organization in early- and late-onset Alzheimer's disease, Neurobiol. Aging 62 (2018) 231–242.
[94] I. Costa, D.J. Barbosa, S. Benfeito, et al., Molecular mechanisms of ferroptosis and their involvement in brain diseases, Pharmacol. Ther. (2023), https://doi.org/10.1016/j.pharmthera.2023.108373.
[95] S. Swarbrick, N. Wragg, S. Ghosh, A. Stolzing, Systematic Review of miRNA as Biomarkers in Alzheimer's Disease, Mol. Neurobiol. 56 (2019) 6156–6167.
[96] H. Zetterberg, B.B. Bendlin, Biomarkers for Alzheimer's disease—preparing for a new era of disease-modifying therapies, Mol. Psychiatry 26 (2021) 296–308.
[97] N. El Kadmiri, N. Said, I. Slassi, B. El Moutawakil, S. Nadifi, Biomarkers for Alzheimer Disease: Classical and Novel Candidates' Review, Neuroscience 370 (2018) 181–190.
[98] O. Hansson, Biomarkers for neurodegenerative diseases, Nat. Med. 27 (2021) 954–963.
[99] H. Hong, Y. Li, B. Su, Identification of circulating miR-125b as a potential biomarker of Alzheimer's disease in APP/PS1 transgenic mouse, J Alzheimer's Dis 59 (2017) 1449–1458.
[100] A. Vergallo, S. Lista, Y. Zhao, et al., MiRNA-15b and miRNA-125b are associated with regional Aβ-PET and FDG-PET uptake in cognitively normal individuals with subjective memory complaints, Transl. Psychiatry (2021), https://doi.org/10.1038/s41398-020-01184-8.
[101] L. Gaetani, K. Blennow, P. Calabresi, M. Di Filippo, L. Parnetti, H. Zetterberg, Neurofilament light chain as a biomarker in neurological disorders, J. Neurol. Neurosurg. Psychiatry (2019) 1–12.
[102] Q. Shen, D.A. Loewenstein, W.Z. Elizabeth Potter, J. Appel, M.T. Greig, A. Acevedo, E. Schofield, W. Barker, W.H. Yougui, R.D. Potter, Volumetic and visual rating of MRI scans in the diagnosis of amnestic MCI and Alzheimer's disease, Alzheimers Dement. 7 (2011) 1–17.
[103] T. Toyonaga, A. Fesharaki-Zadeh, S.M. Strittmatter, R.E. Carson, Z. Cai, PET Imaging of Synaptic Density: Challenges and Opportunities of Synaptic Vesicle Glycoprotein 2A PET in Small Animal Imaging, Front. Neurosci. 16 (2022) 1–7.
[104] M. Gratuze, C.E.G. Leyns, D.M. Holtzman, New insights into the role of TREM2 in Alzheimer's disease, Mol. Neurodegener. 13 (2018) 1–16.
[105] Y. Lee, Y. Park, H. Nam, J.W. Lee, S.W. Yu, Translocator protein (TSPO): The new story of the old protein in neuroinflammation, BMB Rep. 53 (2020) 20–27.
[106] H. Hampel, S.E. O'Bryant, J.L. Molinuevo, H. Zetterberg, C.L. Masters, S. Lista, S. J. Kiddle, R. Batrla, K. Blennow, Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic, Nat. Rev. Neurol. 14 (2018) 639–652.
[107] S. Palmqvist, P.S. Insel, E. Stomrud, et al., Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease, EMBO Mol. Med. 11 (2019) 1–13.
[108] E.H. Thijssen, R. La Joie, A. Strom, et al., Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study, Lancet Neurol. 20 (2021) 739–752.
[109] K. Blennow, H. Zetterberg, Biomarkers for Alzheimer's disease: current status and prospects for the future, J. Intern. Med. 284 (2018) 643–663.
[110] A. Lleó, H. Zetterberg, J. Pegueroles, et al., Phosphorylated tau181 in plasma as a potential biomarker for Alzheimer's disease in adults with Down syndrome, Nat. Commun. 12 (2021) 1–8.

[111] T.K. Karikari, A. Emeršič, A. Vrillon, et al., Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis, Alzheimer's Dement 17 (2021) 755–767.
[112] L. Ismaili, B. Refouvelet, M. Benchekroun, et al., Multitarget compounds bearing tacrine- and donepezil-like structural and functional motifs for the potential treatment of Alzheimer's disease, Prog. Neurobiol. 151 (2017) 4–34.
[113] J. Weller, A. Budson, Current understanding of Alzheimer's disease diagnosis and treatment. F1000Research 7 (2018) 1–9.
[114] J. Cummings, A. Ritter, K. Zhong, Clinical Trials for Disease-Modifying Therapies in Alzheimer's Disease: A Primer, Lessons Learned, and a Blueprint for the Future, J. Alzheimer's Dis. 64 (2018) S3–S22.
[115] J.L. Cummings, T. Morstorf, K. Zhong, Cummings, Jeffrey L_Alzheimer's_drug development candidates failures_2014, Alzheimers Res. Ther. 6 (2014) 1–7.
[116] Q. Li, H. Yang, Y. Chen, H. Sun, Recent progress in the identification of selective butyrylcholinesterase inhibitors for Alzheimer's disease, Eur. J. Med. Chem. 132 (2017) 294–309.
[117] A. Iraji, O. Firuzi, M. Khoshneviszadeh, H. Nadri, N. Edraki, R. Miri, Synthesis and structure-activity relationship study of multi-target triazine derivatives as innovative candidates for treatment of Alzheimer's disease, Bioorg. Chem. 77 (2018) 223–235.
[118] M. Bortolami, F. Pandolfi, D. De Vita, et al., New deferiprone derivatives as multi-functional cholinesterase inhibitors: design, synthesis and in vitro evaluation, Eur. J. Med. Chem. 198 (2020) 1–17.
[119] J. Gupta, M.T. Fatima, Z. Islam, R.H. Khan, V.N. Uversky, P. Salahuddin, Nanoparticle formulations in the diagnosis and therapy of Alzheimer's disease, Int. J. Biol. Macromol. 130 (2019) 515–526.
[120] G. Liu, P. Men, P.L.R. Harris, R.K. Rolston, G. Perry, M.A. Smith, Nanoparticle iron chelators: A new therapeutic approach in Alzheimer disease and other neurologic disorders associated with trace metal imbalance, Neurosci. Lett. 406 (2006) 189–193.
[121] I. Celardo, J.Z. Pedersen, E. Traversa, L. Ghibelli, Pharmacological potential of cerium oxide nanoparticles, Nanoscale 3 (2011) 1411–1420.
[122] H.J. Kwon, M.Y. Cha, D. Kim, D.K. Kim, M. Soh, K. Shin, T. Hyeon, I. Mook-Jung, Mitochondria-Targeting Ceria Nanoparticles as Antioxidants for Alzheimer's Disease, ACS Nano 10 (2016) 2860–2870.
[123] M. Bilal, M. Barani, F. Sabir, A. Rahdar, G.Z. Kyzas, Nanomaterials for the treatment and diagnosis of Alzheimer's disease: An overview, NanoImpact 20 (2020), 100251.
[124] E.E. Congdon, E.M. Sigurdsson, Tau-targeting therapies for Alzheimer disease, Nat. Rev. Neurol. 14 (2018) 399–415.
[125] T. Guo, W. Noble, D.P. Hanger, Roles of tau protein in health and disease, Acta Neuropathol. 133 (2017) 665–704.
[126] W. Grabowska-Pyrzewicz, A. Want, J. Leszek, U. Wojda, Antisense oligonucleotides for Alzheimer's disease therapy: from the mRNA to miRNA paradigm, EBioMedicine 74 (2021), 103691.
[127] M. Vaz, S. Silvestre, Alzheimer's disease: Recent treatment strategies, Eur. J. Pharmacol. 887 (2020), 173554.
[128] C. Zhang, Y. Xu, A. Chowdhary, et al., Memory enhancing effects of BPN14770, an allosteric inhibitor of phosphodiesterase-4D, in wild-type and humanized mice, Neuropsychopharmacology 43 (2018) 2299–2309.
[129] F. Panza, M. Lozupone, G. Logroscino, B.P. Imbimbo, A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease, Nat. Rev. Neurol. 15 (2019) 73–88.
[130] M.S. Rafii, R.A. Sperling, M.C. Donohue, et al., The AHEAD 3–45 Study: Design of a prevention trial for Alzheimer's disease, Alzheimer's Dement (2022) 1–7.
[131] V. Walia, D. Kaushik, V. Mittal, et al., Delineation of Neuroprotective Effects and Possible Benefits of AntioxidantsTherapy for the Treatment of Alzheimer's Diseases by Targeting Mitochondrial-Derived Reactive Oxygen Species: Bench to Bedside, Mol. Neurobiol. 59 (2022) 657–680.
[132] K. Youn, C.T. Ho, M. Jun, Multifaceted neuroprotective effects of (-)-epigallocatechin-3-gallate (EGCG) in Alzheimer's disease: an overview of pre-clinical studies focused on β-amyloid peptide, Food Sci. Hum. Wellness 11 (2022) 483–493.
[133] L. Cassidy, F. Fernandez, J.B. Johnson, M. Naiker, A.G. Owoola, D.A. Broszczak, Oxidative stress in alzheimer's disease: A review on emergent natural polyphenolic therapeutics, Complement. Ther. Med. 49 (2020), 102294.
[134] F. He, X. Ru, T. Wen, NRF2 , a Transcription Factor for Stress Response and Beyond. 1–23 (2020).
[135] D.A. Davies, A. Adlimoghaddam, B.C. Albensi, Role of nrf2 in synaptic plasticity and memory in alzheimer's disease, Cells 10 (2021) 1–22.
[136] W. Chen, Y. Hu, D. Ju, Gene therapy for neurodegenerative disorders: advances, insights and prospects, Acta Pharm. Sin. B 10 (2020) 1347–1359.
[137] L. Guan, Y. Han, C. Yang, S. Lu, J. Du, H. Li, J. Lin, CRISPR-Cas9-Mediated Gene Therapy in Neurological Disorders, Mol. Neurobiol. 59 (2022) 968–982.
[138] M. Grundman, R. Morgan, J.D. Lickliter, et al., A phase 1 clinical trial of the sigma-2 receptor complex allosteric antagonist CT1812, a novel therapeutic candidate for Alzheimer's disease, Alzheimer's Dement Transl. Res. Clin. Interv. 5 (2019) 20–26.
[139] N.J. Izzo, C.M. Yuede, K.M. LaBarbera, et al., Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification, Alzheimer's Dement 17 (2021) 1365–1382.
[140] K. Yang, C. Zeng, C. Wang, M. Sun, D. Yin, T. Sun, Sigma-2 receptor—a potential target for cancer/alzheimer's disease treatment via its regulation of cholesterol homeostasis, Molecules (2020), https://doi.org/10.3390/molecules25225439.
[141] Q. Behfar, A.R. Zuniga, Aging, Senescence, and Dementia. 3 (2022) 523–531.
[142] J. Kaur, J.N. Farr, Cellular senescence in age-related disorders, Transl. Res. 226 (2020) 96–104.
[143] R. Liu, Aging , Cellular Senescence , and Alzheimer ' s Disease (2022).
[144] E.J. Novais, V.A. Tran, S.N. Johnston, K.R. Darris, A.J. Roupas, G.A. Sessions, I. M. Shapiro, B.O. Diekman, M.V. Risbud, Long-term treatment with senolytic drugs Dasatinib and Quercetin ameliorates age-dependent intervertebral disc degeneration in mice, Nat. Commun. 12 (2021) 1–17.
[145] M.M. Gonzales, V.R. Garbarino, E. Marques Zilli, R.C. Petersen, J.L. Kirkland, T. Tchkonia, N. Musi, S. Seshadri, S. Craft, M.E. Orr, Senolytic Therapy to Modulate the Progression of Alzheimer's Disease (SToMP-AD): A Pilot Clinical Trial, J. Prev. Alzheimer's Dis. 9 (2022) 22–29.
[146] A. Guerrero, B. De Strooper, I.L. Arancibia-Cárcamo, Cellular senescence at the crossroads of inflammation and Alzheimer's disease, Trends Neurosci. 44 (2021) 714–727.
[147] N. Musi, J.M. Valentine, K.R. Sickora, E. Baeuerle, C.S. Thompson, Q. Shen, M. E. Orr, Tau protein aggregation is associated with cellular senescence in the brain, Aging Cell (2018), https://doi.org/10.1111/acel.12840.
[148] J.N. Justice, A.M. Nambiar, T. Tchkonia, et al., Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study, EBioMedicine 40 (2019) 554–563.
[149] A.A. Oliva, M. Brody, M. Agronin, et al., Safety and efficacy of Lomecel-B in patients with mild Alzheimer's disease: Results of a double-blinded, randomized, placebo-controlled phase 1 clinical trial, Alzheimer's Dement 17 (2021) 1–3.
[150] M. Brody, M. Agronin, B.J. Herskowitz, et al., Results and insights from a phase I clinical trial of Lomecel-B for Alzheimer's disease, Alzheimer's Dement 19 (2023) 261–273.
[151] J.L. Kirkland, T. Tchkonia, Senolytic drugs: from discovery to translation, J. Intern. Med. 288 (2020) 518–536.
[152] J. Cummings, G. Lee, K. Zhong, J. Fonseca, K. Taghva, Alzheimer's disease drug development pipeline: 2021, Alzheimer's Dement Transl. Res. Clin. Interv. 7 (2021) 1–24.
[153] X.Y. Liu, L.P. Yang, L. Zhao, Stem cell therapy for alzheimer's disease, World J. Stem Cells 12 (2020) 787–802.
[154] A.U. Pradhan, O. Uwishema, H. Onyeaka, I. Adanur, B. Dost, A review of stem cell therapy: An emerging treatment for dementia in Alzheimer's and Parkinson's disease, Brain Behav. 12 (2022) 1–5.
[155] A. Rasheed, Z.A. Bin, A. Munawwar, Z. Sarfraz, A. Sarfraz, K. Robles-Velasco, I. Cherrez-Ojeda, The Allosteric Antagonist of the Sigma-2 Receptors—Elayta (CT1812) as a Therapeutic Candidate for Mild to Moderate Alzheimer's Disease: A Scoping Systematic Review, Life 13 (2023) 1–15.
[156] G.M. Rishton, G.C. Look, Z.J. Ni, et al., Discovery of Investigational Drug CT1812, an Antagonist of the Sigma-2 Receptor Complex for Alzheimer's Disease, ACS Med. Chem. Lett. 12 (2021) 1389–1395.