Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-179921
IOS Press

Review

Multifactorial Hypothesis and Multi-Targets for Alzheimer's Disease

Cheng-Xin Gong*, Fei Liu and Khalid Iqbal
Department of Neurochemistry, Inge Grundke-Iqbal Research Floor, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA

Abstract. The amyloid cascade hypothesis has been dominating drug discovery for Alzheimer's disease (AD) for the last two decades. The failure of the development of effective drugs for slowing down or reversing the progression of AD warrants the AD field to consider out-of-the-box thinking and therapeutic approaches. We propose the multifactorial hypothesis of AD, emphasizing that AD is caused by multiple etiological factors, which may result in common brain pathology and functional consequences through several separate but integrated molecular pathways. More than one etiological factor and mechanistic pathway may be involved in a single individual with sporadic AD, and different individuals may have different etiological factors, involving different mechanisms/pathways. We urge the recognition of the multifactorial nature of AD and the paradigm shift of AD drug development from a single target to multiple targets, either with the multitarget-directed ligands approach or the cocktail therapy approach. We believe that patient stratification and the use of the precision medicine model will also benefit AD drug discovery.

Keywords: Alzheimer's disease, cocktail therapy, multifactorial hypothesis, multitarget-directed ligands, patient stratification, precision medicine model

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and is characterized by chronic, progressive neurodegeneration that leads to cognitive impairment and eventually to dementia. In familial, early onset AD, the disease is caused by certain mutations in the genes of presenilins or amyloid-β protein precursor (AβPP). Over 95% of AD cases are sporadic in nature and are not caused by any known gene mutations. Both familial and sporadic AD are characterized by two important brain lesions: aggregation of amyloid-β (Aβ) into amyloid plaques and of hyperphosphorylated microtubule-associated protein tau into neurofibrillary tangles. The presence of amyloid plaques, neurofibrillary tangles, and neuronal/synaptic loss in the brain are the characteristic histopathological hallmarks of AD.

The modern era of AD research at the molecular level began in 1980s. During the last three decades, many molecular pathways involved in or relevant to the mechanisms of AD have been learned. However, modern AD research has not yet led to the development of any drug that can slow down the progression of AD or cure the disease. Only one drug, memantine, was developed that is still symptomatic and has moderate efficacy in temporarily reducing symptoms for only moderate or severe AD [1].

The failure of developing good effective drugs for AD, despite enormous amounts of resources and effort invested in the last 2-3 decades, led the AD field to think seriously what we have done, where we

*Correspondence to: Cheng-Xin Gong, Department of Neurochemistry, Inge Grundke-Iqbal Research Floor, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA. Tel.: +1 718 494 5248; Fax: +1 718 698 7916; E-mail: Chengxin.gong@csi.cuny.edu.

stand now, and where we should head in AD research. This reconsideration is obviously seen in the last several years and has led many to doubt or even abandon the amyloid cascade hypothesis and shift their efforts to other possible mechanisms or targets, such as tau pathology, for their research and drug development [2–5]. At this transition time for AD research as well as the 110th year anniversary of the first publication of AD by Alois Alzheimer, it is especially timely and important to have a collection of ideas and opinions from AD experts, as organized by the *Journal of Alzheimer's Disease*, regarding the new beginnings of AD research.

The failure of AD clinical trials to date could result from many reasons, which have been discussed recently [6–8]. These reasons include the complex nature of the disease, limits of animal models for preclinical studies, inadequate designs of clinical trials, and many others. One important reason is probably the lack of appreciation and understanding of the multifactorial nature and mechanisms of the disease. To date, most AD clinical trials have been based on a single mechanism or pathway.

In addition to the dominant amyloid cascade hypothesis [9, 10], several other hypotheses have been proposed for the mechanisms of sporadic AD. These hypotheses include the cholinergic hypothesis [11, 12], tau hypothesis [13, 14], mitochondrial hypothesis [15, 16], oxidative stress hypothesis [17, 18], neuroinflammation hypothesis [19], brain insulin resistance hypothesis [20, 21], brain metabolic hypothesis [22–24], calcium hypothesis [25], innate immunity hypothesis [26, 27], and others. All these AD hypotheses are backed by substantial support from research data. This is actually not surprising because, as an age-associated neurodegenerative disease, many factors may initiate the development of AD and many molecular pathways may mediate the progression of the disease in the aged brain. However, a common problem of these hypotheses is that they intend to overemphasize the specific mechanism/pathway proposed and undervalue other mechanisms and heterogeneity. Such a narrow focus appears to attribute to the failure of AD drug development during the last decades.

Sporadic AD is caused by multiple etiological factors, which may result in common pathological brain damage and functional consequences through several separate but integrated molecular pathways. The multiple etiological factors and mechanistic pathways are likely involved in a single individual with sporadic AD, and different individuals may have different etiological factors and involve somewhat different mechanisms/pathways. This article discusses the multifactorial mechanism and multi-targets for AD.

THE MULTIFACTORIAL HYPOTHESIS OF AD

The development, growth, and maturation of a human body reaches its peak in the third decade of life. Human brain, as a special organ, may further mature for decades due to continuous learning and new experience. However, wearing and aging of the human brain starts at middle age. Normal aging is a constant balancing between physiological aging plus pathological risks/insults and the natural defense mechanisms (Fig. 1A). There are many risks and insults that occur and accumulate during aging, including genetic risks, epigenetic and metabolic factors, and environmental insults. The human body also responds to these factors/insults with its defense mechanisms, which could include general defense and those specific to individual insults. The balance between aging/insults and the defense mechanisms is dynamic and can shift within a certain range under physiological conditions. During normal aging, although the right side of the balance shown in Fig. 1A can be heavier as the accumulation of factors/insults, such as factor A to G, the balance tilts to the right side but still maintains within the normal range. However, as one or more of these factors/insults get heavier or new factors/insults (e.g., factor H, I, etc.) are added up, the imbalance eventually reaches the threshold and breaks the balance, i.e., initiation of the development of AD. These factors/insults collectively result in neurodegeneration, leading to cognitive impairment and eventually dementia, through individual molecular pathways (Fig. 1B). Some of these pathways involve in Aβ overproduction/aggregation and tau hyperphosphorylation/aggregation, leading the formation of amyloid plaques and neurofibrillary tangles as the two hallmark brain lesions of AD.

Our proposed multifactorial hypothesis can perfectly explain why aging is the most important risk factor for AD, as the defense mechanisms on the left side of the balance shown in Fig. 1A becomes weaker during aging. On the other side, healthy lifestyle, such as physical and intellectual exercises and healthy diet, can help the defense mechanisms and thus inhibit or delay the onset of the disease.

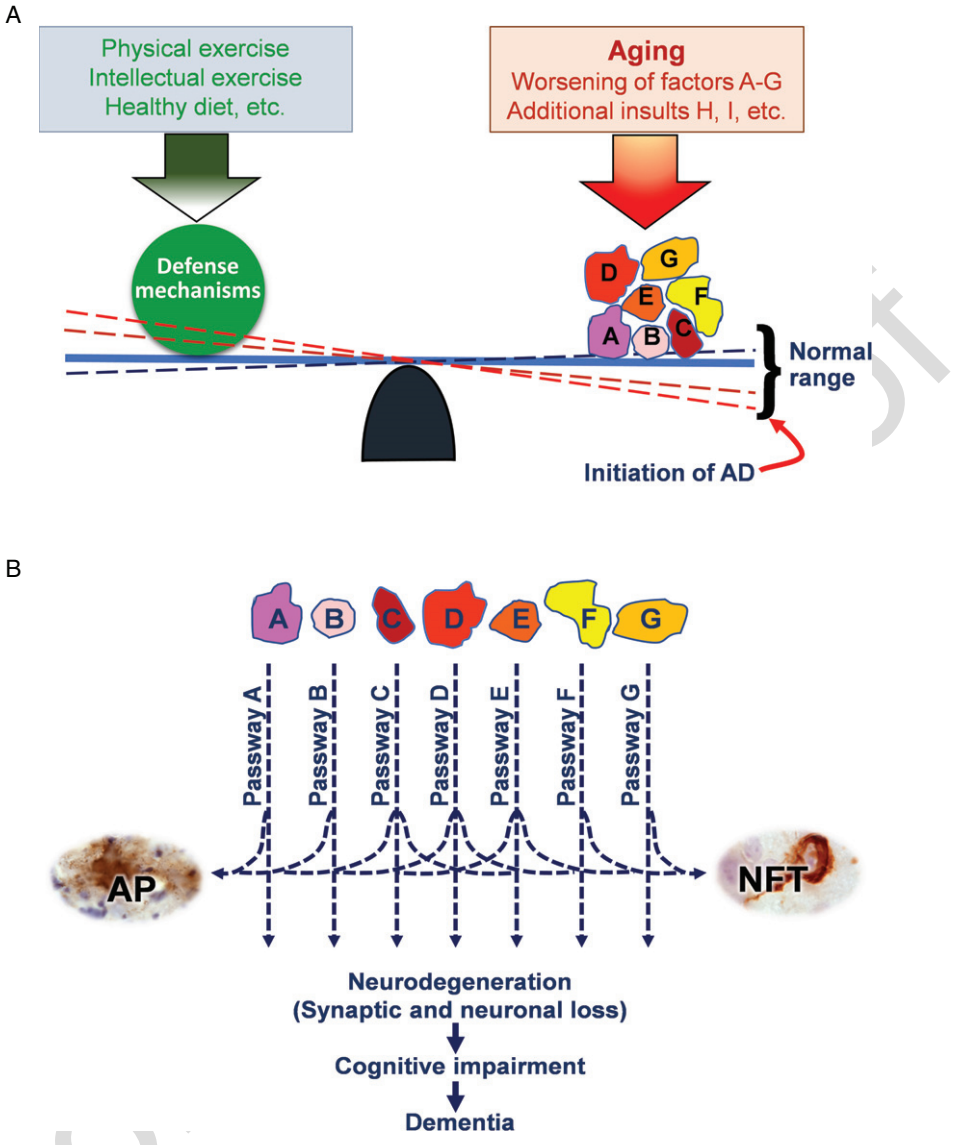

Fig. 1. The proposed multifactorial hypothesis of AD. A) The balance between the potential factors/insults accumulated during normal aging and the defense mechanisms. Worsening of these factors/insults (such as A to G) and/or adding of additional insults (such as H, I, etc.) can initiate the onset of AD. B) The multifactorial insults collectively cause neurodegeneration through multiple molecular mechanisms/pathways and consequently cognitive impairment and dementia. Some of these pathways also lead to the formation of amyloid plaques (AP) and neurofibrillary tangles (NFT), which are part of the end products of these pathways and also hallmark brain lesions of AD.

Multifactorial mechanisms of AD have been proposed previously, which state that more than one etiopathological factors and mechanisms are involved in the pathogenesis of AD [28–30]. However, the multifactorial hypothesis of AD that we proposed here is different from those proposed previously. Our hypothesis emphasizes two key concepts for the development of AD. First, we emphasize that the development and onset of sporadic AD result from the *collective* effects of multiple factors/insults that are not restricted to one or more specific insults. This emphasis warrants targeting more than one insults/pathways simultaneously for effective AD therapy. Second, we emphasize that each individual may have a different combination of etiological factors/insults that cause the onset of AD in this particular individual. This emphasis recognizes the diversity of etiological factors and molecular mechanisms among individual AD cases and justifies the stratification of AD patients and the use of precision

medicine concept for the treatment of AD, which will be discussed below.

The previously proposed hypotheses of AD, such as the amyloid cascade hypothesis, tau hypothesis, and neuroinflammation hypothesis, are all supported by more or less experimental and clinical evidence. Our proposed multifactorial hypothesis does not conflict with those hypotheses but include each of them as one factor/insult for the disease development. Instead, we believe that we need to consider all parts of the issues of AD simultaneously when designing and testing new AD therapeutics. One major problem with previously proposed hypotheses is to emphasize one pathway but overlook or even ignore all others. This problem, in our opinion, partially accounts for the failure of all AD drug development so far.

Each factor/insult of the right side of the balance of Fig. 1 may have different weights and contribute differently to the initiation and development of sporadic AD, and in different individuals the weight of each factor/insult may be different. Extensive research accumulated during the last three decades suggest many important factors/insults. They include aging, as well as genetic, epigenetic, metabolic, and environmental factors. Some of these factors/insults, such as mutations of presenilins and AβPP, appear to be so strong that they can lead to early onset familial AD without co-existing of other insults. However, in most AD cases, amyloid or tau pathology is insufficient to lead to sporadic AD, as these pathologies can also be seen in the brains of individuals without cognitive impairment.

STRATEGY TO TARGET THE MULTIFACTORIAL MECHANISM OF AD

An overview of the AD clinical trial data indicated that, over the last decade, more than 50 drug candidates have successfully passed phase II clinical trials, but none has passed phase III [31]. According to our proposed multifactorial AD hypothesis, it is not surprising that all the clinical trials targeting to a single pathway or mechanism critical to AD have failed so far. This is obvious because sporadic AD is caused by an imbalance of a *collective* action of several insults, as shown in Fig. 1. Inhibiting or removing only one of them is unlikely to be sufficient to restore the balance to the normal range. It is time for us to take a paradigm shift for AD drug development to a multi-targets approach on the basis of the multifactorial AD hypothesis (Fig. 2).

Fig. 2. Proposed strategies for AD drug development on the basis of the multifactorial hypothesis.

It is generally much more difficult to design a drug that can act at multiple targets. However, this is not impossible. A few groups in Europe and China have started the approach of multitarget-directed ligands (MTDL) for AD drug development [32–36]. The aim of MTDL design is to combine features that can interact with two or more of the desired targets. The MTDL molecules can be conceived to directly interact with multiple targets associated with AD by the molecular hybridization of different pharmacophore moieties from already identified bioactive molecules [37, 38]. Each pharmacophore of the new hybrid drug can preserve the capacity of interacting with their specific sites on the targets and thus generate multiple specific pharmacological responses, which would enable the treatment of multifactorial AD. The development of MTDLs can prevent the challenge of simultaneously administering multiple drugs with potentially different degrees of bioavailability, pharmacokinetics, and metabolism. Thus, this pharmacological approach can also provide patients with a simplification of the therapeutic regimen.

Another approach, which is probably more practical, is to select substances of multiple actions against various insults/mechanisms involved in AD from nature sources. There are several natural compounds, such as isaindigotone, chelerythrine, chalcone, coumarin, huprine, curcumin, rhein, berberine, and resveratrol derivatives, that deserve investigation for AD drug development. This approach may indicate new directions for the development of new anti-AD drugs.

The third strategy is to consider simultaneous treatments with more than one drugs targeting various insults/mechanisms according to the multifactorial AD hypothesis. Such an approach had been used effectively in chemotherapy and in fighting against HIV/AIDS as the cocktail therapy. The cocktail therapy is proved to be essential to such an infective disease with a clear single cause, infection with the HIV virus. It actually makes more sense to employ such an approach for fighting

against AD, a disease with multiple etiologies and mechanisms.

Another important strategy for AD drug discovery is to stratify AD patients based on their likely factors/insults and test AD drug candidates in the stratified population of AD patients. Because the sporadic AD can be caused by a combination of various etiological factors and different molecular mechanisms/pathways may dominate in different populations, AD can be categorized into different subgroups, and different subgroups likely represent different etiopathogenic mechanisms and possibly also somewhat different clinical profiles. On the basis of the levels of tau, ubiquitin, and $A\beta_{1-42}$ in the cerebrospinal fluid, we were able to stratify AD patients into at least five subgroups [39]. Importantly, each of these five subgroups presented a different clinical profile. A recent study demonstrated structural variation in Aβ fibrils from AD clinical subtypes [40], suggesting some molecular and structural basis for AD subgroups. Therefore, testing a specific drug candidate in the stratified subgroup of AD patients, rather than the mixed populations of all AD patients, will certainly increase the likelihood of success in clinical trials. With the latest advances of brain imaging techniques and AD biomarkers, stratification of AD cases is now already feasible and will soon become more practical.

Precision medicine is a new medical model that proposes the customization of medical treatment and care to the individual patients. This model has been used successful for treating certain cancers [41]. In light of the unsuccessful investment of vast amount of effort and resources for AD drug discovery in the last two decades, it is time to make a paradigm shift and consider the precision medicine model for AD drug discovery and for future management of AD patients. Our knowledge of the disease-causing mutations of *PSEN1, PSEN2*, and *APP* for familial AD and of *ApoE* alleles and polymorphisms of some genes, such as *TREM2*, as risk factors for sporadic AD already make the use of precision medicine model for treating AD possible. Brain imaging and biomarker data can add additional values for customization of individual AD patients.

MULTI-TARGETS FOR TREATING AD: CURRENT STATUS

The multifactorial nature of AD means that there are many potential therapeutic targets. Targeting these targets individually with current drugs has been ineffective for AD in clinical trials. A possible answer lies in a polypharmacological approach to modify activities of several of these targets simultaneously, especially those associated with the pathogenesis of the disease. The main therapeutic targets currently under investigation for treating AD include key proteins (Aβ and tau) and their processing, receptors (cholinergic, glutamatergic, serotoninergic, dopaminergic, noradrenergic, histaminergic), enzymes (cholinesterase [ChE], α-, β- and γ-secretase, monoamine oxidases [MAO], O-GlcNAcase), and pathways/processes (insulin signaling, excitotoxicity, neuroinflammation, oxidative stress, neurogenesis, calcium and metal homeostasis, endoplasmic reticulum, and mitochondrial damage), all of which have been shown to be involved in the pathogenesis of AD.

Initial efforts on the multi-target strategy for treating AD are mainly focused on the development of compounds that have ChE inhibitor activity (tacrine- and donepezil-related derivatives) plus one or more properties of anti-Aβ aggregation, β-secretase inhibition, promotion of non-amyloidogenic cleavage of AβPP, MAO inhibition, neuroprotection, anti-oxidation, metal-chelating, NMDA (N-Methyl-D-aspartate) antagonist, nitric oxide-releasing, anti-inflammatory, tau hyperphosphorylation inhibition, and binding to serotonin receptors or opioid sigma 1 receptors. Tacrine is among the most popular pharmacophores used for the design of MTDLs since it is very active cholinesterase inhibitor. There is also a number of hybrid compounds containing fragments of donepezil, galantamine, or memantine. The pharmacologies and initial evaluations of these compounds have been recently reviewed by Guzior et al. [42] and Ismaili et al. [43] and thus are not discussed here in detail.

Examples of these hybrid compounds under investigation comprise the dual binding site of ChE inhibitors with additional properties such as anti-Aβ aggregating activity [44, 45], neuroprotective and antioxidant activity [46, 47], calcium channel blocking [48, 49], cannabinoid CB1 receptor antagonism [50], BACE-1 inhibition [51, 52], histamine H3 receptor antagonism [53], NMDA receptor channel blocking [54], serotonin 5-HT3 receptor antagonism [55], or serotonin transporter inhibition [56]. Other examples of dual-acting ligands are MAO-B inhibitors with iron-chelating agents [57], metal chelators with BACE-1 inhibitors [58], metal chelators with antioxidants [59], and modulators of

γ-secretase with PPARγ activities [60]. Most of these multifunctional ligands have been shown to display biological activity *in vitro* and require verification in animal models. However, several compounds like bis(7)-tacrine [61], ladostigil [62, 63] and memoquin [64] showed promising activity *in vivo* and in preclinical or even clinical studies.

Several groups have synthesized and assessed compounds bearing the N-benzylpiperidine group present in donepezil and the N-propargylamine motif present in PF9601N, a potent and selective MAO-B inhibitor with neuroprotective activities *in vitro* and *in vivo* [34]. Both scaffolds were linked by different heterocyclic ring systems, such as pyridine, indole or 8-hydroxyquinoline, allowing facile synthesis of different MTDL molecules for AD therapy. In addition to inhibiting ChE and MAO, some of these new MTDL molecules also have antioxidant, anti-Aβ-aggregating, anti-inflammatory, anti-apoptotic, and metal-chelating properties. Preclinical studies suggest that these MTDL compounds can target the multiple pathways involved in the pathogenesis of AD and thus represent a potential improvement of the current pharmacological therapy of AD. One example of MTDL model that progressed to clinical trials against AD is ladostigil, designed to inhibit MAO and ChE but also incorporating potent anti-apoptotic and neuroprotective activities [63]. The MTDL attempt combining activities of MAO and ChE has been reviewed recently [65].

The use of the well-known AD drugs donepezil, tacrine, or rivastigmine [47, 66] and bioactive natural products such as curcumin [67], berberine [68, 69], or 8-hydroxyquinoline [70]; as structural scaffolds for the development and search of new chemical entities with multiple properties for the treatment of AD has been investigated. These new hybrid compounds should be considered as simplified versions or lead drugs possessing potential as real alternatives to the current unsuccessful drugs for treating AD.

Another approach for the multi-target AD drug development is repurposing, i.e., the development of existing or abandoned drugs for new indications, related to the original purpose or after off-target effects are identified by data mining. Repurposing can reduce the time to launch, cost of development, and the uncertainty associated with safety and pharmacokinetics. Data mining is a way of using pre-existing knowledge about molecules and applying it to develop new drugs [71]. The most promising drug currently being investigated for repurposing is rasagiline, a selective, irreversible MAO-B inhibitor for the treatment of Parkinson's disease. The repurposing for AD was due to its ability to regulate the non-amyloidogenic processing of AβPP [72]. Rasagiline also has a neuroprotective activity due to the propargylamine moiety that activates Bcl-2 and downregulates the Bax proteins [73]. One phase II trial of rasagiline sponsored by Teva Pharmaceutical Industries was completed without publication of the results, and another phase II trial sponsored by the Cleveland Clinic is undergoing (https://www.clinicaltrials.gov/ct/show/NCT02359552).

Another example of repurposing for AD treatment is anti-diabetic drugs. Diabetes is a known risk factor of AD, and brain insulin signaling is deregulated in AD [74, 75]. Our preclinical studies using AD mouse models indicate that several anti-diabetic drugs, including insulin sensitizers and intranasal insulin, are promising for reduction of AD-like brain pathologies and cognitive impairment [76, 77]. Studies on the repurposing of anti-diabetic drugs for the treatment of AD was reviewed recently in detail [78]. A phase II clinical trial of intranasal insulin administration in amnestic mild cognitive impairment (MCI) and mild to moderate AD showed improved delayed memory and preserved caregiver-rated functional ability and general cognition [79]. Long-acting intranasal insulin detemir also improves cognition for adults with MCI or early-stage AD [80]. A recent randomized, double-blind, placebo-controlled phase II trial also found that the treatment of MCI or mild to moderate AD patients with daily intranasal regular insulin for two to four months improved memory associated with preserved brain volume on MRI and reduction in the tau-P181/Aβ_{42} ratio [81]. Three GLP-1 (glucagon-like peptide 1) analogs, which are used for treating diabetes, have shown *in vivo* benefits in mouse AD models [82] and potential therapeutic value in AD [83]. Liraglutide is a GLP-1 receptor agonist that can cross the blood-brain barrier [84], ameliorate AD-associated brain pathologies and improve learning and memory in animal models [85–87]. This anti-diabetic drug prevented the decline of brain glucose metabolism, synaptic dysfunction, and disease evolution of AD in a 6-month small clinical trial [88]. A multicenter randomized double-blind placebo-controlled phase IIb clinical trial for AD is currently undergoing (https://www.clinicaltrials.gov/ct/show/NCT01469351). The GLP-1 analog Exendin-4 was also evaluated in a Phase II clinical trial (see https://www.clinicaltrials.gov/ct/show/NCT01255163), but the results has not been published at the time of preparation of this article.

A few clinical trials have started to evaluate drug candidates in stratified AD patients with benefits. For example, a small clinical trial testing intranasal insulin in MCI and AD patients found that the treatment facilitated recall on two measures of verbal memory in memory-impaired ApoE4 carriers [89]. Insulin also differentially modulated plasma Aβ according to ApoE genotype. Another trial testing the long-acting intranasal insulin, detemir, in MCI and AD cases found that the treatment enhanced memory for ApoE4 carriers but worsened it for non-carriers [80]. In a recent prevention trial for MCI and dementia, the subjects were stratified into four cohorts on the basis of age, ApoE genotype, sex, education, family history of dementia, vascular risk, subjective memory concerns, and baseline cognitive performance [90].

CONCLUSIONS AND PERSPECTIVES

To date, most pharmacological research is driven to discover highly selective drugs. This strategy has failed to develop any drugs that can slow down or stop the progression of AD. The recognition of the multifactorial nature of AD warrants a paradigm shift of AD drug development from a single target into multiple targets, either with the MTDL approach or the cocktail approach. The therapeutic potential of multi-targets for the treatment of complex neurodegenerative diseases like AD must be recognized. Patient stratification and the use of precision medicine model will certainly benefit both single and multi-targets AD drug discovery. While there are many potential targets for disease-modifying drugs, it is important to prioritize and test which combinations will work. It seems logical that the pathways involved in synaptic and neuronal loss, rather than the deficiencies caused by cell death or AD lesions, must be targeted in order to slow down or reverse the disease progression. Of course, targeting a combination of both would theoretically relieve symptoms and prevent further neuronal loss. Therefore, combination of pharmacophores interacting with both symptomatic and disease-modifying targets is highly justified for the initial research of MTDLs for AD.

Co-administration of several drugs is an alternative approach to treat multifactorial diseases like AD. It could be a more useful therapeutic option than designed multiple ligands. This approach might even have to be employed for AD drug clinical trials, since all single AD drug clinical trials have failed to date.

The completion of human genome study and recent advances of brain imaging and biomarkers have made the stratification of AD patients for both clinical trials and future treatments not only possible but also practical. Computational and mathematical models based on individual genomic, epigenomic, neuroimaging, and biomarker data can optimize the stratification of AD patients for better therapeutic outcomes. These models can also serve for the precision medicine model for individual AD patients for customized medical treatment.

An international Alzheimer's Precision Medicine Initiative (APMI) was recently established through a collaboration of leading interdisciplinary clinicians and scientists devoted to the implementation of precision medicine model for fighting against AD [91, 92]. The successful implementation of this model in AD will likely result in breakthrough therapies with optimized safety profiles, better responder rates and treatment responses.

Development of an effective drug for treating AD is clearly very challenging. Our experience indicates that there is no simple way of searching for AD therapy. The recognition of the multifactorial hypothesis of AD and the consideration of using the multi-targets approach gives hope for developing new and effective therapy for AD.

ACKNOWLEDGMENTS

We are grateful to Ms. G. Ramirez for secretarial assistance and GRAMS of our institute for preparation of the figure. The authors work for the New York State Office for People with Developmental Disabilities.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/17-9921).

REFERENCES

[1] Cummings JL (2004) Treatment of Alzheimer's disease: Current and future therapeutic approaches. *Rev Neurol Dis* **1**, 60-69.

[2] Giacobini E, Gold G (2013) Alzheimer disease therapy–moving from amyloid-beta to tau. *Nat Rev Neurol* **9**, 677-686.

[3] Iqbal K, Liu F, Gong CX (2014) Alzheimer disease therapeutics: Focus on the disease and not just plaques and tangles. *Biochem Pharmacol* **88**, 631-639.

[4] Moreno-Trevino MG, Castillo-Lopez J, Meester I (2015) Moving away from amyloid Beta to move on in Alzheimer research. *Front Aging Neurosci* **7**, 2.

[5] Harrison JR, Owen MJ (2016) Alzheimer's disease: The amyloid hypothesis on trial. *Br J Psychiatry* **208**, 1-3.
[6] Knopman DS (2008) Clinical trial design issues in mild to moderate Alzheimer disease. *Cogn Behav Neurol* **21**, 197-201.
[7] Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B (2015) Prevention of sporadic Alzheimer's disease: Lessons learned from clinical trials and future directions. *Lancet Neurol* **14**, 926-944.
[8] McGhee DJ, Ritchie CW, Zajicek JP, Counsell CE (2016) A review of clinical trial designs used to detect a disease-modifying effect of drug therapy in Alzheimer's disease and Parkinson's disease. *BMC Neurol* **16**, 92.
[9] Hardy JA, Higgins GA (1992) Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256**, 184-185.
[10] Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* **8**, 595-608.
[11] Perry EK, Perry RH, Blessed G, Tomlinson BE (1977) Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* **1**, 189.
[12] Bartus RT, Dean 3rd RL, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408-414.
[13] Iqbal K, Grundke-Iqbal I (1996) Molecular mechanism of Alzheimer's neurofibrillary degeneration and therapeutic intervention. *Ann N Y Acad Sci* **777**, 132-138.
[14] Iqbal K, Liu F, Gong CX (2016) Tau and neurodegenerative disease: The story so far. *Nat Rev Neurol* **12**, 15-27.
[15] Swerdlow RH, Burns JM, Khan SM (2010) The Alzheimer's disease mitochondrial cascade hypothesis. *J Alzheimers Dis* **20**(Suppl 2), S265-279.
[16] Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G (2010) Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochim Biophys Acta* **1802**, 2-10.
[17] Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science* **262**, 689-695.
[18] Zhu X, Raina AK, Perry G, Smith MA (2004) Alzheimer's disease: The two-hit hypothesis. *Lancet Neurol* **3**, 219-226.
[19] McGeer PL, Rogers J, McGeer EG (1994) Neuroimmune mechanisms in Alzheimer disease pathogenesis. *Alzheimer Dis Assoc Disord* **8**, 149-158.
[20] de la Monte SM (2009) Insulin resistance and Alzheimer's disease. *BMB Rep* **42**, 475-481.
[21] Deng Y, Li B, Liu Y, Iqbal K, Grundke-Iqbal I, Gong CX (2009) Dysregulation of insulin signaling, glucose transporters, O-GlcNAcylation, and phosphorylation of tau and neurofilaments in the brain: Implication for Alzheimer's disease. *Am J Pathol* **175**, 2089-2098.
[22] Hoyer S (2000) Brain glucose and energy metabolism abnormalities in sporadic Alzheimer disease. Causes and consequences: An update. *Exp Gerontol* **35**, 1363-1372.
[23] Iqbal K, Grundke-Iqbal I (2005) Metabolic/signal transduction hypothesis of Alzheimer's disease and other tauopathies. *Acta Neuropathol* **109**, 25-31.
[24] Gong CX, Liu F, Iqbal K (2016) O-GlcNAcylation: A regulator of tau pathology and neurodegeneration. *Alzheimers Dement* **12**, 1078-1089.
[25] Khachaturian ZS (1994) Calcium hypothesis of Alzheimer's disease and brain aging. *Ann N Y Acad Sci* **747**, 1-11.
[26] Guillot-Sestier MV, Doty KR, Town T (2015) Innate Immunity Fights Alzheimer's Disease. *Trends Neurosci* **38**, 674-681.
[27] Sims R, van der Lee SJ, Naj AC, Bellenguez C, Badarinarayan N, Jakobsdottir J, Kunkle BW, Boland A, Raybould R, Bis JC, Martin ER, Grenier-Boley B, Heilmann-Heimbach S, Chouraki V, Kuzma AB, Sleegers K, Vronskaya M, Ruiz A, Graham RR, Olaso R, Hoffmann P, Grove ML, Vardarajan BN, Hiltunen M, Nothen MM, White CC, Hamilton-Nelson KL, Epelbaum J, Maier W, Choi SH, Beecham GW, Dulary C, Herms S, Smith AV, Funk CC, Derbois C, Forstner AJ, Ahmad S, Li H, Bacq D, Harold D, Satizabal CL, Valladares O, Squassina A, Thomas R, Brody JA, Qu L, Sanchez-Juan P, Morgan T, Wolters FJ, Zhao Y, Garcia FS, Denning N, Fornage M, Malamon J, Naranjo MCD, Majounie E, Mosley TH, Dombroski B, Wallon D, Lupton MK, Dupuis J, Whitehead P, Fratiglioni L, Medway C, Jian X, Mukherjee S, Keller L, Brown K, Lin H, Cantwell LB, Panza F, McGuinness B, Moreno-Grau S, Burgess JD, Solfrizzi V, Proitsi P, Adams HH, Allen M, Seripa D, Pastor P, Cupples LA, Price ND, Hannequin D, Frank-Garcia A, Levy D, Chakrabarty P, Caffarra P, Giegling I, Beiser AS, Giedraitis V, Hampel H, Garcia ME, Wang X, Lannfelt L, Mecocci P, Eiriksdottir G, Crane PK, Pasquier F, Boccardi V, Henandez I, Barber RC, Scherer M, Tarraga L, Adams PM, Leber M, Chen Y, Albert MS, Riedel-Heller S, Emilsson V, Beekly D, Braae A, Schmidt R, Blacker D, Masullo C, Schmidt H, Doody RS, Spalletta G Jr WTL, Fairchild TJ, Bossu P, Lopez OL, Frosch MP, Sacchinelli E, Ghetti B, Yang Q, Huebinger RM, Jessen F, Li S, Kamboh MI, Morris J, Sotolongo-Grau O, Katz MJ, Corcoran C, Dunstan M, Braddel A, Thomas C, Meggy A, Marshall R, Gerrish A, Chapman J, Aguilar M, Taylor S, Hill M, Fairen MD, Hodges A, Vellas B, Soininen H, Kloszewska I, Daniilidou M, Uphill J, Patel Y, Hughes JT, Lord J, Turton J, Hartmann AM, Cecchetti R, Fenoglio C, Serpente M, Arcaro M, Caltagirone C, Orfei MD, Ciaramella A, Pichler S, Mayhaus M, Gu W, Lleo A, Fortea J, Blesa R, Barber IS, Brookes K, Cupidi C, Maletta RG, Carrell D, Sorbi S, Moebus S, Urbano M, Pilotto A, Kornhuber J, Bosco P, Todd S, Craig D, Johnston J, Gill M, Lawlor B, Lynch A, Fox NC, Hardy J, Consortium A, Albin RL, Apostolova LG, Arnold SE, Asthana S, Atwood CS, Baldwin CT, Barnes LL, Barral S, Beach TG, Becker JT, Bigio EH, Bird TD, Boeve BF, Bowen JD, Boxer A, Burke JR, Burns JM, Buxbaum JD, Cairns NJ, Cao C, Carlson CS, Carlsson CM, Carney RM, Carrasquillo MM, Carroll SL, Diaz CC, Chui HC, Clark DG, Cribbs DH, Crocco EA, DeCarli C, Dick M, Duara R, Evans DA, Faber KM, Fallon KB, Fardo DW, Farlow MR, Ferris S, Foroud TM, Galasko DR, Gearing M, Geschwind DH, Gilbert JR, Graff-Radford NR, Green RC, Growdon JH, Hamilton RL, Harrell LE, Honig LS, Huentelman MJ, Hulette CM, Hyman BT, Jarvik GP, Abner E, Jin LW, Jun G, Karydas A, Kaye JA, Kim R, Kowall NW, Kramer JH, LaFerla FM, Lah JJ, Leverenz JB, Levey AI, Li G, Lieberman AP, Lunetta KL, Lyketsos CG, Marson DC, Martiniuk F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Morris JC, Murrell JR, Myers AJ, O'Bryant S, Olichney JM, Pankratz VS, Parisi JE, Paulson HL, Perry W, Peskind E, Pierce A, Poon WW, Potter H, Quinn JF, Raj A, Raskind M, Reisberg B, Reitz C, Ringman JM, Roberson ED, Rogaeva E, Rosen HJ, Rosenberg RN, Sager MA, Saykin AJ, Schneider JA, Schneider LS, Seeley WW, Smith AG, Sonnen JA, Spina S, Stern RA, Swerdlow RH, Tanzi RE, Thornton-Wells TA, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Van Eldik LJ, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer

KA, Wilhelmsen KC, Williamson J, Wingo TS, Woltjer RL, Wright CB, Yu CE, Yu L, Garzia F, Golamaully F, Septier G, Engelborghs S, Vandenberghe R, De Deyn PP, Fernadez CM, Benito YA, Thonberg H, Forsell C, Lilius L, Kinhult-Stahlbom A, Kilander L, Brundin R, Concari L, Helisalmi S, Koivisto AM, Haapasalo A, Dermecourt V, Fievet N, Hanon O, Dufouil C, Brice A, Ritchie K, Dubois B, Himali JJ, Keene CD, Tschanz J, Fitzpatrick AL, Kukull WA, Norton M, Aspelund T, Larson EB, Munger R, Rotter JI, Lipton RB, Bullido MJ, Hofman A, Montine TJ, Coto E, Boerwinkle E, Petersen RC, Alvarez V, Rivadeneira F, Reiman EM, Gallo M, O'Donnell CJ, Reisch JS, Bruni AC, Royall DR, Dichgans M, Sano M, Galimberti D, St George-Hyslop P, Scarpini E, Tsuang DW, Mancuso M, Bonuccelli U, Winslow AR, Daniele A, Wu CK, Gerad/Perades CAE, Peters O, Nacmias B, Riemenschneider M, Heun R, Brayne C, Rubinsztein DC, Bras J, Guerreiro R, Al-Chalabi A, Shaw CE, Collinge J, Mann D, Tsolaki M, Clarimon J, Sussams R, Lovestone S, O'Donovan MC, Owen MJ, Behrens TW, Mead S, Goate AM, Uitterlinden AG, Holmes C, Cruchaga C, Ingelsson M, Bennett DA, Powell J, Golde TE, Graff C, De Jager PL, Morgan K, Ertekin-Taner N, Combarros O, Psaty BM, Passmore P, Younkin SG, Berr C, Gudnason V, Rujescu D, Dickson DW, Dartigues JF, DeStefano AL, Ortega-Cubero S, Hakonarson H, Campion D, Boada M, Kauwe JK, Farrer LA, Van Broeckhoven C, Ikram MA, Jones L, Haines JL, Tzourio C, Launer LJ, Escott-Price V, Mayeux R, Deleuze JF, Amin N, Holmans PA, Pericak-Vance MA, Amouyel P, van Duijn CM, Ramirez A, Wang LS, Lambert JC, Seshadri S, Williams J, Schellenberg GD (2017) Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. *Nat Genet* **49**, 1373-1384.

[28] Iqbal K, Wang X, Blanchard J, Liu F, Gong CX, Grundke-Iqbal I (2010) Alzheimer's disease neurofibrillary degeneration: Pivotal and multifactorial. *Biochem Soc Trans* **38**, 962-966.

[29] Calza L, Baldassarro VA, Giuliani A, Lorenzini L, Fernandez M, Mangano C, Sivilia S, Alessandri M, Gusciglio M, Torricella R, Giardino L (2013) From the multifactorial nature of Alzheimer's disease to multitarget therapy: The contribution of the translational approach. *Curr Top Med Chem* **13**, 1843-1852.

[30] Nixon RA (2017) Amyloid precursor protein and endosomal-lysosomal dysfunction in Alzheimer's disease: Inseparable partners in a multifactorial disease. *FASEB J* **31**, 2729-2743.

[31] Bachurin SO, Bovina EV, Ustyugov AA (2017) Drugs in clinical trials for Alzheimer's disease: The major trends. *Med Res Rev* **37**, 1186-1225.

[32] Leon R, Garcia AG, Marco-Contelles J (2013) Recent advances in the multitarget-directed ligands approach for the treatment of Alzheimer's disease. *Med Res Rev* **33**, 139-189.

[33] Rochais C, Lecoutey C, Gaven F, Giannoni P, Hamidouche K, Hedou D, Dubost E, Genest D, Yahiaoui S, Freret T, Bouet V, Dauphin F, Sopkova de Oliveira Santos J, Ballandonne C, Corvaisier S, Malzert-Freon A, Legay R, Boulouard M, Claeysen S, Dallemagne P (2015) Novel multitarget-directed ligands (MTDLs) with acetylcholinesterase (AChE) inhibitory and serotonergic subtype 4 receptor (5-HT4R) agonist activities as potential agents against Alzheimer's disease: The design of donecopride. *J Med Chem* **58**, 3172-3187.

[34] Unzeta M, Esteban G, Bolea I, Fogel WA, Ramsay RR, Youdim MB, Tipton KF, Marco-Contelles J (2016) Multi-target directed donepezil-like ligands for Alzheimer's disease. *Front Neurosci* **10**, 205.

[35] Yahiaoui S, Hamidouche K, Ballandonne C, Davis A, de Oliveira Santos JS, Freret T, Boulouard M, Rochais C, Dallemagne P (2016) Design, synthesis, and pharmacological evaluation of multitarget-directed ligands with both serotonergic subtype 4 receptor (5-HT4R) partial agonist and 5-HT6R antagonist activities, as potential treatment of Alzheimer's disease. *Eur J Med Chem* **121**, 283-293.

[36] Yan J, Hu J, Liu A, He L, Li X, Wei H (2017) Design, synthesis, and evaluation of multitarget-directed ligands against Alzheimer's disease based on the fusion of donepezil and curcumin. *Bioorg Med Chem* **25**, 2946-2955.

[37] Wang Z, Wang Y, Wang B, Li W, Huang L, Li X (2015) Design, synthesis, and evaluation of orally available clioquinol-moracin M hybrids as multitarget-directed ligands for cognitive improvement in a rat model of neurodegeneration in Alzheimer's disease. *J Med Chem* **58**, 8616-8637.

[38] Zha X, Lamba D, Zhang L, Lou Y, Xu C, Kang D, Chen L, Xu Y, Zhang L, De Simone A, Samez S, Pesaresi A, Stojan J, Lopez MG, Egea J, Andrisano V, Bartolini M (2016) Novel tacrine-benzofuran hybrids as potent multitarget-directed ligands for the treatment of Alzheimer's disease: Design, synthesis, biological evaluation, and X-ray crystallography. *J Med Chem* **59**, 114-131.

[39] Iqbal K, Flory M, Khatoon S, Soininen H, Pirttila T, Lehtovirta M, Alafuzoff I, Blennow K, Andreasen N, Vanmechelen E, Grundke-Iqbal I (2005) Subgroups of Alzheimer's disease based on cerebrospinal fluid molecular markers. *Ann Neurol* **58**, 748-757.

[40] Qiang W, Yau WM, Lu JX, Collinge J, Tycko R (2017) Structural variation in amyloid-beta fibrils from Alzheimer's disease clinical subtypes. *Nature* **541**, 217-221.

[41] Shrager J, Tenenbaum JM (2014) Rapid learning for precision oncology. *Nat Rev Clin Oncol* **11**, 109-118.

[42] Guzior N, Wieckowska A, Panek D, Malawska B (2015) Recent development of multifunctional agents as potential drug candidates for the treatment of Alzheimer's disease. *Curr Med Chem* **22**, 373-404.

[43] Ismaili L, Refouvelet B, Benchekroun M, Brogi S, Brindisi M, Gemma S, Campiani G, Filipic S, Agbaba D, Esteban G, Unzeta M, Nikolic K, Butini S, Marco-Contelles J (2017) Multitarget compounds bearing tacrine- and donepezil-like structural and functional motifs for the potential treatment of Alzheimer's disease. *Prog Neurobiol* **151**, 4-34.

[44] Tumiatti V, Minarini A, Bolognesi ML, Milelli A, Rosini M, Melchiorre C (2010) Tacrine derivatives and Alzheimer's disease. *Curr Med Chem* **17**, 1825-1838.

[45] Viayna E, Sabate R, Munoz-Torrero D (2013) Dual inhibitors of beta-amyloid aggregation and acetylcholinesterase as multi-target anti-Alzheimer drug candidates. *Curr Top Med Chem* **13**, 1820-1842.

[46] Fernandez-Bachiller MI, Perez C, Campillo NE, Paez JA, Gonzalez-Munoz GC, Usan P, Garcia-Palomero E, Lopez MG, Villarroya M, Garcia AG, Martinez A, Rodriguez-Franco MI (2009) Tacrine-melatonin hybrids as multifunctional agents for Alzheimer's disease, with cholinergic, antioxidant, and neuroprotective properties. *ChemMedChem* **4**, 828-841.

[47] Samadi A, Valderas C, de los Rios C, Bastida A, Chioua M, Gonzalez-Lafuente L, Colmena I, Gandia L, Romero A, Del Barrio L, Martin-de-Saavedra MD, Lopez MG, Villarroya M, Marco-Contelles J (2011) Cholinergic and neuroprotective drugs for the treatment of Alzheimer and neuronal

vascular diseases. II. Synthesis, biological assessment, and molecular modelling of new tacrine analogues from highly substituted 2-aminopyridine-3-carbonitriles. *Bioorg Med Chem* **19**, 122-133.

[48] Leon R, de los Rios C, Marco-Contelles J, Huertas O, Barril X, Luque FJ, Lopez MG, Garcia AG, Villarroya M (2008) New tacrine-dihydropyridine hybrids that inhibit acetylcholinesterase, calcium entry, and exhibit neuroprotection properties. *Bioorg Med Chem* **16**, 7759-7769.

[49] Tomassoli I, Ismaili L, Pudlo M, de Los Rios C, Soriano E, Colmena I, Gandia L, Rivas L, Samadi A, Marco-Contelles J, Refouvelet B (2011) Synthesis, biological assessment and molecular modeling of new dihydroquinoline-3-carboxamides and dihydroquinoline-3-carbohydrazide derivatives as cholinesterase inhibitors, and Ca channel antagonists. *Eur J Med Chem* **46**, 1-10.

[50] Lange JH, Coolen HK, van der Neut MA, Borst AJ, Stork B, Verveer PC, Kruse CG (2010) Design, synthesis, biological properties, and molecular modeling investigations of novel tacrine derivatives with a combination of acetylcholinesterase inhibition and cannabinoid CB1 receptor antagonism. *J Med Chem* **53**, 1338-1346.

[51] Piazzi L, Cavalli A, Colizzi F, Belluti F, Bartolini M, Mancini F, Recanatini M, Andrisano V, Rampa A (2008) Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds. *Bioorg Med Chem Lett* **18**, 423-426.

[52] Zhu Y, Xiao K, Ma L, Xiong B, Fu Y, Yu H, Wang W, Wang X, Hu D, Peng H, Li J, Gong Q, Chai Q, Tang X, Zhang H, Li J, Shen J (2009) Design, synthesis and biological evaluation of novel dual inhibitors of acetylcholinesterase and beta-secretase. *Bioorg Med Chem* **17**, 1600-1613.

[53] Incerti M, Flammini L, Saccani F, Morini G, Comini M, Coruzzi M, Barocelli E, Ballabeni V, Bertoni S (2010) Dual-acting drugs: An *in vitro* study of nonimidazole histamine H3 receptor antagonists combining anticholinesterase activity. *ChemMedChem* **5**, 1143-1149.

[54] Rook Y, Schmidtke KU, Gaube F, Schepmann D, Wunsch B, Heilmann J, Lehmann J, Winckler T (2010) Bivalent beta-carbolines as potential multitarget anti-Alzheimer agents. *J Med Chem* **53**, 3611-3617.

[55] Cappelli A, Gallelli A, Manini M, Anzini M, Mennuni L, Makovec F, Menziani MC, Alcaro S, Ortuso F, Vomero S (2005) Further studies on the interaction of the 5-hydroxytryptamine3 (5-HT3) receptor with arylpiperazine ligands. development of a new 5-HT3 receptor ligand showing potent acetylcholinesterase inhibitory properties. *J Med Chem* **48**, 3564-3575.

[56] Toda N, Kaneko T, Kogen H (2010) Development of an efficient therapeutic agent for Alzheimer's disease: Design and synthesis of dual inhibitors of acetylcholinesterase and serotonin transporter. *Chem Pharm Bull (Tokyo)* **58**, 273-287.

[57] Fernandez-Bachiller MI, Perez C, Gonzalez-Munoz GC, Conde S, Lopez MG, Villarroya M, Garcia AG, Rodriguez-Franco MI (2010) Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. *J Med Chem* **53**, 4927-4937.

[58] Huang W, Lv D, Yu H, Sheng R, Kim SC, Wu P, Luo K, Li J, Hu Y (2010) Dual-target-directed 1,3-diphenylurea derivatives: BACE 1 inhibitor and metal chelator against Alzheimer's disease. *Bioorg Med Chem* **18**, 5610-5615.

[59] Zheng H, Youdim MB, Fridkin M (2009) Site-activated multifunctional chelator with acetylcholinesterase and neuroprotective-neurorestorative moieties for Alzheimer's therapy. *J Med Chem* **52**, 4095-4098.

[60] Hieke M, Ness J, Steri R, Dittrich M, Greiner C, Werz O, Baumann K, Schubert-Zsilavecz M, Weggen S, Zettl H (2010) Design, synthesis, and biological evaluation of a novel class of gamma-secretase modulators with PPARgamma activity. *J Med Chem* **53**, 4691-4700.

[61] Li W, Mak M, Jiang H, Wang Q, Pang Y, Chen K, Han Y (2009) Novel anti-Alzheimer's dimer Bis(7)-cognitin: Cellular and molecular mechanisms of neuroprotection through multiple targets. *Neurotherapeutics* **6**, 187-201.

[62] Weinreb O, Amit T, Bar-Am O, Youdim MB (2012) Ladostigil: A novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. *Curr Drug Targets* **13**, 483-494.

[63] Youdim MB (2013) Multi target neuroprotective and neurorestorative anti-Parkinson and anti-Alzheimer drugs ladostigil and m30 derived from rasagiline. *Exp Neurobiol* **22**, 1-10.

[64] Capurro V, Busquet P, Lopes JP, Bertorelli R, Tarozzo G, Bolognesi ML, Piomelli D, Reggiani A, Cavalli A (2013) Pharmacological characterization of memoquin, a multi-target compound for the treatment of Alzheimer's disease. *PLoS One* **8**, e56870.

[65] Ramsay RR, Majekova M, Medina M, Valoti M (2016) Key targets for multi-target ligands designed to combat neurodegeneration. *Front Neurosci* **10**, 375.

[66] Bolognesi ML, Cavalli A, Valgimigli L, Bartolini M, Rosini M, Andrisano V, Recanatini M, Melchiorre C (2007) Multi-target-directed drug design strategy: From a dual binding site acetylcholinesterase inhibitor to a trifunctional compound against Alzheimer's disease. *J Med Chem* **50**, 6446-6449.

[67] Malar DS, Devi KP (2014) Dietary polyphenols for treatment of Alzheimer's disease–future research and development. *Curr Pharm Biotechnol* **15**, 330-342.

[68] Jiang H, Wang X, Huang L, Luo Z, Su T, Ding K, Li X (2011) Benzenediol-berberine hybrids: Multifunctional agents for Alzheimer's disease. *Bioorg Med Chem* **19**, 7228-7235.

[69] Shan WJ, Huang L, Zhou Q, Meng FC, Li XS (2011) Synthesis, biological evaluation of 9-N-substituted berberine derivatives as multi-functional agents of antioxidant, inhibitors of acetylcholinesterase, butyrylcholinesterase and amyloid-beta aggregation. *Eur J Med Chem* **46**, 5885-5893.

[70] Gomes LM, Vieira RP, Jones MR, Wang MC, Dyrager C, Souza-Fagundes EM, Da Silva JG, Storr T, Beraldo H (2014) 8-Hydroxyquinoline Schiff-base compounds as antioxidants and modulators of copper-mediated Abeta peptide aggregation. *J Inorg Biochem* **139**, 106-116.

[71] Corbett A, Williams G, Ballard C (2013) Drug repositioning: An opportunity to develop novel treatments for Alzheimer's disease. *Pharmaceuticals (Basel)* **6**, 1304-1321.

[72] Yogev-Falach M, Amit T, Bar-Am O, Youdim MB (2003) The importance of propargylamine moiety in the anti-Parkinson drug rasagiline and its derivatives in MAPK-dependent amyloid precursor protein processing. *FASEB J* **17**, 2325-2327.

[73] Youdim MB, Maruyama W, Naoi M (2005) Neuropharmacological, neuroprotective and amyloid precursor

processing properties of selective MAO-B inhibitor antiparkinsonian drug, rasagiline. *Drugs Today (Barc)* **41**, 369-391.

[74] Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J Alzheimers Dis* **7**, 63-80.

[75] Liu Y, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX (2011) Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. *J Pathol* **225**, 54-62.

[76] Chen Y, Zhao Y, Dai CL, Liang Z, Run X, Iqbal K, Liu F, Gong CX (2014) Intranasal insulin restores insulin signaling, increases synaptic proteins, and reduces Abeta level and microglia activation in the brains of 3xTg-AD mice. *Exp Neurol* **261**, 610-619.

[77] Yu Y, Li X, Blanchard J, Li Y, Iqbal K, Liu F, Gong CX (2015) Insulin sensitizers improve learning and attenuate tau hyperphosphorylation and neuroinflammation in 3xTg-AD mice. *J Neural Transm (Vienna)* **122**, 593-606.

[78] Chen Y, Zhang J, Zhang B, Gong CX (2016) Targeting insulin signaling for the treatment of Alzheimer's disease. *Curr Top Med Chem* **16**, 485-492.

[79] Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B (2012) Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: A pilot clinical trial. *Arch Neurol* **69**, 29-38.

[80] Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, Callaghan M, Arbuckle M, Behl C, Craft S (2015) Long acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. *J Alzheimers Dis* **45**, 1269-1270.

[81] Craft S, Claxton A, Baker LD, Hanson AJ, Cholerton B, Trittschuh EH, Dahl D, Caulder E, Neth B, Montine TJ, Jung Y, Maldjian J, Whitlow C, Friedman S (2017) Effects of regular and long-acting insulin on cognition and Alzheimer's disease biomarkers: A pilot clinical trial. *J Alzheimers Dis* **57**, 1325-1334.

[82] Gengler S, McClean PL, McCurtin R, Gault VA, Holscher C (2012) Val(8)GLP-1 rescues synaptic plasticity and reduces dense core plaques in APP/PS1 mice. *Neurobiol Aging* **33**, 265-276.

[83] Holscher C (2014) Central effects of GLP-1: New opportunities for treatments of neurodegenerative diseases. *J Endocrinol* **221**, T31-41.

[84] Hunter K, Holscher C (2012) Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. *BMC Neurosci* **13**, 33.

[85] McClean PL, Parthsarathy V, Faivre E, Holscher C (2011) The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *J Neurosci* **31**, 6587-6594.

[86] Yang Y, Zhang J, Ma D, Zhang M, Hu S, Shao S, Gong CX (2013) Subcutaneous administration of liraglutide ameliorates Alzheimer-associated tau hyperphosphorylation in rats with type 2 diabetes. *J Alzheimers Dis* **37**, 637-648.

[87] McClean PL, Holscher C (2014) Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. *Neuropharmacology* **76**(Pt A), 57-67.

[88] Gejl M, Gjedde A, Egefjord L, Moller A, Hansen SB, Vang K, Rodell A, Braendgaard H, Gottrup H, Schacht A, Moller N, Brock B, Rungby J (2016) In Alzheimer's disease, 6-month treatment with GLP-1 analog prevents decline of brain glucose metabolism: Randomized, placebo-controlled, double-blind clinical trial. *Front Aging Neurosci* **8**, 108.

[89] Reger MA, Watson GS, Green PS, Baker LD, Cholerton B, Fishel MA, Plymate SR, Cherrier MM, Schellenberg GD, Frey 2nd WH, Craft S (2008) Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. *J Alzheimers Dis* **13**, 323-331.

[90] Qian J, Wolters FJ, Beiser A, Haan M, Ikram MA, Karlawish J, Langbaum JB, Neuhaus JM, Reiman EM, Roberts JS, Seshadri S, Tariot PN, Woods BM, Betensky RA, Blacker D (2017) APOE-related risk of mild cognitive impairment and dementia for prevention trials: An analysis of four cohorts. *PLoS Med* **14**, e1002254.

[91] Hampel H, O'Bryant SE, Durrleman S, Younesi E, Rojkova K, Escott-Price V, Corvol JC, Broich K, Dubois B, Lista S, Alzheimer Precision Medicine Initiative (2017) A Precision Medicine Initiative for Alzheimer's disease: The road ahead to biomarker-guided integrative disease modeling. *Climacteric* **20**, 107-118.

[92] Chiesa PA, Cavedo E, Lista S, Thompson PM, Hampel H, Alzheimer Precision Medicine Initiative (2017) Revolution of resting-state functional neuroimaging genetics in Alzheimer's disease. *Trends Neurosci* **40**, 469-480.