Ligands for Alzheimer’s Disease therapy

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Despite tremendous research efforts at every level, globally, there is still a lack of effective drugs for the treatment of Alzheimer's disease (AD). The biochemical mechanisms of this devastating neurodegenerative disease are not yet clearly understood. This review analyses the relevance of multiple ligands in drug discovery for AD as a versatile toolbox for a polypharmacological approach to AD. Herein, we highlight major targets associated with AD, ranging from acetylcholine esterase (AChE), beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1), glycogen synthase kinase 3 beta (GSK-3β), N-methyl-D-aspartate (NMDA) receptor, monoamine oxidases (MAOs), metal ions in the brain, 5-hydroxytryptamine (5-HT) receptors, the third subtype of histamine receptor (H_3 receptor), to phosphodiesterases (PDEs), along with a summary of their respective relationship to the disease network. In addition, a multitarget strategy for AD is presented, based on reported milestones in this area and the recent progress that has been achieved with multitargeted-directed ligands (MTDLs). Finally, the latest publications referencing the enlarged panel of new biological targets for AD related to the microglia are highlighted. However, the question of how to find meaningful combinations of targets for an MTDLs approach remains unanswered.

multitarget drug discovery MTDLs tacrine donepezil AChE inhibitors BACE-1 inhibitors GSK-3β nhibitors

Despite tremendous research efforts at every level, globally, there is still a lack of effective drugs for the treatment of Alzheimer's disease (AD). The biochemical mechanisms of this devastating neurodegenerative disease are not yet clearly understood. This review analyses the relevance of multiple ligands in drug discovery for AD as a versatile toolbox for a polypharmacological approach to AD.

1. Introduction

In 1906, Alois Alzheimer presented his first signature case and the pathological features of the disease which, from 1910, became known as Alzheimer’s disease (AD). AD is clinically characterized by a loss of memory, the retardation of thinking and reasoning, and changes in personality and behaviours [1][2]. Nowadays, approximately 40 million people over the age of 60 suffer from AD worldwide, and the number of patients is increasing, with the perspective of cases doubling every 20 years [3]. AD is a progressive and irreversible neurological disorder occurring in the central nervous system (CNS) mainly confined within the hippocampus and the cerebral cortex, domains of the forebrain related to memory and higher cognitive functions. The histological manifestation of AD presents extracellular deposits of β-amyloid peptide (Aβ) and the intracellular formation of neurofibrillary tangles

consisting of paired helical filaments of hyperphosphorylated tau protein [4][5]. AD is a complex and multifactorial disease, which means that it is influenced by a combination of multiple genes and environmental/risk factors. In the early 1990s, mutations in the genes of amyloid-beta A4 precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) were determined for familial AD [6][7][8]. Presenilins are components of the γ-secretase complex which, when mutated, can affect amyloid precursor protein (APP) processing to form toxic forms of Aβ. In addition to genes, genetic risk loci for AD were determined and one of them, apolipoprotein E, type ε4 allele (APOE ε4), is associated with late-onset familial AD [9][10]. Studies on the binding of apoE (a peptide corresponding to the low-density lipoprotein receptor binding domain) to APP, showed that blocking of the interaction of apoE with *N*-terminal APP reduces Alzheimer's-associated Aβ accumulation and tau pathologies in the brain [11]. Known risk factors include age, having a family history of AD, APOE ε4, vascular problems (heart disease, stroke, high blood pressure), diabetes, and obesity [12][13]. However, the reasons why sporadic AD occurs is still unknown. There are various descriptive hypotheses regarding the causes of sporadic AD, including the cholinergic hypothesis [13], amyloid hypothesis [14][15][16], tau propagation hypothesis [17][18], mitochondrial cascade hypothesis [19][20], calcium homeostasis hypothesis [21][22], inflammatory hypothesis [23][24][25], neurovascular hypothesis [26], metal ion hypothesis [27][28][29], and lymphatic system hypothesis [30][31]. Moreover, there are many factors that may associated with AD, such as various microbes (triggering amyloidosis), viral pathogens (*Herpesviridae* family) [32][33], decreased expression of microRNAs-107 (miRNA-107) [34][35][36][37][38] and RAS-RAF-MEK signalling pathway (atrophy of neurons) [39], regional hypometabolism [40][41], and mitochondrial dysfunction [42][43][44]. In summary, the aetiology of AD is recognized but not fully understood.

Currently, there are a total of five therapies in the clinic, and four drugs approved by the FDA for AD (Table 1), which mostly aim at restoring physiological ACh levels. The inhibition of the enzyme acetylcholinesterase (AChE), responsible for the hydrolysis of Ach, is the main biochemical mechanism of action of donepezil, rivastigmine and galantamine. In turn, memantine, through noncompetitive antagonism of the *N*-methyl-d-aspartate (NMDA) receptor, blocks current flow (especial calcium) and reduces the excitotoxic effect of glutamate [45].

Table 1. Drugs approved for AD therapy.

Drug Structure	Targets	Therapeutic Effects
	-AChE inhibitor -5-HT_{2A} inducer -ChE inducer -NOS inhibitor/inducer	-selectively and reversibly inhibits AChE -improves the cognitive and behavioral signs and symptoms of AD -neuroprotective

	-TNF inhibitor -IL-1β inhibitor/inducer -NMDAR downregulator	
	-AChE inhibitor -ChE inhibitor	-parasympathomimetic and a reversible cholinesterase inhibitor -inhibits both BuChE and AChE -enhances cholinergic function
	-AChE competitive and reversible inhibitor -AChR subunit alpha-7 allosteric modulator -N AChR allosteric modulator -ChE inhibitor	-enhances cholinergic function -improve cognitive performance in AD -not considered as a disease-modifying drug
	-NMDAR uncompetitive (open-channel) antagonist -Alpha-7 nicotinic cholinergic receptor subunit antagonist	-inhibits calcium influx into cells that is normally caused by chronic NMDAR activation by glutamate -enhances neuronal synaptic plasticity

Drugs only provide temporary symptomatic relief among patients with mild-to-moderate symptoms of AD, but do not provide a cure or protection. Thus, one of the largest unmet medical needs is a modifying treatment for AD. The complicated pathogenesis of AD, in association with the various descriptive hypotheses involved in the onset and development of the disease and along with the imperfect single-target drugs available, form an excellent rational basis for the implementation of multi-target strategies as part of the drug discovery pipeline against AD. The multi-target-directed ligands (MTDLs) strategy foresees the development of a single molecule able to affect several key targets/pathways, which can have a synergistic effect on the AD network, leading to superior improvement on

memory and cognition. While the idea of MDTLs is simple at a glance, the rational design of a compound in which two or more pharmacophores are combined in a single molecular entity is a challenge. Herein, we highlight major targets associated with AD and collate the latest publications which have resulted in an enlarged panel of drug targets.

2. AD-Related Targets

Exploration of hypotheses regarding the causes of AD focus on major pathogenesis factors and pathways (Figure 1). Firstly, cholinergic deficit, which led to the discovery of the primary AD targets of acetylcholinesterase (AChE) and butyrylocholinosterase (BuChE). Next, for amyloid aggregation, where the main target is beta-secretase 1 (BACE-1), whereas for the hyperphosphorylation of tau protein, glycogen synthase kinase 3 beta (GSK-3β) and cyclin dependent kinase 5 (Cdk5) are the key targets. Increased oxidative damage and inflammation and unbalanced homeostasis of biometals in the course of AD led to the discovery of further potential targets for AD treatment.

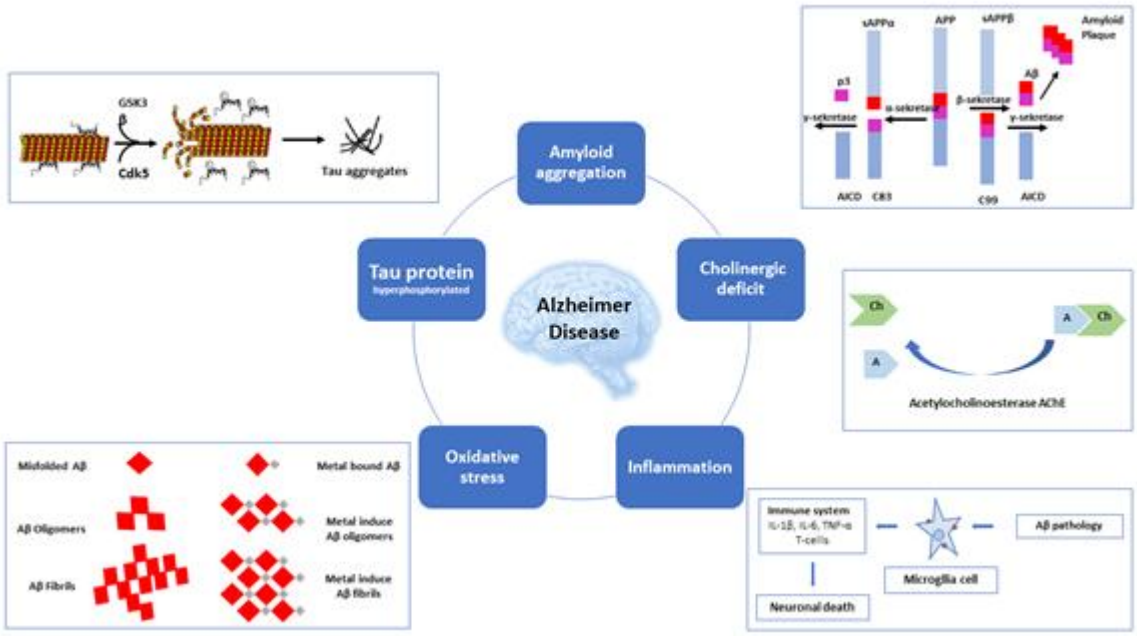

Figure 1. Multiple pathological pathways of AD.

Moreover, the pathogenesis of AD involves numerous receptors, namely, (*N*-methyl-d-aspartate (NMDA), 5-hydroxytryptamine (5-HT) serotonin, the third subtype of histamine receptor (H_3 receptor), and enzymes, namely monoaminoxidases (MAOs), and phosphodiesterases (PDEs). The first AChE inhibitor (AChEI) for AD treatment, tacrine, was approved in 1993. However it was withdrawn shortly after release due to liver toxicity [46] (Figure 2). Currently, the inhibition of AChE is a fundamental property of drugs approved by the FDA which are principally AChE inhibitors (AChEIs), such as donepezil, galantamine, and rivastigmine. Even so, tacrine and donepezil are still the targets for modifications or are used as positive controls in enzyme or pharmacological activity tests. The cholinergic hypothesis, combining ACh and AChE as a common modality is emerging as a promising approach in

designing MTDLs for AD. The first clinical example of this approach was caproctamine, which was reported in 1998 as a compound with noncovalent inhibitory activity against the cholinergic system and acetylcholinesterase [47].

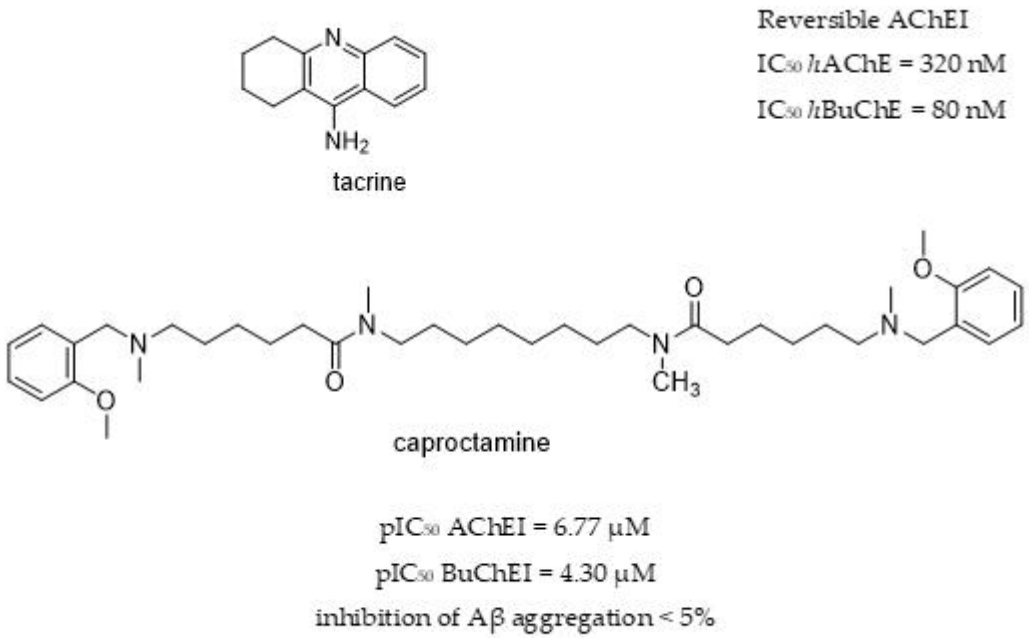

Figure 2. Tacrine and captopramine.

Later, the amyloid cascade hypothesis was proposed to explore the mechanism of AD and has been the gold-standard-beta-amyloid dogma for almost 30 years [48] and has become one of the most dominant research focuses conducted in academia and the pharmaceutical industry.

The typical hallmarks of AD-synaptic dysfunction and senile plaques—are consequences of the production, oligomerization and self-aggregation of beta-amyloid (Aβ). Amyloid precursor protein (APP) is degraded via the non-amyloidogenic (catalyzed by α-secretase and γ-secretase) and amyloidogenic pathways, where the degradation by β-secretase (BACE-1) and γ-secretase generate Aβ species. The Aβ species are composed of 37–49 amino acid residues, with the major species being Aβ40, whereas the major type from the minor species is Aβ42. Aβ40 is the more common metabolite and may actually be anti-amyloidogenic [49], whereas Aβ42 and other longer peptides are highly self-aggregating and lead to profound Aβ deposition [50][51]. Studies of AD-causing mutations in APP, presenilin 1 (PSEN1), and presenilin 2 (PSEN2) genes demonstrate that the vast majority of these mutations alter APP processing in a manner that either increases the absolute or relative levels of Aβ42 [52].

The key histopathological hallmark of AD is the senile plaque-intracellular neurofibrillary tangles in the brain. Tangles are composed of paired helical filaments and straight filaments which are mainly caused by hyperphosphorylated tau protein [53]. The tau protein hypothesis of AD is based on hyperphosphorylation of the tau protein (from the 2–3 to the 5–9 phosphate groups) by the threonine-serine kinase, GSK-3β. Such hyperphosphorylated tau protein is separated from microtubules and subsequently aggregates into insoluble

intracellular neurofibrillary tangles which ultimately cause cell death [54][55]. GSK-3β is a pivotal kinase in neurodevelopment and involved in both physiological and pathological aging. In the non-amyloidogenic pathway, GSK-3β may down-regulate the activity of the α-secretase complex through inhibition of metalloproteinase (ADAM) activity [56] and regulates Aβ production by interfering with APP cleavage at the γ-secretase complex [57]. In turn, in the amyloidogenic pathway, GSK-3β inhibition reduces BACE1-mediated cleavage of APP through a nuclear factor kappa-light-chain-enhancer of an activated B cell (NF-κB) signaling-mediated mechanism. This observation thus suggests that the inhibition of GSK-3β reduces Aβ pathology [58][59][60] and GSK-3β plays a key role in choline metabolism, which involves the regulation of choline acetyltransferase (ChAT) and AChE [61][62]. Further, GSK-3β has the capacity to phosphorylate several mitogen-activated protein kinases (MAPKs), thus regulating axonal stability. Inhibitors of GSK-3β provide protection from intrinsic apoptotic signaling, but potentiate that of extrinsic apoptosis [63].

GSK3β mediates an interaction between two major forms of synaptic plasticity in the brain, *N*-methyl-d-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) and NMDA receptor-dependent long-term depression (LTD). LTP and LTD of hippocampal synaptic transmission represent the principal experimental models underlying learning and memory. In mouse models of AD, early impairments in synaptic transmission were caused, among other factors, by Aβ, which leads to impairment of LTP via tau protein [64]. In the normal brain, activation of GSK3β is essential for NMDA receptor-dependent LTD, and its activity can be regulated by LTP. Following the induction of LTP, there is inhibition of GSK3β activity, whereas GSK3β inhibitors block the induction of LTD. In addition, GSK-3β has been identified as a prominent regulator of inflammation via the promotion of the production of proinflammatory cytokines (interleukin-6(IL-6), IL-1β) and tumor necrosis factor (TNF), or by decreasing the production of the anti-inflammatory cytokine, IL-10 [65][66]. To conclude, GSK-3β inhibition is a popular target for small molecule compounds, mainly based on the three therapeutic approaches, namely the inhibition of phosphorylation, prevention of the aggregation of tau protein, and stabilization of microtubules.

NMDA receptor plays a crucial role in modifying major forms of synaptic plasticity, certain types of learning and memory formation, as well as consolidation of short-term memory into long-term memory under physiological conditions [67]. The glutamatergic (NMDA) hypothesis of AD is based on the observation that inhibition of the NMDA receptor would ameliorate the overall condition of AD patients. However, NMDA cannot be fully antagonized since it exerts important bio-functions in normal synaptic transmission, whereas glutamate-related excitotoxicity and cell death could be caused when NMDA receptors are overstimulated by excess glutamate [68]. Thus, establishing an optimal balance between glutamate stimulation and glutamate-related excitotoxicity is crucial to achieve the most effective treatment of AD.

Other targets for AD are the monoamine oxidase (MAO) enzymes, a group of enzymes consisting of two distinct isoforms (MAO-A and MAO-B) which, by deamination, lead to the metabolism of amine neurotransmitters (e.g., monoamine neurotransmitters). In AD patients, the activity and gene expression of MAO-A is up-regulated in different brain areas [69][70] as well as MAO-B [71]. High levels of MAOs catalyze oxidative deamination, increasing the production of hydrogen peroxide and reactive oxygen species (ROS), which are responsible for oxidative

injuries and the toxic environment characteristic of neurodegeneration [72][73] and increased MAO-B levels can enhance astrogliosis in the brain [74][75].

AD may be implicated by high levels and dysregulation of Cu^{2+}, Fe^{2+}, Zn^{2+}, and Ca^{2+}, which are important biometal ions [76][77]. Cu^{2+} and Zn^{2+} are known to induce the generation of toxic Aβ oligomers by binding to Aβ peptides and influencing the Aβ aggregation pathway [78][79], the redox-active metals, Cu(I/II) and Fe(II/III) generate cytotoxic reactive oxygen species (ROS) [80]. Thus, the use of biometal chelators that can down-regulate high levels of biometals could be a potential therapeutic strategy for the treatment of AD. The serotoninergic neurotransmitter and histaminergic systems play a critical role in the regulation of the CNS. Brain functions mediated by 5-HT_4R and 5-HT_6R require a synergistic effect from cholinergic neurotransmission. Activation of 5-HT_4R can enhance the release of ACh in the hippocampus, whereas 5-HT_6R blockade enhance cholinergic neurotransmission [81][82]. Moreover, 5-HT_4R agonists could promote the nonamyloidogenic cleavage of APP, which releases a soluble sAPPα fragment which, in contrast to Aβ, has putative neurotrophic and neuroprotective properties [83]. The selective partial 5-HT_4 agonist, RS-67333, which is a potent cognitive and learning function enhancer, may reduce Aβ production and has a neuroprotective activity in a cellular model of AD [84][85][86]. Activation of the histamine H_3 receptor decreases the presynaptic release of ACh, and its blockade augments the presynaptic release of Ach, resulting in improved cholinergic neurotransmission in the cortex. However, in clinical trials, the H_3 receptor antagonists failed to achieve cognitive improvement in AD patients [87].

Impaired signaling pathways of cyclic-3′,5′- adenosine monophosphate (cAMP) and cyclic-3′,5′-guanosine monophosphate (cGMP) may contribute to the development and progression of AD. Thus, phosphodiesterase inhibitors (PDEIs), such as rolipram and roflumilast (PDE4Is), vinpocetine (PDE1I), cilostazol and milrinone (PDE3Is), sildenafil and tadalafil (PDE5Is) were found to be involved in the phosphorylation of tau, aggregation of Aβ, neuroinflammation as well as regulation of cognition, mood, and emotion processing. Despite rational arguments, the clinical data do not demonstrate efficacy of selective PDEIs in improving cognition in patients with prodromal and mild AD, and a number of these have been *discontinued due* to failure to meet efficacy *endpoints* in a phase I/II clinical *trial* [88][89][90]. However, the combination therapy of donepezil with cilostazol showed positive effects on patients with mild or moderate-to-severe Alzheimer's patients [91][92]. To conclude, there are many biological targets and signalling pathways involved in AD pathology [93][94]. However, the complex interactions between them is unclear.

3. Physicochemical Properties of MTDLs for AD

MTDLs are designed by combining two (or more) pharmacophoric structural scaffolds, thus physicochemical properties such as molecular weight, solubility, permeability and the ability of MTDLs to permeate cell membranes have to optimized for absorption and distribution within the body [95]. The 'Lipinski rule of five' (RO5)[96] defined the optimal physicochemical properties for perorally administered drugs, which are preferred for perspective long-term treatment of AD patients. RO5 postulated desirable physicochemical property space by setting up limits for molecular weight < 500Da, logarithm of partition coefficient (log*P*; a value classifying the lipophilicity of a compound) < 5, number of hydrogen bond donors (HBDs)< 5, and number of hydrogen bond acceptors (HBAs)<

10. Although RO5 indicates possibility of absorption of drug after oral administration, the rule is not specific for CNS drugs. It turns out that AD drugs need to fulfil strict physicochemical criteria defined for blood–brain barrier (BBB) penetration by transcellular passive diffusion, which is typical for most CNS drugs. Blood-to-brain influx is mediated by the receptor for advanced glycation end products (RAGE). The BBB efflux pumps are transmembrane P-glycoprotein (P-gp), which mediate brain-to-blood transport. Cordon-Cardo et al. first suggested that P-gp might play a role in vivo in limiting brain penetration of xenobiotics [97]. The high expression of P-gp is one of the main reasons that a lot of lipophilic drugs could not penetrate CNS for brain disease therapy.

Extensive studies on physicochemical properties for CNS drugs laid out that molecular weight should not exceed 430–450 Da, log*P* values should be < 4 and the number of HBD and HBA must be < 2 and < 7, respectively [98]. Next, characterisation has been completed with topological polar surface area (TPSA), Log D and acid dissociation constant (pK_a). TPSA counts the surface sum over all polar atoms in molecule, and for most CNS drugs to be < 70 Å^2. LogD is a pH dependent lipophilicity indicator displaying the distribution of a chemical compound between the lipid and aqueous phase. For CNS drugs logD is expected to be <3. The strength of acidic functional groups in the molecule expressed by pKa values for CNS drugs should be 7–9. For streamlining the design of proper physicochemical properties of MTDLs for AD, several powerful predicting techniques, such as BBB-score and the CNS multiparameter optimization approach, have been developed. However, the most of MTDLs have been tested in a parallel artificial membrane permeation assay (PAMPA), which experimentally determine permeability values which would enable them to cross the BBB by passive diffusion [99][100][101][102][103]. The current chemical methods for crossing the BBB could be divided into chemical modification of the drug to form a prodrug, coupling the drugs with mannitol or aromatic substances or using appropriate chemical drug delivery system or drug carrier with the ability to cross BBB [104].

4. Perspectives for MTDLs in the Treatment of AD

At present, only symptomatic treatments exist for AD, namely 3 cholinesterase inhibitors and memantine, which mainly block the progression of the disease and are supposed to interfere with the pathogenic steps responsible for the clinical symptoms. AD is a complex disease, thus the rational way for searching for pathway-target-drug-disease relationships seemed to be a genome-based analysis [105][106]. Kwok et al. [107] used a gene-based test for the genetic validation of potential AD drugs, to provide an initial screening tool for the identification of drugs that are unlikely to be successful. This study, however, provides no evidence that any approved or investigational AD drugs target products of genes strongly associated with late-onset AD, which might explain the lack of efficacy to date.

MTDLs toward AD have to confirm their simultaneous multitarget engagement in both in vitro and in vivo assay systems. Thus, the use of cellular models (cell-screening systems) of AD, based on human induced pluripotent stem cell (hiPSCs) technologies could change preclinical research [108]. In addition, the animal models currently available cannot fully reflect the multifactorial nature of human AD for multiple ligand testing. Age-dependent neurodegeneration mouse models that mimic AD rely mostly on the neuronal overexpression of human proteins carrying a familial AD-causing mutation in presenilin, which increases Aβ42 production and oligomerization [109][110]. In fact, with respect to neurodegenerative diseases generally, mouse mutations corresponding to human

disease-linked mutations rarely result in neurodegenerative phenotypes, mostly because age is the single greatest risk factor for neurodegeneration, and mice have much shorter lifespans than humans.

Up to 2019, over 2000 clinical trials in AD drug development had been reported in which various hypotheses for AD were tested. Liu et al. [25] reported that the amyloid hypothesis was the most heavily tested (22.3% of trials), with the neurotransmitter hypothesis being the second most tested (19.0% of trials). In turn, a systematic review of the AD drug development pipeline in 2019, showed that there were 132 agents in clinical trials and 90 agents in trials targeting cognitive enhancement, and a further 14 trials intended to treat neuropsychiatric and behavioural aspects of AD [109]. Results suggested that there is a conceptual supremacy for disease-modifying therapies, as opposed to symptomatic-disease approaches [48]. From 96 agents reported in disease modification trials, 40% have amyloid as the primary target, or as one of several effects, whereas seven small molecules and 10 biologics have tau as a primary or combination, target (18%). Until recently, AD drug development has been largely focused on beta amyloid plaques and tau tangles in the brain. These attempts have yielded less successful results than had been anticipated. Currently, some of the most novel non-amyloid approaches were put to the discovery and development of drugs for Alzheimer's and related dementias. Firstly, repurposing an existing drug accelerates the drug development timeline. An example of this is the repurposing of a Parkinson's drug, rasagiline, that holds promise for slowing the progression of Alzheimer's disease in patients with mild cognitive impairment [110]. In addition, new drug candidates could restore lost cognitive function and lead to neuroprotective therapies for AD and other forms of dementia [111].

The diversification of targets and the entry of combination therapies into the potential therapeutic pipeline is particularly noticeable between phase 3 and 2 of clinical trials. The using of new biomarkers allow early assessments of the impact of candidate interventions on disease biology [109], however there is lack of accurate biomarkers to identify and track the progression of AD, which has slowed therapeutic development in tauopathies.

So far, a wide range of new biological targets are under consideration as microglial targets (Table 3). Microglia are a class of innate immune cells (macrophages) within the CNS. Microglia fulfil a number of varied roles within the CNS including the immune response, maintenance of homeostasis, extracellular signalling, phagocytosis, antigen presentation and synaptic pruning [112]. In AD, microglia reaction was initially thought to be incidental and triggered by Aβ deposits and dystrophic neurites. Moreover, recent genome-wide association studies have established that the majority of AD risk loci are found in or near genes that are highly and sometimes uniquely expressed in microglia [209]. On the other hand, a multitude of independent sources have suggested that neuroinflammation contributes to the pathogenesis of AD. Therefore, a hypothesis has been proposed that microglia could be critically involved in the early steps of AD and microglial targets are now being considered as the next important potential therapeutic targets

Table 3. Emerging microglial targets in AD based on [112][113][114][115][116][117].

Pathway.	Targets	Evidence
Purinergic signalling	-ionotropic P2XRs -metabotropic P2Y -adenosine P1Rs -P2YR-dependent calcium signaling	-P2 × 7R is up-regulated in AD -P2Y2R protective role -P2Y6R - small molecule from GliaCure claims to promote microglial phagocytosis through binding of the microglial purinergic P2Y6 receptor [118] -P2Y12 plays an important role in homeostatic microglia
NOD-like receptor family pyrin domain containing 3 (NLRP3)	-triggers TREM2 receptor expressed in myeloid cells 2	-NLRP3 inflammasome activation is a pathophysiological pathway in AD
Toll-like receptors (TLRs)	-TLR2 -TLR4	-triggers the neuroinflammatory response -downstream TLR signalling through NFκB, activator protein 1 and IFN regulatory factor (IRF) pathways lead to proinflammatory gene transcription
microglial fractalkine receptor	-CX3CL1/CX3CR1	-CX3CL1 exerts an inhibitory signal, maintaining microglia in a resting state
Receptor- interacting serine/threonine-protein kinase 1 (RIPK1)	-RIPK1 inhibitor	-enzyme downstream of TNFα signalling, has been shown to mediate microglial responses in AD

It is also worth mentioning the innovative nanotechnology-based approaches in the treatment of AD, an approach that is seen as a great hope for developing new treatment strategies. The achievements of nanotechnology are

particularly encouraging because they provide many benefits while overcoming the limitations of conventional formulations.

Recently, Chen et al. [119] reported the preparation of a methylene blue loaded multifunctional nanocomposite. The constructed nanocomposite has ultra-small ceria nanocrystals and iron oxide nanocrystals assembled onto the surface of mesoporous silica nanoparticles, methylene blue loaded into its pores and, additionally, a tau tracer, Amino-T807, grafted onto the surface. The adopted strategy allowed for the achievement of a promising synergistic effect as a result of ROS scavenging ability of ultra-small ceria nanocrystals, ameliorating mitochondrial oxidative stress-induced damage and use of a tau aggregation inhibitor, methylene blue, which could be released in neurons to prevent hyperphosphorylated tau aggregation. On the other hand, Guo et al. [120] designed and optimized a novel fusion peptide (TPL) comprising a BBB-penetrating peptide, TGN, and a neuron binding peptide, Tet1, through a four-glycine linker. Interestingly, TPL-modified nanoparticles led to an increase in BBB-penetration and neuron targeting efficacy, in comparison to the nanoparticles co-decorated with the two mono-ligands. Moreover, after the encapsulation of a neuroprotective peptide, NAP, the TPL nanoparticles reduced the intracellular ROS, showed protection of microtubules against Aβ25-35-induced toxicity, and rescued the OA-induced tau aggregation and neuronal apoptosis.

Another interesting study concerns the development of a polymeric micelle drug delivery system consisting of the RAGE targeting peptide (Ab) derived from Aβ protein, an amphiphilic polymer with ROS responsiveness and scavenging ability, and curcumin, a natural compound which has been described to target to Aβ aggregation [121]. Such a type of nanosystem has the ability to accumulate in the AD brain and initiate subsequent microglia-based microenvironment modulation. More importantly, it is possible that multiple targets within the AD microenvironment could be controlled to reeducate the hyperactive microglia and protect damaged neurons. The examples listed above confirm the need for further intensive studies to obtain better clinical translation of multifunctional nanosized drug delivery systems

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