**Combination Therapy in Alzheimer’s Disease: Is it time?**

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**Running title:** Combination therapy in Alzheimer’s disease

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**Abstract**

Alzheimer’s disease (AD) is the commonest cause of dementia globally. There is increasing evidence showing AD has no single pathogenic mechanism, and thus treatment approaches focusing only on one mechanism are unlikely to be meaningfully effective. With only one potentially disease modifying treatment approved, targeting Aβ, AD is underserved regarding effective drug treatments**.** Combining multiple drugs or designing treatments that target multiple pathways could be an effective therapeutic approach. Considering the distinction between added and combination therapies, one can conclude that most trials fall under the category of added therapies. For combination therapy to have an actual impact on the course of AD, it is likely necessary to target multiple mechanisms including but not limited to Aβ and tau pathology. Several challenges have to be addressed regarding combination therapy, including choosing the correct agents, the best time and stage of AD to intervene, designing and providing proper protocols for clinical trials. This can be achieved by a cooperation between the pharmaceutical industry, academia, private research centers, philanthropic institutions, and the regulatory bodies. Based on all the available information -the success of combination therapy to tackle complicated disorders such as cancer, and the blueprint already laid out on how to implement combination therapy and overcome its challenges- an argument can be made that the field has to move cautiously but quickly toward designing new clinical trials, further exploring the pathological mechanisms of AD, and re-examining the previous studies with combination therapies so that effective treatments for AD may be finally found.

**Keywords**: Alzheimer’s disease, combination therapy, treatment, clinical trials

**Introduction**

Alzheimer’s disease (AD) accounts for the largest number of cases among dementia. The clinical hallmark of the disease is progressive cognitive and memory impairment [[1](#_ENREF_1)]. The exact pathophysiological mechanism behind AD is still unclear, and numerous hypotheses have been suggested to depict AD pathology at a molecular level. However, none of them have satisfactorily painted the whole picture. Nevertheless, most of the proposed mechanisms have in common the concept that AD results from multiple factors acting together, and thus no single mechanistic treatment approach would likely be effective [[2-4](#_ENREF_2)]. Important pathological mechanisms of AD include extracellular and intracellular accumulation of fibrillary proteins, amyloid-beta (Aβ) and hyperphosphorylated Tau [[5](#_ENREF_5), [6](#_ENREF_6)]. Other mechanisms suggested to contribute to AD pathology include various neurotransmission pathway alterations (e.g., cholinergic, glutamatergic, serotoninergic, histaminergic, dopaminergic, noradrenergic), excitotoxicity, oxidative stress, neuroinflammation and immune system dysfunction, mitochondrial damages vascular abnormalities impairing the brain blood supply, reduced production of nerve growth factors and lipid metabolism. A myriad of metabolic and genetic risk factors are known to be involved in AD development such as diabetes, hypertension, cholesterol, allelic variation in apolipoprotein E (ApoE) and in the case of early onset/ familial AD, mutations in amyloid precursor protein (APP) and presenilin (PSEN) genes [[6-10](#_ENREF_6)]. It is noteworthy that studies on environmental factors such as oral and gut microbiota and their effect on neuroinflammation and amyloid plaque formation are gaining momentum [[11](#_ENREF_11), [12](#_ENREF_12)].

Cholinesterase inhibitors rivastigmine, donepezil and galantamine, and the glutamate receptor antagonist memantine, along with the monoclonal antibody aducanumab (Aduhelm), are the currently available Food and Drug Administration (FDA) approved agents to treat AD. However, those drugs targeting neurotransmitter pathways do not modify the course of the disease, as they do not halt the progression of neurodegeneration, but rather, lead to a temporary slowdown of the cognitive decline by altering neurotransmitter activity. In the case of aducanumab, an anti-Aβ monoclonal antibody, the drug selectively targets amyloid aggregates to facilitate their removal. Presently, with the exception of aducanumab, the effects of which remain to be confirmed [[13](#_ENREF_13)], no new treatment to halt or slow AD progression has been developed. Indeed almost all clinical trials have failed to show real improvement in the course of the disease [[14](#_ENREF_14), [15](#_ENREF_15)]. The intricate labyrinth of AD pathology, parts of which are still uncharted territory, is likely the reason behind the failure of treatment trials [[16](#_ENREF_16)]. Lack of an effective treatment is not limited to AD and many multifactorial diseases share this problem, which is partly caused by their multiple pathogenic pathways which makes a multidrug approach targeting more than one mechanism the optimal method in managing them [[17-19](#_ENREF_17)]. Such approaches have been implemented in several conditions with effective results, namely acquired immunodeficiency syndrome (AIDS) [[19-21](#_ENREF_19)], autoimmune diseases such as rheumatoid arthritis and systemic sclerosis [[18](#_ENREF_18), [22](#_ENREF_22)], cancer [[17](#_ENREF_17), [23-29](#_ENREF_23)], Parkinson’s disease [[30](#_ENREF_30), [31](#_ENREF_31)], and hypertension [[32-34](#_ENREF_32)]. Interestingly, mathematical models have shown the added benefit of combination of therapies as opposed to single therapy [[10](#_ENREF_10), [26](#_ENREF_26), [27](#_ENREF_27), [35-39](#_ENREF_35)], which highlights the question why this approach has not yet been implemented for AD.

In summary, AD involves multiple complex interconnected pathological mechanisms, and thus requires a multitarget treatment approach. Of note, the temporal course of the disease should also be considered and could be addressed by implementing a multi-step combination therapy [[4](#_ENREF_4)]. All the aforementioned reasons for this approach led the authors to examine the pathological mechanisms of AD and to debate current treatments and selected clinical trials. Finally, we discuss in detail the rationale for combination therapy and how it would be beneficial in stepping towards an effective treatment approach for AD, without omitting the obstacles for its application as well as the surrounding ethical issues. We propose that combination therapy might be the best approach and that we might be at the crossroad to reinforce this therapeutic approach to tackle AD.

**Pathological pathways of AD**

The hallmarks of AD pathology are deposition of Aβ as plaques in the neocortex and neurofibrillary tangles (NFTs) in limbic and cortical association areas [[40](#_ENREF_40)]. Also observable are, neuropil threads, dystrophic neurites, astrogliosis an microglial activation, along with the frequent coexistence of cerebral amyloid angiopathy [[41](#_ENREF_41)]. All the aforementioned pathologies lead to a progressive neurodegeneration with synaptic and neuronal loss resulting in dementia. Mixed pathologies including vascular disturbances and Lewy bodies are frequently observed among older patients [[42](#_ENREF_42)]. The entangled mechanisms involved in AD (Figure 1) have been reported by numerous studies [[43](#_ENREF_43)], and the assumption that any single cause explains AD as the original amyloid hypothesis suggests has been ruled out and is no longer pursued [[44](#_ENREF_44)]. In the next sections, we discuss different pathological mechanisms of AD to provide the grounds for the needs for combination of therapies [[45](#_ENREF_45)].

*A pathology*

Numerous genetic studies indicate that mutations in the genes encoding amyloid precursor protein (APP) or in enzymes that generate Aβ induce autosomal dominantly inherited AD, making Aβ a significant disease initiator. APP is cleaved by β-secretases (BACE1) and γ-secretases. The resulting fragments are then polymerized and turned into insoluble amyloid fibrils, aggregation of which produces amyloid plaques. Aβ also oligomerizes and diffuses in synaptic clefts interfering with synaptic signals [[46](#_ENREF_46), [47](#_ENREF_47)]. Current AD disease models propose that Aβ plaques or non-fibrillar, soluble, or oligomeric forms of Aβ, initiate a cascade leading to tau misfolding and assembly spreading throughout the cortex, leading to neural system failure, neurodegeneration and cognitive decline [[48](#_ENREF_48), [49](#_ENREF_49)]. For example, it is hypothesized that Aβ polymerization leads to activation of microglia, culminating in hyperphosphorylation of the microtubule-associated tau protein, and its polymerization into insoluble NFTs [[4](#_ENREF_4)]. Microglial activation and local inflammatory responses further contribute to neurotoxicity [[50](#_ENREF_50)]. As more than 90% of Aβ is found in an apolipoprotein complex [[51](#_ENREF_51)], the question of the lipoprotein-mediated axis for Aβ has been raised by Lam et al. [[52](#_ENREF_52)] who reported causal evidence of a lipoprotein- Aβ/capillary axis as a mechanism involved in neurodegeneration, in a pre-clinical study using a non-AD model. Regarding the genetic basis for AD pathology, possession of the *APOE ε4* allele is the major risk factor for late-onset AD [[8](#_ENREF_8)], with those homozygous for ApoE ε4 having a 50% chance of developing the disease. A major effect of ApoE ε4 is to alter clearance of Aβ from the central nervous system [[53](#_ENREF_53)]. Although the precise mechanisms for interactions between Aβ and tau remain unclear, there is evidence that Aβ continues to drive tau pathology through the course of the disease, rather than simply being an initiating factor [[54-56](#_ENREF_54)]. Thus, a logical approach to slow AD might be to combine anti-Aβ and anti-tau agents. It is noteworthy to mention that anti-tau immunotherapies and antisense therapies for tau are already in clinical trials, making combination therapy a realistic possibility both in experimental and clinical aspects [[40](#_ENREF_40)].

*Tau pathology*

There is a consensus on the significant role of tau in AD pathogenesis. As reported by a multitude of studies, AD progression is correlated with NFTs and neuropil threads formed with phosphorylated tau species [[57](#_ENREF_57)]. Phosphorylated tau has a significantly reduced capacity of binding to microtubules [[58-61](#_ENREF_58)] giving rise to aggregation of the protein into NFTs [[62](#_ENREF_62)]. It is noteworthy to mention that the formation of tangles better correlates with the progressive loss of neurons and synapses, making NFT pathology more concordant with the clinical aspects and the severity of AD [[41](#_ENREF_41)]. In comparison, Aβ pathology plateaus quite early on during the symptomatic phase of AD [[63](#_ENREF_63)]. Missplicing of tau which results in longer tau isoforms could play a part in tau pathology of AD [[64](#_ENREF_64)]. Furthermore, N- and C-terminal truncation of tau can possibly impact the way tau interacts with microtubules, NFT formation and cellular localization [[65](#_ENREF_65), [66](#_ENREF_66)]. Proteolytic cleavage of tau has been suggested to be significant in the development and progression of AD as it could lead to neurotoxic fragments [[67-69](#_ENREF_67)]. Despite the formerly held assumption that tau aggregates act as the toxic species, studies reported that in concordance with Aβ, oligomeric tau, and not tau aggregates, constitute the toxic species, precipitating synaptic dysfunction and neurodegeneration [[70](#_ENREF_70)].

*The neuroinflammatory pathway*

The third main pathological mechanism of AD, which is putatively a connector between the other two main pathologies, is neuroinflammation defined as a sustained inflammatory response in the AD brain [[71-74](#_ENREF_71)]. Evidence of neuroinflammation as a key pathology has been observed and reported in numerous post-mortem studies [[75-78](#_ENREF_75)]. The hypothesis is that an imbalance of pro- and anti-inflammatory signals in AD causes persistent neuroinflammation which contributes to both Aβ and tau pathologies and exacerbates neurodegeneration [[4](#_ENREF_4), [79-82](#_ENREF_79)]. Genetic studies have highlighted the role of the immune system in AD. An analysis of two large independent genome-wide association studies of late-onset AD strongly implicated genetic variation in the functions of the immune system as cause of late-onset AD susceptibility. These studies define the immune processes aetiologically relevant and suggest that immunity may be a suitable target for novel and existing therapeutic approaches [[83](#_ENREF_83)]. Several genetic risk factors have been identified to be involved in AD neuroinflammation, namely, triggering receptor expressed on myeloid cells 2 (TREM2), cluster of differentiation 33 (CD33), component receptor 1 gene (CR1), adenosine triphosphate binding cassette subfamily a member 7 (ABCA7) and SH-2 containing inositol 5' polyphosphatase 1 (SHIP1). TREM2 has been reported to have a significant role in AD pathology [[84](#_ENREF_84)]. Based on the evidence presented, combination therapy targeting Aβ and/or tau added with agents targeting inflammation may slow AD progression [[85](#_ENREF_85)].

*Neurotransmitter alterations*

A significant amount of evidence has confirmed the interference of Aβ with mechanisms involved with neurotransmitters, namely acetylcholine and glutamate. These neurotransmitters are key in processes of leaning and memory, which explain the pathophysiology of neurochemical defects seen in AD [[86](#_ENREF_86)]. The ‘cholinergic hypothesis’ was suggested over two decades ago, stating the insufficient cholinergic function in the CNS plays a significant role in the cognitive impairment that comes with old age and AD [[87](#_ENREF_87)]. The decreased functioning of the cholinergic system is accompanied by a decrease in choline acetyltransferase (ChAT), muscarinic and nicotinic acetylcholine binding and the concentration of acetylcholine in the synaptic cleft [[88](#_ENREF_88)]. Glutamate metabolism also influences cognition [[87-90](#_ENREF_87)], with evidence for the role of glutamatergic overstimulation of the postsynaptic N-methyl-D-aspartate (NMDA) receptors in the pathogenesis of AD, resulting in neuronal damage [[90-92](#_ENREF_90)]. Owing to the nonspecific degeneration associated with the accumulation of Aβ, many types of synaptic dysfunctions have been reported affecting cholinergic, monoaminergic and glutamatergic systems [[93](#_ENREF_93)].

*Nutritional factors*

Several epidemiological studies have reported associations between nutritional and dietary factors with the onset of AD [[94-97](#_ENREF_94)], implicating insulin resistance, cardiovascular disease, obesity, and other metabolic syndromes as environmental risk factors for AD [[80](#_ENREF_80), [96](#_ENREF_96), [98-101](#_ENREF_98)]. Healthy diets with a high amount of plant-based meals -soy beans, nuts, omega-3 polyunsaturated fatty acids- and a low amount of saturated fat animal-based proteins and refined sugar, have been reported to reduce neuroinflammation, insulin resistance and the risk of neurodegeneration and thus the development of AD [[97](#_ENREF_97), [102-104](#_ENREF_102)]. Nutritional effects have been observed on mitochondrial dysfunction, oxidative stress, and membrane lipid dysregulation known to contribute to AD physiopathology [[100](#_ENREF_100), [105](#_ENREF_105), [106](#_ENREF_106)]. Indeed, dyslipidemia and impaired glycemia have been reported to increase lipid peroxidation leading to oxidative stress and ultimately to neural damage [[107](#_ENREF_107)]. Epidemiological studies suggested that healthy diets such as the Mediterranean diet, the DASH diet (dietary approaches to stop hypertension) or theMIND diet (Mediterranean-DASH diet intervention for neurodegenerative delay) are associated with lower risk of dementia and AD [[108](#_ENREF_108)]. Unhealthy diets can also unbalance the circadian modulation of cortisol resulting in a poor sleep quality [[109](#_ENREF_109)]. This in turn will affect the glymphatic system, a waste clearance system described in the brain connecting the lymphatic system to the dural sinuses and [meningeal](https://en.wikipedia.org/wiki/Meninges) arteries and facilitated by the astrocytes [[110](#_ENREF_110)]. The glymphatic system has been reported to be involved in the clearance of amyloid from the brain, and thus a dysfunctional system will result in A aggregation [[111-113](#_ENREF_111)]. Particular diets and nutrients can impact the gut microbiota and alter its composition [[114](#_ENREF_114), [115](#_ENREF_115)], leading to increased bacteria-produced amyloids and thus contributing to the onset of AD as described below [[113](#_ENREF_113), [116-118](#_ENREF_116)].

*Microbiota factors*

The gut and the brain are deeply interconnected via the gut-brain axis [[119](#_ENREF_119), [120](#_ENREF_120)] which is modulated by the gut microbiome. A perturbed gut microbiome leads to chronic inflammation and numerous immune responses that can potentially play a role in AD pathology [[121-123](#_ENREF_121)]. Indeed, gut microbiome changes and the associated inflammation can increase the permeability of the intestines leading to insulin resistance, a pathological risk factor for AD as mentioned above [[124](#_ENREF_124)]. The gut microbiome is a producer of bacterial amyloids that have been hypothesized to activate pathological pathways causing inflammation, accumulation of Aβ and neurodegeneration [[116](#_ENREF_116), [125-127](#_ENREF_125)]. The idea is that the amyloid excreted by the gut microbiome will act as a cerebral seed generating a prion cascade-like Aβ aggregation in the brain, compromising brain function [[128](#_ENREF_128), [129](#_ENREF_129)]. Dysbiosis of gut microbiota can occur as a result of the low-grade state of inflammation in the elderly, dietary changes, antibiotic treatments, non-steroidal anti-inflammatory drugs, etc.. This will generate a breakdown of the gut barrier leading to pro-inflammatory cytokines and bacterial products which will leak into the circulation crossing the impaired blood-brain barrier [[113](#_ENREF_113), [115](#_ENREF_115), [130-135](#_ENREF_130)].

Studies of the oral microbiota also reported an association with AD pathology [[136](#_ENREF_136)], especially as a result of chronic periodontal disease [[137](#_ENREF_137)]. The underlying suggested pathomechanism is that altered oral microbiota will excrete pro-inflammatory mediators migrating to the blood stream, resulting in systemic and central inflammation and AD pathology [[137-139](#_ENREF_137)].

**Current therapies in AD**

As of September 2021, there are six Food and Drug Administration (FDA) approved treatments for AD in the US (donepezil, galantamine, rivastigmine, memantine, a combination of donepezil and memantine, commonly referred to as the standard treatment for AD, and the newly approved aducanumab). It is also noteworthy that the approval of aducanumab has not been without controversy [[140-142](#_ENREF_140)]. With only one potentially disease modifying treatment approved, AD is significantly underserved regarding effective drug treatments. This lack of effective therapeutic options might be partly explained by a focus on only monotherapy trials namely cholinesterase inhibitors or memantine, targeting one specific mechanism without considering many other contributing pathogenesis pathways that cause AD [[16](#_ENREF_16)]. Current clinical trials mostly implement symptom targeting drugs (cholinesterase inhibitors or memantine) or a new disease modifying or symptomatic agent. Of note, the DOMINO-AD protocol was performed to explore combining standard therapy agents. This clinical trial used a two-by-two factorial design to assess the combination of donepezil and memantine [[143](#_ENREF_143)]. Combination therapy was not substantially better than donepezil alone in this trial. This demonstrates that symptomatic agents by nature cannot be the answer to this disease. If there is going to be a way forward, it has to be agents which target underlying pathological mechanisms.

Clinical trials studying the effectiveness of treatment strategies compared to the standard symptomatic drugs, such as inhibiting BACE1 or Aβ immunization, as is the newly approved aducanumab, will be a step forward in finding the best way to stop AD progression. The recent failure of many treatment trials for AD to meet primary study endpoints hints that there is a need for changing how we approach treating AD. Keeping in mind the complexity of the pathogenesis of AD, combining multiple drugs, or designing treatments that target multiple pathways could be a much more effective approach in the treatment of AD [[141](#_ENREF_141), [144](#_ENREF_144), [145](#_ENREF_145)].

*Single therapies*

So far the FDA approved treatments and most clinical trials in AD are single therapies, which can be categorized into immunotherapies and pharmacotherapies. A complete list of all single therapies in trial as of September 2021 based on the FDA website ClinicalTrials.gov is provided in supplementary tables S1-S3, classified by the pathways investigated or the therapeutic approach.

*Combination therapy*

Combination therapy refers to the administering of two or more therapeutic agents, in combination with each other or with a placebo. Combination therapy differs from “added therapy” which is adding a new agent to the standard and often symptomatic AD treatment comparing the results with placebo [[146](#_ENREF_146)]. Considering the distinction between added and combination therapies, one can conclude that most trials fall under the category of added therapies, defined by adding a new agent to patients already taking the standard symptom targeting AD treatment as opposed to studying the effects of these new agents in combination. An important aspect of combination therapy is the potential synergistic effects of the agents which can be studied as the agents are tested both separately and in combination [[16](#_ENREF_16)]. For combination therapy to have an actual impact on the course of AD, it is likely necessary to target multiple mechanisms including but not limited to Aβ and tau pathology. So far, combination therapies have assessed combining two amyloid-targeting agents e.g., Aβ secretase inhibitor (BACE1) and anti-Aβ monoclonal antibody (mAb). Other ongoing evaluations include: BACE1 with anti-tau mAb, anti-Aβ mAb with anti-tau mAb, or BACE1 inhibitor with anti-Aβ mAb and anti-tau mAb together. Other compounds in early stages of development that may be used in combination target tau modulators and inhibitors; RNA interference and antisense approaches towards tau; inflammatory markers such as cytokine and chemokine inhibitors as well as key microglial proteins (e.g., TREM2, CX3CR1), mitochondrial modulators, free radical inhibitors, and vascular modulators including those that interfere with APOE pathways [[146](#_ENREF_146)]. We have listed current clinical trials of added therapies in supplementary table S4, complementing the detailed review on this therapeutic approach [14]. However, the main focus of this review is combination therapies thus the added therapies are not discussed in detail.

**Current combination trials in AD**

Unlike single and added therapies, combination therapy trials in AD are very limited and only a few trials have been or are being conducted. Many combinations of agents targeting different aspects of AD pathology could be adopted. Very few have so far been considered in designing clinical trials, the first one being a phase 2 trial (NCT03367403), called TRAILBLAZER-ALZ, from Eli Lilly aiming to evaluate the safety, tolerability and efficacy of an Aβ antibody (LY3002813) with and without a BACE1 inhibitor (LY3202626) in subjects with early symptomatic AD. This strategy explores the possibility that stimulating an immune response against Aβ while at the same time decreasing additional production of Aβ by inhibiting BACE1 will impact Aβ pathology more than targeting either mechanism alone [[16](#_ENREF_16)]. In October 2018, Lilly decided to continue the trial but without the addition of LY3202626 [[147](#_ENREF_147)]. Another strategy was combining human albumin with an intravenous human immunoglobin (Gamunex, Grifols Therapeutics, Clayton, NC, USA). The rationale behind this strategy is that Aβ exits the blood-brain barrier and binds to both albumin and the injected immunoglobulin to finally be cleared out of the circulation via plasma exchange. Based on the peripheral sink hypothesis of amyloid [[148](#_ENREF_148)], the study suggested that repeating the process will increase Aβ clearance from the brain since it implements a dual transport of Aβ (albumin and immunoglobulin) [[149](#_ENREF_149)]. Scheduled to be completed in December 2017, the trial was terminated because the phase 3 did not demonstrate efficacy on the co-primary endpoints.

Combination therapies can also be one agent targeting multiple mechanisms of AD pathology as opposed to multiple agents targeting a single mechanism. An example is the receptor for advanced glycation end-products (RAGE) which is expressed by a variety of brain cells and binds and transports Aβ from blood to brain. Studies have implied the involvement of RAGE in multiple mechanisms of AD pathology [[150](#_ENREF_150)]. Consequently, RAGE inhibitors will have a combination of therapeutic functions, namely, lowering brain Aβ levels, slowing neuroinflammation and cognitive decline. However, a phase 3 clinical trial for the RAGE inhibitor azeliragon (also known as PF-04494700 and TTP488) did not meet the co-primary efficacy endpoint (improvement in cognitive or functional outcomes) and was terminated [[151](#_ENREF_151)].

Targeting neuroinflammation and Aβ is another promising approach as both pathologies are significantly present in AD. The ALZT-OPT1 phase 1/2 trial combines two FDA approved drugs, cromolyn (targeting Aβ) and ibuprofen (targeting inflammation), and is currently recruiting subjects with mild to moderate AD. Another ALZT-OPT1 phase 3 trial on early AD patients has been completed with no posted results. Cromolyn is a mast-cell stabilizer used to treat asthma and is structurally similar to anti-amyloid agents and can potentially cross the blood-brain barrier. Experimental studies showed that cromolyn possessed inhibitory effects against Aβ monomer aggregation into oligomers and fibrils without any impact on Aβ production, and that one week of daily administration of cromolyn reduced soluble Aβ concentration by more than 50% [[152](#_ENREF_152)]. Another study reported cromolyn to stimulate microglial recruitment to Aβ deposits with increased microglia phagocytosis; however, ibuprofen alone did not have any effect [[153](#_ENREF_153)]. Table 1 lists all combination therapy trials on AD until November 2021, with the trials categorized by their combination strategy and the targeted mechanisms against AD. Figure 2 shows the targeted pathways by combination therapies via highlighting the national clinical trial (NCT) number onto the targeted pathology.

**Potential combination therapy not explored in AD**

Combination therapy can target a single or multiple mechanisms involved in a specific or multiple pathological pathways, acting via multiple therapeutic agents, each targeting one mechanism or one agent aiming at multiple mechanisms. Rasagiline, a drug to treat Parkinson’s disease, is an agent targeting multiple pathological pathways including inhibiting monoamine oxidase (MAO)-B, with neuroprotective properties and repercussion on amyloid processing [[154](#_ENREF_154), [155](#_ENREF_155)], and thus could be suggested for AD. Combination therapy could sequentially act against a certain pathway, an example would be removing amyloid plaques using monoclonal antibodies followed by preventing further accumulation of plaques via inhibiting BACE1 [[156](#_ENREF_156)]. Approaching AD in this manner has great potential in treating the disease as there is an ever-growing understanding of causative mechanisms involved in AD pathogenesis, and can address the complicated connection between various pathological pathways [[157](#_ENREF_157)]. A novel combination therapy approach by Denali Therapeutics implements a bispecific antibody designed to better cross the blood-brain barrier via binding to transferrin receptors on endothelial cells, and then targeting Aβ and tau in the brain. This method of using antibody transport vehicles has been reported effective in delivering antibodies into the brain. It has resulted in reduced Aβ levels and its accumulation into plaques in APP transgenic mice via anti-BACE1 antibodies or acting against tau pathology in tau transgenic mice using anti-tau antibodies [[158](#_ENREF_158), [159](#_ENREF_159)]. A bispecific antibody transport vehicle is currently being engineering by the company to target both Aβ and tau simultaneously. Based on evidence that Aβ accelerates tau propagation [[155](#_ENREF_155)], this bispecific antibody may have the potential for synergism.

Other interventions, such as neurostimulation and novel approaches using stem cells, clustered regularly interspaced short palindromic repeats (CRISPR) gene editing, and antisense oligonucleotides may one day be proven to be beneficial to prevent or treat AD either as monotherapy or in combination with other treatments. To advance these treatments and determine when they might be most appropriate to use across the disease course, study populations need to be better characterized with a wide range of biomarkers to determine, for example, whether there are differential benefits to those with or without pathological markers of AD [[146](#_ENREF_146)]. As becoming more apparent, combination therapy has a vast potential and merits further research and trials exploring this relatively new territory of AD therapy. In addition, based on the European Union-North American Clinical Trials in Alzheimer’s Disease Task Force (EU/US CTAD Task Force), combination therapy approach to target various mechanisms and pathways are likely to be required for successful treatment of AD [[160](#_ENREF_160)]. It should be noted that only a limited number of approaches of combination therapy have been or are being studied, and while it is gaining momentum, combination therapy has many issues and challenges that need to be addressed in order for its future success.

**Challenges associated with combination therapy in AD**

Though challenging, it could be plausible to combine therapeutic agents which have not been approved and/or their effects or lack thereof has not been well documented. Choosing which pathogenic mechanisms and effective compounds to target precise mechanisms, whether to administer the compounds at the same time or in a specific order, the dosage ratio, and at what stage of the disease to intervene are significant but not all of the challenges faced when designing a combination therapy trial for AD [[146](#_ENREF_146)]. The absence of reliable animal models mimicking the complicated pathogenesis of AD with very little preclinical documentation is another issue of combination therapy. Furthermore, adding to the difficulties is the missing cooperation between pharmaceutical companies, academic researchers, and private organizations. However, the EU/US CTAD Task Force is a great example of a good collaboration. The task force has successfully formed an international collaboration of investigators from academia, industry, non-profit foundations, and regulatory agencies. Since 2014, they have explored several options for AD, providing guidance for future treatments and clinical trials [[161-163](#_ENREF_161)]. This re-emphasises the need for such collaborations in AD. However, even if all these problems are overcome, regulatory and ethical issues remain.

From an industrial perspective, designing and conducting combination trials is a particularly complicated task. Indeed, recruiting sufficient eligible participants at a specific stage of the disease with the same prominent pathological markers for the combined agents to be effective, and willing to undergo an unprecedented clinical trial with no guarantee of success or complete safety, is not within the realm of possibility for most companies [[146](#_ENREF_146)]. Such combination trials would be large-scale, multicentered, and international, with the aim of studying the effects of multiple drugs administered to treat a highly complex condition. The studied factors would include any possible synergistic beneficial impact of the combined agents as well as their independent therapeutic effects in order to provide robust evidence that the combination therapy’s benefits would supersede it is possible harms. Such an operation would be very complex and expensive and will most likely require sample sizes greater than the current phase 2/3 trials.

*What agents to combine and at what stage of the disease?*

To reach an acceptable therapeutic response resulting from additive or synergistic impact of combination therapy, the stage of the disease at which clinical trials intervene is of great significance. In the earliest stages of AD (i.e., more than 20 years before disease onset), targeting A alone with a monotherapy may prove to be effective. As amyloid plaque burden grows (approximately 10 to 20 years before onset), combining an amyloid plaque removal agent with a soluble Aβ production modulator might be indicated; and when biomarkers demonstrate an increase in soluble tau isoform production, a tau production inhibitor might be added. As hypometabolism becomes apparent, multiple drugs may be appropriate including those that remove plaques, protect neuronal function, inhibit tau seeding and production, and improve brain perfusion [[146](#_ENREF_146)]. The overlapping temporal sequence of these various pathways suggests that multi-target combinations may vary by disease stage [[164](#_ENREF_164)]. For primary prevention, targeting genetic and other modifiable risk factors may be an ideal approach. Once pathology has been triggered, and assuming that cortical tau pathology is dependent on Aβ, treatment targeting Aβ alone (with sequential or combination therapy, ideally involving two or more mechanisms of action) may be sufficient. Once tau pathology has been initiated, both Aβ and tau-directed therapies, possibly in combination with disease-modifying agents directed at other targets, may be necessary [[4](#_ENREF_4)]. For prodromal or symptomatic AD, combinations including treatments directed at downstream events and concomitant pathologies will almost certainly be needed for maximum benefit [[146](#_ENREF_146)].

*Regulatory issues*

An understanding is forming, though not yet a consensus, regarding the importance and benefits of combination therapy, as well as the necessity of regulating it specifically. One such regulation is the 21st Century Cures Act which deems necessary the efficiency of combination therapy by developing a process that considers market demand, coordinates between research facilities, and complies with good manufacturing processes [[165](#_ENREF_165)]. Regulatory bodies do support combination therapy studies and trials [[166](#_ENREF_166)], both the FDA and European Medicines Agency have released guidelines, specifying the non-clinical and clinical data required for combination therapy development [[155](#_ENREF_155), [167](#_ENREF_167), [168](#_ENREF_168)]. According to these guidelines, combination trials should provide sufficient evidence justifying the pharmacological and medical rationale and highlighting the efficacy with a satisfactory risk benefit ratio for the combination therapy. For diseases that are not sufficiently controlled using single-therapies, a randomized controlled superiority trial should be conducted to provide data pointing to better therapeutic results of the combination therapy compared to that of single-therapies, and they should do so by implementing a 3-arm A versus B versus AB trial design [[8](#_ENREF_8)]. The FDA recommendations to co-develop two novel drugs, is a 4-arm A versus B versus AB versus placebo (or approved standard-of-care agent) design in phase 2 trial and a 2-arm AB versus placebo (or standard-of-care agent) design in phase 3 trial [[3](#_ENREF_3)]. The International Conference of Harmonization has also established regulatory guidance across countries which touch on potential drug combination scenarios suggesting that instead of conducting an entire toxicology study on the combination of agents, a bridging study of up to 90 days giving the combination to an appropriate species would be sufficient. Regarding novel experimental agents, the efficacy of which has not been backed by adequate evidence, the FDA has outlined criteria based on which the need for a toxicity study will be determined, including the organs targeted by the drugs, pharmacodynamic or pharmacokinetic interaction between the drugs, prior human or animal-based studies regarding the combination, and the effects of the agents on one another, be it a synergistic reaction, a suppressive impact on the side-effects or in fact any possible alteration of the effectiveness of each drug [[146](#_ENREF_146)]. From an industrial standpoint, the treatment exposure time and the numbers of subjects needed before an assessment can be made as whether combination is clinically better than single therapy is the most significant issue in meeting regulatory needs.

Cognitive measures are not sensitive enough to identify early subtle changes which makes functional markers the best bet to gain evidence of efficacy. Regulatory bodies are especially keen on data suggesting synergistic impact of a combination, demonstrating added value over single therapies, which begs the question of whether trials can be conducted with the combination only compared to placebo or if single therapy arms are required. Reliable biomarkers that reflect the progression of AD are of great importance and it is necessary that all of these biomarkers be validated in the populations in which they will be used [[146](#_ENREF_146)]. The FDA promotes combination therapy development only for “serious diseases,” defined as “a disease or condition associated with morbidity that has substantial impact on day-to-day functioning” [[3](#_ENREF_3)] and has issued specific guidelines on combination therapy development for cancer, tuberculosis, and HIV infection [[6](#_ENREF_6), [53](#_ENREF_53), [140](#_ENREF_140)]. From a regulatory standpoint, the success and potential benefit of combination treatment in the said disorders have paved the way for efficient development of effective combination therapy for AD [[165](#_ENREF_165)].

**Conclusion**

The pathogenesis of AD is complex, with multiple pathways evolving in the course of the disease, many of which affect or cause each other resulting in a very complicated pattern of different mechanisms interacting with one another; thus, making AD progression very difficult to stop or slow with almost all of the single and added clinical therapies failing to show promising results. The FDA-approved drugs for AD include cholinesterase inhibitors and memantine or the combination of these agents along with the newly approved, and the only potentially disease-modifying agent, aducanumab. Adding to the frustration in AD treatment is the failure of many promising new drugs in larger phase 3 trials, not meeting efficacy endpoints during the past decades. Only the combination of cholinesterase inhibitors and memantine has had success, and very limited at that, in the treatment of AD, and the studied benefits of aducanumab though promising have not proved it to be sufficient in halting or slowing its progression. Therefore, now more than ever, it is important to acknowledge that targeting multiple pathologic mechanisms in a very complicated disorder may very well be the best if not the only way for successful treatment of AD. Combination therapy, by targeting multiple different pathological mechanisms, provides the possibility of effective synergistic impact on treating AD. Very few such trials with combination strategies have been or are being tested to date. Combination therapy can also potentially lower the doses of the individual agents which in turn can reduce costs and adverse effects. There is a myriad of combination strategies, almost all of which have not yet been tested, making this approach an almost uncharted territory with great potential and immense possibilities. Challenges of treating AD have pushed researchers and investigators toward new approaches such as an added therapy to the standard AD treatment and repurposing already approved drugs intended for other conditions, or combining two such drugs, each targeting a different pathway, following in the footsteps of successful past experiences for other serious diseases and conditions, such as cancer and HIV [[141](#_ENREF_141)]. The rationale is clear, however serious challenges have to be addressed and dealt with, including choosing the correct agents, the best time and stage of AD to intervene, coordinating the academia and pharmaceutical companies, designing and providing proper protocols for clinical trials and better experimental models. As is the case with most scientific advancements, making a strong case regarding profits and market demand, can go a long way in making combination therapies with all their challenges a reality. This can be achieved by a cooperation between the pharmaceutical industry, the academia, private research centers, philanthropic institutions, and the regulatory bodies. Such cooperation would be the best way in advancing the science and solving matters such as regulatory challenges, ethical issues, intellectual property and data sharing. Based on all available information, the success already achieved of combination therapy to tackle complicated disorders such as cancer and the blueprint already laid out on how to implement combination therapy and overcome its challenges, all with the great need and urge finally to control AD, a strong argument can be made that the field has to move cautiously but quickly toward designing new clinical trials, further exploring the pathological mechanisms of AD, and re-examining the previous studies all with combination therapies in mind, so that it may finally find the treatment for AD.

**Conflict of interest**

None to declare

**References**

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**Figure 1.** Pathophysiomechanisms involved in Alzheimer's disease (AD).

Summary of the key pathological mechanisms of AD, Aβ pathology (in blue), tau pathology (in purple), and immune system mediated mechanisms (in pink), are depicted to emphasize the importance of a multitarget approach for AD treatment.

Abbreviations: APP, Amyloid Precursor Protein; Aβ, Amyloid-beta; ApoE4, Apolipoprotein E4; NMADR, N-methyl-D-aspartate receptor; PrP, Prion Protein; IL1β, Interleukin 1-beta; TNFα, Tumor Necrosis Factor-alpha; CCL3, Chemokine (C-C motif) Ligand 3; AMPK, Adenosine Monophosphate-activated Protein Kinase; PP2A, Protein Phosphatase 2A; PSD-95, Postsynaptic Density Protein 95*;* CDK5*,* Cyclin-dependent kinase 5; GSK-3β, Glycogen Synthase Kinase 3-beta;



**Figure 2.** The AD pathophysiomechanisms targeted by combination therapy and identified by the national clinical trial number. The mode of action is identified by a color code as follow: multiple pathways (green), same pathway (light purple) ; one agent targeting multiple pathways (light blue). The figure highlights the importance for targeting multiple mechanisms, the limited number of combination therapy trials performed, and the mechanisms potentially to target in future trials.

Abbreviations: APP, Amyloid Precursor Protein; Aβ, Amyloid-beta; ApoE4, Apolipoprotein E4; NMADR, N-methyl-D-aspartate receptor; PrP, Prion Protein; IL1β, Interleukin 1-beta; TNFα, Tumor Necrosis Factor-alpha; CCL3, Chemokine (C-C motif) Ligand 3; AMPK, Adenosine Monophosphate-activated Protein Kinase; PP2A, Protein Phosphatase 2A; PSD-95, Postsynaptic Density Protein 95;CDK5*,* Cyclin-dependent kinase 5; GSK-3, Glycogen Synthase Kinase 3-beta;

**Table 1:** Combination therapy trials in Alzheimer’s disease

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Agent** | **Type** | **AD Stage** | **Number Enrolled** | **Age** | **Phase/Status** | **Baseline Therapy\*** | **outcome** | **Clinical Trials** |
| **BACE1 inhibitor + amyloid immunization** | | | | | | | | |
| LY3202626 | Intravenous amyloid immunization plus BACE1 inhibitor | Early AD | 266 | 60-85 | II  Active, not recruiting |  | The trial continued without the addition of LY3202626 | NCT03367403 |
| **IVIG + human albumin** | | | | | | | | |
| Flebogamma® 5% DIFand Albutein® 20% | Intravenous human Immunoglobulin + human albumin | mmAD | 347 | 55-85 | II/III  completed | AChEIs and/or memantine | Patients administered both IVIG and albumin had less reduction in brain glucose metabolism than sham-treated patients | NCT01561053 |
| Gamunex | Intravenous human  Immunoglobulin + human albumin | Mild to Moderate | 508 | 50-89 | III |  | The study was terminated because the first Phase 3 did not demonstrate efficacy on the co-primary endpoints | NCT01524887 |
| **The receptor for advanced glycation end-products (RAGE) inhibitor** | | | | | | | | |
| Azeliragon | RAGE inhibitor | mAD. | 880 | 50 ≤ | III | AChEIs and/or memantine | did not meet either coprimary  efficacy endpoint (improvement in cognitive  or functional outcomes) | NCT02080364 |
| 43 | 50-85 | II | * ChEIs or memantine | no results posted | NCT03980730 |
| **Combination targeting neuroinflammation** | | | | | | | | |
| ALZT-OP1 | Anti-amyloid + anti-inflammatory | Early AD | 620 | 55-79 | III  completed | * AChEIs and/or memantine | no results posted | NCT02547818 |
| Healthy + mmAD | 56 | I/II  recruiting | * Standard | N/A | NCT04570644 |
| **Other combinations** | | | | | | | |  |
| Insulin (Humulin® R U-100)  Empagliflozin 10 MG | intranasal insulin + sodium-glucose cotransporter type 2 inhibitor (SGLT2i) | pAD /  Early AD | Recruiting | 55-85 | II |  | no results posted/ Recruiting | NCT05081219 |
| Dasatinib + Quercetin | Tyrosine kinase  inhibitor  (dasatinib);  flavonoid  (quercetin) | AD | 5 | 65 ≤ | I/ II | cholinesterase inhibitors | No study results posted/ Recruiting | NCT04063124 |
| Dasatinib + Quercetin | Tyrosine kinase  inhibitor  (dasatinib);  flavonoid  (quercetin) | AD | 20 | 55 ≤ | I/II | On a stable dose of cholinesterase inhibitors and/or memantine for at least 3 months or not on them at all | No study results posted/ Enrolling by invitation | NCT04785300 |

AD: Alzheimer’s disease; MCI: mild cognitive impairment; mmAD: mild to moderate Alzheimer’s disease; pAD: prodromal Alzheimer’s disease; mAD: mild Alzheimer’s disease; AChEI: Acetylcholinesterase Inhibitor; BACE1: β-site amyloid precursor protein cleaving enzyme 1; ChEI: Cholinesterase inhibitor; NMDA (N-methyl-D-aspartate) receptor antagonists

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