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Pathophysiology of Alzheimer's disease: advances in drug development and therapeutic innovations

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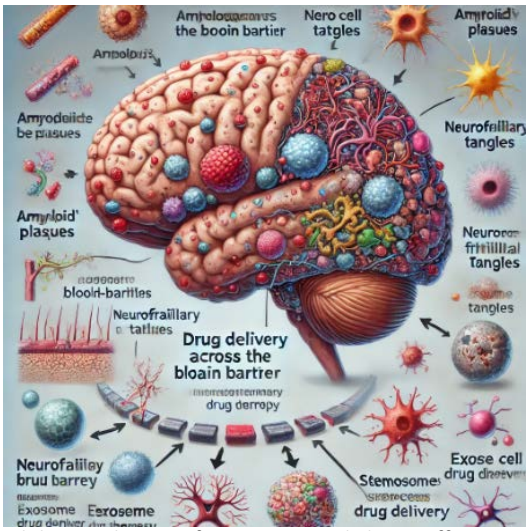
Review Article

ABSTRACT



Alzheimer's disease causes cognitive decline, and drug discovery focuses on its pathophysiology. Current research is concentrating not only on amyloid β markers and tangles but also on synaptic defects, mitochondrial dysfunction, and inflammation as key therapeutic targets. Treatments for the amyloid pathway include monoclonal antibodies (e.g., aducanumab and lecanemab) to remove formed Aβ. Ongoing preliminary studies of small molecules and beta-secretase inhibitors are indeed targeting Aβ production to interrupt this toxic cascade. Small molecule strategies involve using small molecules and antisense oligonucleotides to reduce tau hyperphosphorylation and its levels. Future treatments for neurodegenerative diseases aim to modulate microglial activation and cytokine signaling using potential NLRP3 inhibitors, for example, to balance the immune response. Biological mesenchymal stem cells (MSCs) and neural progenitor cells (NPCs) are being discussed for neurodegenerative treatment and stem cell therapy. Nanotechnology has improved drug delivery across the blood-brain barrier, resulting in better targeting and reduced toxicity. Gene editing via CRISPR-Cas9 gene therapy targeting the genetic basis of Alzheimer's disease is on the horizon. Biomarkers are assisting in identifying and monitoring treatment response in Alzheimer's disease, while novel uses of FDA-approved drugs offer new treatment options, improve management strategies, and potentially enhance patient outcomes.

Keywords: AD, neurodegenerative conditions, innovative strategies, drugs and therapeutics.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease that results in the deterioration of cognitive abilities as well as other aspects of a person's behavior and efficiently. It is closely associated with amyloid-beta plaques, tau protein tangles, and neuroinflammation. AD is the major cause of dementia, revealing the increased vulnerability of the aging population. It represents one of the worldwide social and medical issues that have significant economic consequences. Economically, AD incurs billions of United States dollars in annual health care costs and lost productivity. As longevity becomes a reality in the global society, and considering the fact that AD will continue to affect these older populations in the future, considerable efforts should be made to establish a better understanding of the illness and to develop improved prevention, diagnostic and therapeutic methods. Around 55 million individuals were estimated to have dementia in 2022, 8 million more than in 2015. Predictions indicate that this number could rise to 75 million by 2030 and 132 million by 2050. The global costs of dementia increased to $818 billion in 2015 and are projected to reach $2 trillion by 2030.[1] In 2015, dementia influenced 10.5 million Europeans, projected to rise to 13.42 million by 2030.[2]

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In terms of global gross domestic product, this registers at about 1%, since the yearly costs related to their care amount to over $600 billion due to dementia, primarily caused by Alzheimer's and cerebrovascular diseases, which tend to affect approximately 35 million sufferers.[3] AD is a significant disaster. In addition, some restrictions make Alzheimer's research more difficult, urgent, and important, with social anxiety as one of the possible aftereffects of its complex nature. Although the disease is solely a subject of research only, the 2021 Budget Request from NIH is expected to exceed $700 million.[4]

In 2022, approximately 55 million people worldwide were affected by dementia, up from 47 million in 2015.

AD is known to involve numerous interlinked antipathogenic processes interlinked with one another, which ultimately result in neuronal loss and dementia. The main features of AD pathogenesis are the amyloid plaques, which arise from the accumulation of amyloid-beta (Aβ) peptides in the intercellular space; Aβ peptides originate from the amyloid precursor protein (APP) after it has been cleaved by proteolytic enzymes. Simultaneously, hyperphosphorylated tau protein forms both soluble and insoluble oligomers that accumulate in neurons, creating neurofibrillary tangles (NFTs) that disrupt with microtubule stability and affect intracellular trafficking. The pathology of these events is associated with ongoing neuroinflammation resulting from microglial activation and astrogliosis, alongside increased synthesis and release of pro-inflammatory cytokines, which intensify neuronal injury. Other factors contributing to this relentless disease include synaptic loss, oxidative stress, mitochondrial dysfunction, and a compromised BBB. These factors collectively result in the gradual reduction of synaptic connections, neuronal death, and shrinkage of brain volume, particularly in memory and brain control circuits: the hippocampus and cortex.

Understanding these interconnected pathways is essential for the development of treatment strategies against AD. The clinical manifestation of AD is characterized by the appearance of abnormal aggregates of proteins-amyloid plaques and neurofibrillary tangles, as well as functional disturbances in synaptic plasticity and neuroinflammation.[5] Impaired autophagy, the cellular process by which damaged organelles and misfolded proteins are degraded, is a critical cellular process contributing to the progression of the disorder. The evaluation emphasizes the role of both bulk and selective autophagy in AD pathogenesis and explores potential therapeutic strategies that induce autophagy to address the disease.[6] AD intervention has largely been focused on amyloid-beta, but not much of that has translated into success in the clinic. The new focus seems to be on the neuroinflammatory processes that involve the activities of cytokines and chemokines, the activation and provocation of the complement system, involvement in oxidative stress, and the pathways of cyclooxygenase, among others in progressing AD, in terms of brain protection, microglia, astrocytes, and oligodendrocytes are partners. Learning about these inflammation and immunoregulation pathways may facilitate the development of novel anti-inflammatory therapies for the postponement of AD.[7] The majority of clinical drug studies for AD have been halted mid-trial due to low efficacy or serious side effects, rather than clinical studies on current medications providing only symptomatic relief.

Recent FDA approvals of aducanumab and lecanemab indicate potential disease-modifying effects, although their long-term efficacy and safety require further validation. The assessment highlights the current understanding of AD pathogenesis, advances in diagnostic biomarkers, updates on clinical trials, and emerging drug development technologies, including selective inhibitors and protein-protein interaction modulators.[8] AD is primarily characterized by extracellular neuritic plaques and intracellular neurofibrillary tangles, formed from aggregated β-amyloid (Aβ) and hyperphosphorylated tau protein. Current drug development has focused on Aβ-directed therapeutics with two FDA - cleared drugs, although the efficacy and safety of these drugs are still under dispute. Greater focus is now being placed on tau as a therapeutic target, based on its strong link to cognitive dysfunction.

Alzheimer's disease is linked to neuroinflammation, and there is evidence that shows a bidirectional connection between tau pathology and inflammatory events.[9] Present-day research has principally aimed at probing Aβ assembly and the resulting neurotoxic effects, but little is known about the related biology. The function of other extracellular proteins that deposit in plaques is also discussed; their relation to Aβ and their potential involvement in AD pathogenesis, as well as the techniques used to examine the incorporation of these proteins into plaques, are also described, contrasting macroscopic approaches on post-mortem tissue with molecular biological techniques. The identification of connections among Aβ-interacting proteins appears to reveal significant enrichment of functional and structural keywords; overall, the current findings contribute to the existing literature by providing insight into the relationships inherent in AD.[10]

Neuroinflammation is also associated with AD, and evidence indicates a bidirectional relationship between tau pathology and inflammatory events.

Oxidative stress in AD patients is linked to various pathological phenomena due to three primary factors: the dysregulation of transition metal homeostasis in the brain leading to an increase in Aβ interaction, the increased activity of certain oxidases including NADPH oxidase and MAO-B, and the effects of mitochondrial dysfunction. Aβ peptides are proven to cause ROS production and are responsible for this effect. Aβ oligomers increase ROS production through NADPH oxidase and modulate NMDARs by releasing arachidonate involved in synaptic

plasticity. Furthermore, metal-Aβ complexes like Cu and Fe exhibit the ability to produce ROS via Fenton reactions, according to various investigations. Thus, oxidative stress results in cyto toxicity and further contributes to mitochondrial dysfunction; it has been previously reported that AD patients have reduced numbers of morphologically normal mitochondria (Figure 1). Reduced electron transport chain activities mean that the rate at which ROS accumulates increases , hence increasing oxidative stress.[11]

Pharmacological treatment of AD faces multiple challenges and origins. The greatest challenge is the unclear understanding of the molecular mechanisms behind AD, which hinders the definition and validation of targets. Numerous clinical trials focused on amyloid-beta plaques and tau tangles have proven ineffectual, casting doubt on the efficacy of the aforementioned targets. However, the BBB raises another question of how potential therapeutics can penetrate brain tissue to exert their effects. Adding more confusion to trial design and analysis are patient variability, genetic predispositions, and even aspects of the environment and lifestyle. NeuroAIDS biomarkers for early detection and efficacy of antiretroviral therapy are poorly defined, leading to delayed treatment until extensive neuronal damage occurs. In addition, due to long cycles and high costs of AD drug development, along with the need for regulatory approval, there are many drug failures.

Overcoming these challenges calls for interventions that incorporate various delivery modes, modern drug delivery systems, and tandem medicine approaches based on patient characteristics. Speculation regarding the latest drugs to treat AD has been challenging due to significant preclinical neuronal loss from Aβ, tau protein issues, drug side effects, and poor clinical trial design. New molecular targets, biomarkers, and diagnostic methods, along with nonpharmacological therapies, can be particularly important for early-stage pathological processes. Tomorrow's medicine encompasses targeted ultrasound treatment and deep brain stimulation, stem cells, gene manipulation, and, of course, new drugs and shared daily habits aimed at preventing AD or other neurodegenerative diseases, or even curing them.[12] Problems involving pathogens in the central nervous system, as well as diseases and injuries that impair its function, affect both movement and cognition. Treatment is challenging due to the neurons' inability to regenerate. Biological processes in drug development face obstacles like the BBB, target identification, limited disease knowledge, unresolved

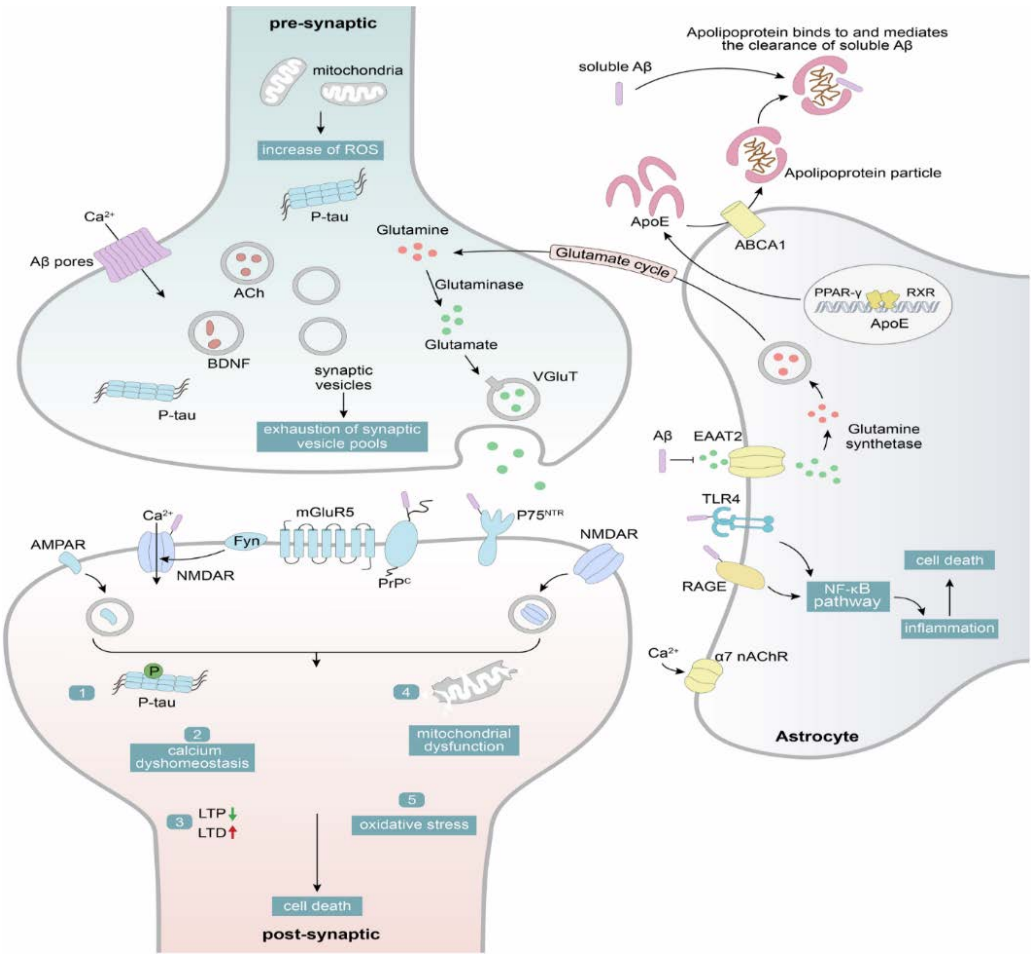

Figure 1. Aβ interacts with synaptic receptors, leading to calcium imbalance, impaired long-term potentiation, tau hyperphosphorylation, mitochondrial dysfunction, oxidative stress, and neuronal death. It also inhibits glutamate reuptake, causing accumulation and hyperactivity. Aβ and pro-inflammatory cytokines may transform astrocytes to the A1 phenotype, affecting their function and interactions, contributing to neuroinflammation and neuronal loss. Additionally, APOE released from astrocytes aids in Aβ clearance via lipoprotein binding. Reprinted (adapted) with permission from.[8]

clinical endpoints, patient variation, unpredictable preclinical models, and scarce biomarkers that require solutions.[13] Efforts should be made to speed up the detection and development of antiestrogen therapies for AD, as its burden on society is alarming. Analysts believe that only late-phase drug candidates may be available by 2025, and high attrition rates will remain an issue. The methods to address these issues include enhancing trial design, developing disease registries, and creating biomarker assays. The targets or goals for AD are enhancing awareness, increasing funding, fostering collaboration, and improving knowledge of AD biology.[14] Despite its wide use, the short-term outcomes are low, with a high prevalence of negative impacts and the incidence of having a rigorous treatment plans; the long-term side effects have not been fully established yet. Population-level access tends to be exhausted by broadening access and the gains per individual person.

Treating specific patient categories requires considerable input and involves costs linked to opportunity costs. Broadening access may dilute individual benefits due to Alzheimer's complexity. Implementing treatment in narrowly defined patient groups requires substantial resources, creating considerable opportunity costs and posing challenges even for well-funded healthcare systems.[15] Future AD research aims to develop targeted, personalized therapeutic strategies by addressing complex disease mechanisms such as tau pathology, synaptic dysfunction, neuroinflammation, and mitochondrial impairment.

Evolving Perceptions into the Pathogenesis of Alzheimer's Disease

Recent advances in the understanding of AD indicate that the disease is related to numerous genetic, molecular, and cellular factors, among which APP, PSEN1, PSEN2 mutations, and APOEε4 are recognized as established risk factors of AD, especially in familial early-onset cases. Amyloid-beta plaques and tau tangles are believed to cause neurodegeneration; however, other mechanisms such as neuroinflammation due to the actions of activated microglia or astrocytes, impaired mitochondrial functioning, oxidant-mediated damage, disrupted protein homeostasis, and reduced synaptic plasticity and integrity of the blood-brain barrier also contribute to neurodegeneration. New research elucidates how neuroimaging techniques such as MRI and FDDNP, along with omics technologies like proteomics, genomics, and metabolomics, contribute to pathological and clinical biomarkers as well as novel insights into AD progression and potential therapies. AD is characterized by amyloid β deposits and neurofibrillary tangles in the hippocampal region, differentiated into familial AD attributable to APP, PSEN1, and PSEN2 gene mutations, and sporadic AD connected to aging, genetic, metabolic, and environmental factors. Understanding AD is vital for developing treatments.[16] The amyloid beta (Aβ) hypothesis, proposed by John Hardy and David Allsop, has been central to AD research for decades.[17] It posits that Aβ deposition leads to neurofibrillary tangles, cell death, vascular damage, and dementia. The Aβ peptide results from the abnormal cleavage of the amyloid precursor protein (APP), a single transmembrane protein that undergoes α-, β-, and γ-secretase cleavage. The β-site APP cleavage enzyme (BACE) , together with γ-secretase , generates non-identical Aβ peptides, predominantly Aβ40 and Aβ42, which are crucial in AD pathology.[18] The main nerve cells that contain of tau are the primary element, and tau is well known as a microtubule-associated protein. Tau plays significant roles stabilizing neurons and the transporting nutrients.[19] In an AD context, tau is first and foremost, excessively phosphorylated, leading to a disruption in function and an abnormal propensity to auto-aggregate.[20] Thus, it generates insoluble paired helical filaments (PHFs) and straight filaments (SFs) which consequently enrich the pathology in the human disease.[21] AD is one of the conditions, which inflammatory processes play a major role in disease development.[22] Chronic immune system activation is another consequence of such neuroinflammation, as immense infection mediators periodically respond against certain cells like microglia and astrocytes, triggering the secretion of cytokines, nitric oxide, and reactive oxygen species, which , in turn, leads to the activation of inflammatory cascades.[23]

Oxidative stress is the imbalance between the production of reactive oxygen stress species in the body and the antioxidant defenses. Among them, oxidative stress has been proven to play a significant role in the pathogenesis and progression of AD. Mitochondrial activity and the resultant ROS formation render mitochondria susceptible to oxidative damage due to increased metabolic activity.[24] Mitochondrial abnormalities found in AD are altered distribution of energy, mitochondrial dynamics changes, reduced antioxidative capability, and amplified ROS production seen both in animals and in human brains.[25] The cognitive blood vessels are highly important in delivering oxygen and other nutrients to the brain, which are required for its functions. Hypoperfusion can result from vascular dysfunction, negatively affecting neuronal function. Vascular aging is a key factor in the pathology of AD.[26] Conditions such as cerebral atherosclerosis and cardiovascular diseases heighten the risk of AD. Chronic hypoperfusion leads to neuronal death and promotes the accumulation of Aβ peptides, which is characteristic of AD, while also impairing Aβ clearance from the brain, and worsening its aggregation.[27] AD includes several processes and alterations in the behavior of cells in the CNS.

Vascular dysfunction may lead to hypoperfusion, resulting in adverse effects on neuronal function.

Evidence shows that levels of Aβ peptide, synthesized in neurons, may increase due to enhanced production and/or reduced phagocytosis by microglial cells and astrogliosis. Apart from its neuronal localization, tau undergoes pathological PTMs and forms aggregates in response to pathogenic seeds. Astrocytes are known to play a role in tau spreading. This holds true by triggering neuronal dysfunction, glial activation, and neuroinflammation, which are governed by specific receptors for Aβ and the accumulation of tau. Among the genes involved are

early-onset APP and PS1/2, as well as late-onset mutations in APOE and TREM related to the development of the disease, along with aging and other factors.

Currently, no effective treatments exist for AD.[28] In systems biology focused on AD proteomics, technical challenges and unresolved scientific questions persist. Sample quality is influenced by confounding factors such as age, gender, postmortem interval (PMI), and ischemia, with modified proteomes being more affected than whole proteomes. Addressing these issues requires control experiments and regression analysis. Additionally, small sample sizes can lead to biased results. Human brains encode approximately 16,000 genes, which lead to hundreds of thousands of proteoforms via RNA splicing and post-translational modifications, such as Aβ and tau in AD.[29]

Insights into amyloid-beta (Aβ) and tau protein pathology

Amyloid-beta (Aβ) and tau protein are the two major protein deposits defining AD and are considered the core molecular mechanisms of the illness. Aβ pathology represents the cleavage of APP by beta- and gamma-secretases and the consequent deposition of Aβ peptides in the extracellular space. These plaques interfere with the physiological functioning of synapses, cause neuroinflammation, and directly lead to neurotoxicity. The hallmark of AD is abnormal phosphorylation; particularly, tau protein, a microtubule - associated protein, which is essential for the stability and organization of axons. Hyperphosphorylated tau is released from microtubules, forming intracellular neurofibrillary tangles (NFTs) that disrupt axonal transport and contribute to neuronal dysfunction. There is growing evidence indicating that Aβ and tau interconnect in a mutually pathological system, wherein Aβ pathology accelerates tau hyperphosphorylation. The spread of tau pathology follows a prion-like model, progressing along connected neural circuits. Understanding these pathological processes is transforming approaches to managing AD, including the design of drugs like secretase inhibitors, anti-aggregation agents, and immunomodulators, which specifically target these pathological processes in the hope of slowing or stopping their damaging effects.

Exploring the molecular structure of amyloid fibrils is vital for developing therapies. Misfolding and amyloidogenic aggregation of amyloid β (Aβ) peptide and tau protein are key features in AD. Despite challenges in obtaining structural information due to dynamic functional intermediates, high-resolution solid- state and solution-state NMR spectroscopy has been crucial in revealing structural characteristics. The brief lifetime of intermediates suggests motifs of therapeutic relevance and may lead to better intervention strategies.[30] Aβ is a small peptide composed of amino acids and is generated by many cell types in the CNS, including neurons, astrocytes, and platelets, highlighting its important endogenous function. Among these, neurons and smooth muscle cells exhibit the highest expression of Aβ. Aβ is produced through the enzymatic cleavage of APP, occurring via two pathways: one is the amyloidogenic pathway that produces Aβ plaques, and the other is the non-amyloidogenic pathway that does not form plaques. The high production of Aβ in the body and its elevated expression in certain cells demonstrate its significance in cellular functions and balance.[31] In AD, the accumulation of amyloid-beta (Aβ) activates kinases such as GSK-3β, CDK5, and MAPKs, leading to abnormal phosphorylation of tau protein and the formation of neurofibrillary tangles (NFT).[32] This process is further exacerbated by the breakdown of phosphatases, which should counteract hyperphosphorylation.

In AD, compared to healthy individuals, there is dysregulation of the α-secretase to β-secretase ratio. Increased β-secretase leads to more APP cleavage and high levels of Aβ, especially the Aβ42 form, which tends to aggregate.[33] The γ-secretase complex then cleaves the transmembrane part of APP, forming Aβ peptides, one of which possesses up to 43 amino acids and may be overly hydrophobic.[34] Aβ oligomers combine with cell membrane components, causing breaks in the membrane integrity to allow the entry of Ca^{2+}, which can stop the maintenance of LTP and lead to allow the eventual death of neurons.[35] Aβ oligomers localize the membranes of GM1 gangliosides and disrupt LTP, while cholesterol-rich lipid rafts support Aβ synthesis and membrane interaction. Increased β- and γ-secretase activity occurs in high cholesterol environments, whereas α-secretase is inhibited (Figure 2).[36] Cholesterol also influences Aβ seeding and aggregation, with cholesterol and GM1-rich rafts accelerating Aβ aggregation. Reducing cholesterol in endosomes or lysosomes improves Aβ aggregation and toxicity in mouse models.[37]
Proteins that belong to the Hsp70 family or are associated with chaperone family proteins have been found at elevated levels.[38] The crossing of the blood-brain barrier is regulated by the BBB, which manages soluble Aβ levels in the CNS, with transportation facilitated by receptors such as LRP1 and RAGE.[39] Additional receptors, including gp330/megalin and P-glycoprotein, also contribute. Vascular clearance and astrocyte activity are relevant to Aβ accumulation and toxicity and may thus influence AD in the cognitively normal elderly population. Strategies for reducing CNS Aβ levels include anti-Aβ antibodies and non-immune approaches.[40] Aβ can lead to dysregulation in tau phosphorylation and, thus, enhances tau oligomerization and aggregation, resulting in the formation of toxic tau oligomers, the immediate precursors of neurofibrillary tangles along this pathway.[41] Tau, when hyperphosphorylated, dissociates from the microtubules and ligates with CDK in a similar manner, allowing for aggregate formation. Aβ concurrently activates caspase-3 functions for tau fragmentation , generating a 17-kDa fragment that is highly aggregated, which results in neuronal damage, neurite degeneration, and cell death.[42] Tau tangles can act as "seeds , " inducing the formation of tau-tangle pathology, and they may represent forms of tau that are transferable between neurons.[43]

As presented earlier, tau oligomers mediate the neuroinflammatory responses, leading us to suggest that targeting their formation, including inhibiting caspase-3 formation may be valuable in abrogating the effects of tau pathology.[44] Brain aging and mitochondrial impairment could have specific effects on the onset of AD by altering the Aβ (amyloid β) and tau relationships with the disease. Evidence shows that the combination of aging,

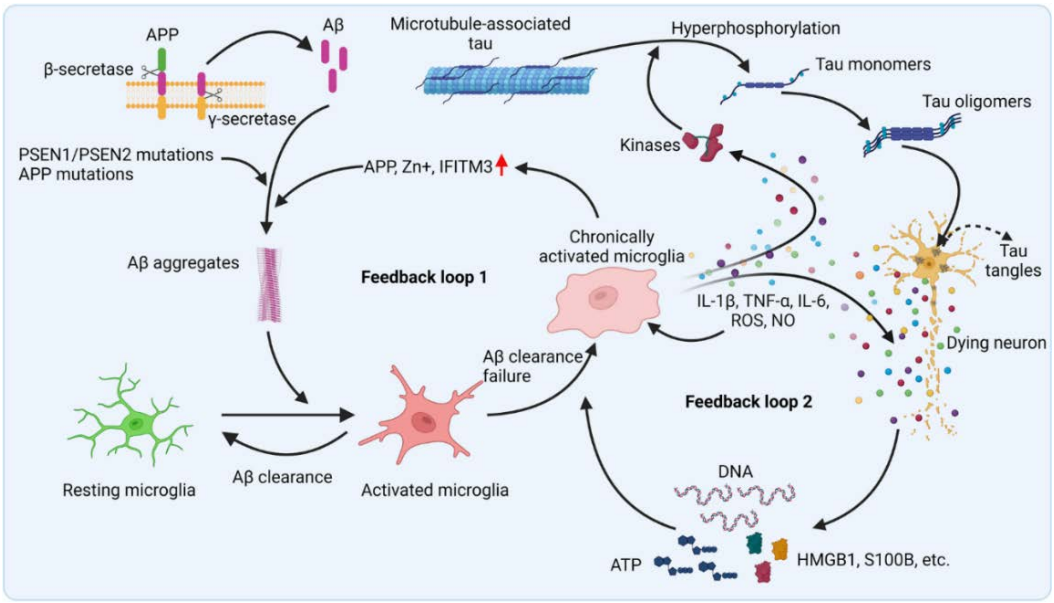

Figure 2. Abnormal cleavage of the amyloid precursor protein (APP) results in the retention of Aβ monomers at the earlier stages of AD. These monomers tend to aggregate because of genetic mutations and give rise to plaques. This aggregation activates microglia to clear Aβ, but if the clearance of Aβ fails, microglia will ultimately become chronically activated, leading to increased APP expression and to the release of metals like Zn+ into the extracellular space, initiating this loop. Hence, continual inflammation ensues. Activated microglia release proinflammatory cytokines and noxious products, culminating in neurotoxicity through an enhancement in tau phosphorylation, forming tau tangles while connecting Aβ aggregation with tau pathology. Reprinted (adapted) with permission from.[52]

Aβ, and tau causes mitochondrial dysfunction via pathways such as impaired oxidative phosphorylation and increased reactive oxygen species.[45] Tau pathology is essential for Aβ toxicity, as demonstrated by studies involving tau knockout mice and hAPPJ20 mice, which overexpress human APP, linked to familial early-onset Alzheimer's. Mice lacking tau genes displayed similar plaque accumulation but were protected from learning deficits and excitotoxicity, suggesting that reducing tau levels could mitigate cognitive decline in AD.[44] Accumulation of Aβ in clinically healthy individuals is not the sole cause of AD. Key roles are played by tau hyperphosphorylation, oxidative stress, and neuroinflammation, which may initiate Aβ accumulation . Recognizing neuroinflammation as a significant factor in AD pathogenesis suggests new therapeutic and preventive stratgies.[46] Cellular biosensors detect early tau pathology; animal models elucidate tau spread mechanisms and the stages of human tauopathy stages.[47] Biomarkers significantly enhance drug development for AD by aiding in diagnosis, predicting outcomes, and assessing treatment responses. Their changes indicate efficacy and safety, while accurate classification ensures valid trial results, emphasizing the need for comprehensive evaluations across multiple trials.[48] Nanoparticles are being explored for therapeutic approaches in AD by coordinating ligands to decrease amyloid aggregation and tau hyperphosphorylation, enhancing PET imaging and developing anti-AD reagents.[49]

AD is a complex neurodegenerative disorder influenced by genetics, aging, inflammation, chronic conditions and lifestyle factors. Early diagnosis and targeted biomarker-informed treatments are vital. Recent FDA approval of anti-Aβ monoclonal antibodies marks progress, highlighting the need for effective therapies.[50]

Role of neuroinflammation and microglial dysfunction

Neuroinflammation and microgliosis are key factors in the pathogenesis of AD. Microglia, the immune cells in the brain, are crucial for development and homeostasis, performing functions such as phagocytosis and synaptic pruning. In AD, microglia are activated by amyloid-beta plaques and tau aggregates, leading to the release of pro-inflammatory cytokines and reactive oxygen species, which exacerbate neuronal damage and disrupt the blood-brain barrier. Changes in the genetic microglial receptors affect the production of cytokines, including TREM2 mRNA, and impair Aβ and tau clearance. Understanding the dual roles played by microglia is vital for developing immunomodulatory therapies for AD.[51] The pathologic and clinical features of NDDs are somewhat similar yet different in some ways; particularly concerning the specific brain regions that are vulnerable and the broader issue of protein deposition.

Diseases such as Parkinson's and Alzheimer's are characterized by chronic inflammation, which was believed to originate from protein clumps. Signaling by a local immune response may initiate the phenomenon of increased protein deposition at disease onset. The immune system, for the most part, is composed of cells that repair tissue and promote the healing response: however, the inability to reverse inflammation might result in chronic disease states, which increase neurotoxic factor production and disease severity. Factors contributing to persistent inflammation include protein aggregates, environmental influences, and genetic susceptibility. Specialized pro-resolving lipid mediators play a crucial role in resolving inflammation. Inflammation appears before protein aggregation in neurodegenerative diseases; active STING expression can induce PD pathology. Anti-inflammatory therapy did not delay

disease progression, highlighting the complex role of inflammatory signaling, which can be both beneficial and detrimental. Effective inflammation-targeted therapies require careful consideration of timing, cell specificity, and target molecule selection.[52] Neuroinflammation is involved in the defense of neuronal integrity and homeostasis, as inflammation can help revive synaptic activity affected by trauma or infection. Its effectiveness is determined by the response that subclasses of cells, such as neurons, glial cells, and astrocytes, demonstrate. This process begins with the production of chemokines, tumor necrosis factors, pro-inflammatory cytokines, and reactive oxygen species (ROS) by astrocytes and microglia; systemic inflammation worsens the effect due to an impaired blood - brain barrier.[53] Both microglia and astrocytes are involved in inflammation in the CNS and can be activated into neurotoxic (M1, A1) or neuroprotective (M2, A2) phenotypes. This simple categorization does not account for the dynamic and variable nature of their phenotypes during the course of neurodegenerative diseases.

Specifically, understanding these roles is necessary for the development of therapeutic interventions.[54] The most abundant glial cells in the brain are astrocytes, and they regulate blood flow, maintain BBB, supply energy to the neurons, and adjust synaptic activities to protect the cellular environment.[55] They maintain and control ion and fluid concentrations, often clear away dead cells, and assist in tissue repair or scar formation. In fluids, the biomarkers include GFAP, S100B, and D-serine; imaging, MRI, and PET scans are utilized. Reactive astrogliosis is associated with CNS pathology , with the initial lesion having an adverse effect on spinal cord injury, and chronic inflammation being a causal agent of neurodegeneration.[54] Indeed, the gene expression of β-plaque-associated microglia is significantly altered through low-level homeostatic gene expression. Unfortunately, it remains unclear if this function of microglia toward neurons is affected by neuronal loss in AD, particularly in the Aβ-loaded zone they inhabit.[56] Such an investigation shows that the severity of the disease is associated with the two different forms of glia. In particular, this emphasizes on the importance of microglial homeostasis following early onset Alzheimer's and the need for validation of microglial gene alterations.[57]

Alzheimer's pathologies have been accelerating for some time, as there is increasing evidence showing that inflammation might be driving the disease. AD patients and AD models require further investigations into microglial signaling and receptors, along with the inflammatory response involving tau pathology onset.[22]

Advances in mitochondrial dysfunction and oxidative stress research

Normally, ROS are involved in intracellular signaling; however, their imbalance results in cell dysfunction and cell death. High levels of Aβ have been associated with mitochondrial damage and oxidative stress, thus playing significant roles in AD.[58] Mitochondrial dysfunction results in pathological cellular processes, encompassing heightened production of ROS and oxidative stress, calcium deregulation and apoptosis.[59] Observably, there are downregulated activities of complexes I, IV, and V along with those of the pyruvate and α-ketoglutarate dehydrogenase complexes in AD.[60] For instance, enzymes such as phosphofructokinase and lactate dehydrogenase often function at a lower rate compared to similarly aged brains that are not afflicted by AD. Concurrently, ROS activity increases. In transgenic mice overexpressing human APP, mitochondrial Aβ accumulates, correlating with decreased activity of ETC complexes III and IV, leading to reduced oxygen consumption.[61]

Oxidative stress is significantly elevated in the brains of individuals with AD compared to age-matched controls, indicating that AD is not a typical aspect of aging. Several factors are thought to contribute to oxidative stress that may increase notably at the onset or during the course of the disease. Mitochondria have a large influence on this process due to their role in energy metabolism and redox homeostasis, and any deviation from mitochondrial dynamics, whether genetic, metabolic, or environmental, can have repercussions for neuronal function.[62] Aging of neurons induced by mitochondrial dysfunction has been incriminated in AD, more especially the sporadic type, where mitochondrial dysfunction may be said to have a direct causal effect. In autosomal dominant AD mutation carriers, reduced oxidative energy production is a profound defect manifesting before clinical signs. Primary and secondary mounting mitochondrial dysfunctions have been evidenced in neurons suffering from energy failure, oxidative stress, and changes in the mitochondrial DNA. These abnormalities may vary from patient to patient but they all intensify one another in a vicious cycle; therefore, the need to develop specific therapeutic strategies.[63]

More and more effective and profound understanding is required to come across regarding research and development in relation to processes and mitochondrial alternations linking neurodegeneration to Alzheimer's disease as well as the molecular direction of therapeutic evolution.

Expressed primarily in muscle and adipose tissue, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is important for the regulation of mitochondrial biogenesis through genes transcribing the mitochondria. The results of the experiments show that PGC-1α levels are decreased in the brains of AD patients, possibly due to a shift in the signal transduction pathways such as the Aβ peptide, which reduces the synthesis of PGC-1 and increases NF-κB-mediated neuronal apoptosis. Furthermore, the transcription factors associated with the nucleus that regulate mitochondrial flicker, known as cardiac-enriched NRF1 and nuclear respiratory factor NRF2, are also decreased in AD; this has a cumulative effect on mitochondrial derived gene signaling.[64] Further and thoroughly understood research must be done regarding the processes and mitochondrial alterations in neurodegeneration associated with Alzheimer's disorder, in addition to directly targeting therapeutic advances. Structural and functional changes in mitochondria are associated with

neurodegenerative disorders, indicating the importance of maintaining mitochondrial dynamics for effective treatment to occur.

Emerging targets: synaptic dysfunction, BBB disruption, and lipid metabolism

Some of the crucial targets identified for the diagnosis and therapy of AD are synaptic dysfunction, damage to the BBB, and disruptions in lipid metabolism. Cognitive decline is also associated with synaptic dysfunction, as a consequence of amyloid toxicity, tau protein, and inflammation, along with reduced synaptic plasticity and a decrease in the number of dendritic spines.[65] BBB damage allows peripheral toxins and immune cells access, contributing to neurodegeneration. Dysregulated lipid metabolism, particularly involving apolipoprotein E (APOE), impacts amyloid formation and neuronal signaling, suggesting the need for polypharmacological treatments.[66] Lipid-related therapeutics for AD efficacy are heavily influenced by genetic factors, particularly the ApoE genotype. Treatments such as PPAR agonists and DHA supplements are more beneficial for ApoE4 carriers, while statins demonstrate greater effectiveness for ApoE4 individuals.

Therapy success may be restricted due to lipid dyshomeostasis in ApoE4 carriers. In fact, changing lifestyles could reduce almost 30-40 percent of dementia cases, benefiting nearly all nanocarriers. Additionally, some peripheral metabolic disorders and vascular profiles affect lipid metabolism in the brain, modulating responses to treatment. Outcomes considering individual genetic and non-hereditary factors should be pursued for optimal therapeutic results.[67] The lipid invasion model is depicted here , whereby increased BBB permeability also allows FFAs bound to albumin to enter the brain, leading to bioenergetic shifts, oxidative stress, and, finally, microglia-mediated neuroinflammation that results in anterograde amnesia. It also responds to the presence of large cholesterol-rich lipoproteins, which lead to endosomal-lysosomal system impairment and Aβ overproduction, forming amyloid plaques and neurofibrillary tangles, known hallmarks of AD. According to the proposed model, the BBB is the barrier preventing lipid access, and here, one can point out the damage to the BBB caused by excessive Aβ and other factors, such as aging, APOE4, and various AD risk factors, including metabolism, diet, and physical activity.[68] Astrocytic disturbances in lipid metabolism affect neuronal health through the imbalance of cholesterol homeostasis, neuroinflammation induced by peroxidation byproducts, and impairment of energy metabolism. They also undermine astrocytic clearance of Aβ and may finesse the pathophysiology of AD.

Targeting astrocytes, along with fatty acid and cholesterol modulation, presents new therapeutic approaches against the progression of AD. Key therapeutic targets include ApoE4 and lipid metabolism enzymes such as monoacylglycerol lipase and CYP46A1. Butyrate, produced by beneficial gut bacteria, shows promise in inhibiting neuroinflammation and lipid accumulation, enhancing mitochondrial fatty acid metabolism, reducing reactive oxygen species, and mitigating pro-inflammatory responses linked to AD pathology.[69] Dysfunction in lipid homeostasis is associated with a higher risk of AD and Parkinson's disease (PD), leading to altered lipid metabolism, protein accumulation, and oxidative damage (Figure 3).

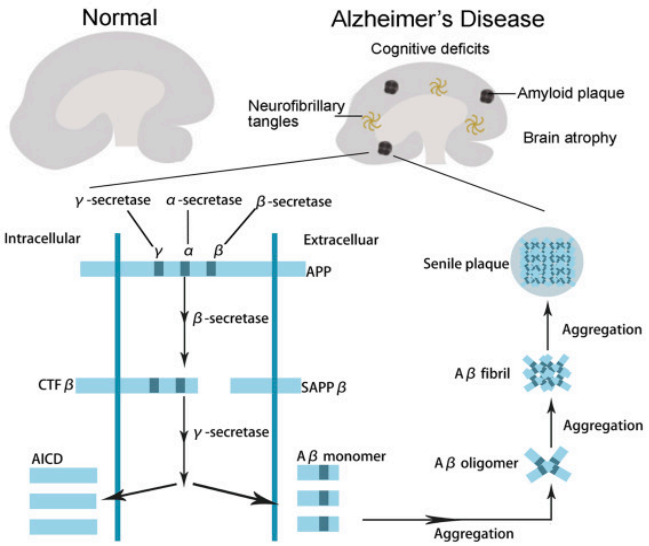

Figure 3. Main pathology of AD includes amyloid-β (Aβ) deposition, tau hyperphosphorylation-induced neurofibrillary tangles (NFT), and neuronal death. Amyloid-β (Aβ) is cleaved from the amyloid-β precursor protein (APP) by secretase cleaving enzymes called β and γ to form plaques in the brain. Excessive collections of Aβ with Tau hyperphosphorylation positively associate with neuronal death often reflected by the development of cognitive deficits and even brain atrophy. Reprinted (adapted) with permission from.[70]

In AD, there is an association between abnormal tau protein and β-amyloid (Aβ) deposition in lipids with a high content of cholesterol and sphingolipids.[71] In the pathogenesis of the disease, α-synuclein aggregation is the primary pathological lesion, with the further accumulation in lipids, including polyunsaturated fatty acids (PUFAs) and cholesterol, exacerbating α-syn misfolding, in relation to harmful effects on the mitochondria due to oxidative interactions with the lipid metabolites of oxidation.[72,73] Lipid peroxidation leads to ferroptosis, which then results in neuronal death. The upshot is the fact that lipid homeostasis is very much in the spotlight regarding to the molecular mechanism of neurodegenerative diseases.[74] Anti-diabetic drugs include medications such as metformin and GLP-1 receptor agonists. The regulation of enzyme activity could directly enhance some or all of the following functions: HMG-CoA reductase activity in the liver, serotonin release, and neuronal firing rates. Monoclonal antibody mechanisms that target lipid metabolism lipoproteins may lead to improved methods for enhancing lipid trafficking capabilities or even for improving microglial functions in preventing or treating AD and PD.[75]

Genetic etiology of AD and related dementias

Two-stage genome-wide association studies in 111,326 dementia cases of AD and 677,663 controls discovered 75 risk alleles, of which 42 are newly identified alleles. Pathway analysis further substantiates the roles ascribed to amyloid/tau and microglial pathways: Gene prioritization has led to the

identification of 31 genes involved in brand - new processes, especially the tumor necrosis factor alpha pathway. A new genetic risk score demonstrated a 1.6- to 1.9-fold increase in AD risk based on deciles, alongside age and the APOE ε4 allele effects.[76] The Aβ hypothesis, established from the identification of Aβ plaques and pathogenic mutations in key AD genes, posits that the accumulation of extracellular Aβ plaques is the primary cause of AD pathology. For the last 25 years, the theory has dominated research, suggesting that Aβ plaques give rise to disease-related processes such as inflammation, tau tangle formation, and synaptic dysfunction. Controversy arose when it became apparent that Aβ plaques were present in some cognitively normal elderly subjects, while absent in a few AD patients, thereby indicating a potential pathology unrelated to Aβ. Additionally, the correlation between Aβ plaque burden and disease severity was weak, and clinical trials targeting Aβ have been unsuccessful. Only 5% to 10% of early-onset AD cases are linked to known familial mutations, hinting at the involvement of alternative pathways in the onset of AD.[77]

Advancements in genetic and genomic technologies over the past thirty years have enhanced the understanding of AD genetic architecture. The most critical genes, such as APP, PSEN1, PSEN2, and APOE, play a vital role in determining an individual's susceptibility to certain diseases, while other critical genes confer less susceptibility to these diseases. Novel technologies today allow the genome-wide sampling of genetic variants and their linkage to biological effects brought about through roles in RNA, protein expression, epigenetics, and genomic interaction. Notably, AD-related genetic variants are concentrated in regulatory elements active in human immune cells.[78] Wightman et al. increased their GWAS sample to 1,126,563 participants, including new biobank and dementia datasets, while Bellenguez et al. expanded their study with data from the European Alzheimer's and Dementia BioBank and additional cohorts, totaling 788,989 participants.[76]

Epigenomics acts as a key moderator for genetic control of gene expression profiling in the genomic context. Consequently, catecholamine concentration is determined in tissue lysates of adrenal glands by the most established analysis.[79] The development of predictive models can be a powerful aid for developing therapeutics to target risk genes, and for validating experiments.[80] Myeloid and microglial cells are influenced by risk variants associated with AD, affecting disease susceptibility through pathways involving cholesterol metabolism, endocytosis, phagocytosis, and the innate immune system. Key genes linked to these pathways include USP6NL, which is involved in endolysosomal membrane trafficking, and rare variants in myeloid-specific genes such as TREM2 and ABCA7, which are critical for efferocytosis and clearance mechanisms in microglia.[81] Rare variation is a potential source of missing heritability in complex traits, as such; variants are under strong selective pressure and remain low in frequency.

Genome-wide association studies (GWAS) commonly revolve around common and low frequency polymorphisms using array-based SNP genotyping and statistical imputation. Second-generation sequencing techniques, such as whole genome sequencing (WGS) and exome sequencing (WES) , are indeed well - suited to detecting rare genetic variants ; however , cost has limited the generation of studies with adequate power.[82] The majority of genetic data is drawn from individuals of European descent, and most research is conducted with low racial and ethnic density populations. This underrepresentation also applies to AD studies, in which African Americans and Hispanic Americans are more likely to develop Alzheimer's than non-Hispanic Whites. This study, like others, suggests that Asian Americans have the same prevalence of Alzheimer's as that of non-Hispanic Whites. These factors may also reduce the generalizability of research findings across populations , given that genetic architecture and linkage disequilibrium patterns may vary across subpopulations, again contributing to health disparities affecting African and African-derived populations that do not benefit from novel Alzheimer's discoveries.[83]

Exploring genetic variations linked to the presentation of AD in traits, observable biomarkers, or other phenotypes increases the statistical power of research due to reduced trait heterogeneity. Genome-wide association studies (GWAS) on cerebrospinal fluid (CSF) and neuroimaging biomarkers, despite sample size limitations, have identified significant genetic loci related to AD. Large blood-based AD biomarker datasets promise improved genetic variant identification.[84] Recent discoveries in AD genetics emphasize microglial efferocytosis and APP metabolism, though much remains unknown. Large-scale whole exome and genome sequencing (WES/WGS) and increased diversity in genome-wide association studies (GWAS) will identify rare variants. Blood-based biomarkers will enhance diagnostic precision and facilitate the discovery of genetic variants linked to AD mechanisms.[80]

Therapeutic Strategies Targeting the Amyloid Pathway

Disease-modifying interventions in AD focus on modulating amyloid burden and the toxicity of apolipoprotein amyloid-beta (Aβ), which is central to the pathophysiological process. Strategies involve preventing the enzymes beta-secretase (BACE1) and gamma-secretase from processing amyloid precursor protein (APP) in order to prevent the formation of Aβ. Monoclonal antibodies (e.g., aducanumab, lecanemab) targeting immune therapies for AD aim to enhance the removal of Aβ plaques or inactivate soluble toxic Aβ oligomers (Figure 4). Small molecules and peptide-based applications that can prevent Aβ aggregation or dissociate preformed plaques have also been explored. Furthermore, Aβ clearance mechanism interventions seek to increase microglial phagocytosis or to manipulate other receptors, such as TREM2, to improve innate immune function. These challenges are why, even now, the amyloid pathway though difficult to prove clinically effective in the later stages of AD remains a primary focus of AD treatment. Identifying factors that can contribute to patient population choices and treatment combinations can increase the effectiveness of amyloid-directed therapies.[85] The approval of aducanumab sparked debate in the AD field over expected benefits, patient suitability, therapy logistics, and risk-benefit ratios.

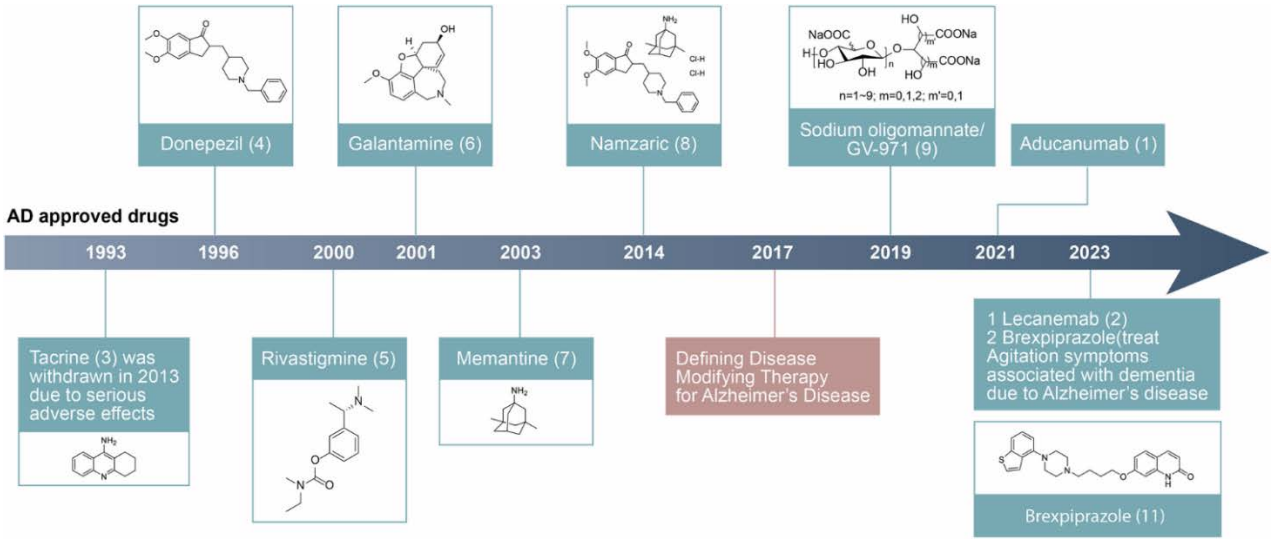

Figure 4. FDA and China's approved Alzheimer's drugs include disease-modifying therapies defined in 2017. Reprinted (adapted) with permission from ref [8].

Unique characteristics of similar anti-amyloid medications complicate comparisons. Key areas for rational drug selection include pharmacodynamics, pharmacokinetics, tolerability, and costs, although evidence for effectiveness remains limited.

Anti-amyloid antibodies (e.g., aducanumab, lecanemab)

Aducanumab and lecanemab belong to a new class of immunotherapeutic agents targeting Aβ in AD. These monoclonal antibodies selectively target Aβ species, which can then be internalized by microglia, phagocytized, and thereby decrease the levels of Aβ burden in the brain. While aducanumab binds to both soluble and amyloid fibrillar Aβ species, including plaque types, lecanemab exhibits a higher binding affinity for soluble Aβ protofibrils. These antibodies have been shown in clinical trials to lower amyloid deposition in the brain, and while there have been modest improvements in slowing cognitive deterioration, controversy remains. Currently, their applicability is hindered by safety issues, namely amyloid-related imaging abnormalities (ARIA), cerebral edema, and microhemorrhages. Nonetheless, the approval of these therapies represents an important advancement in AD treatment, demonstrating the amyloid hypothesis and paving the way for future work in other immunotherapies with greater efficacy and relatively lower toxicity.[86]

To maximize the effectiveness of anti-amyloid drugs for AD, healthcare systems should implement biomarker-guided pathways for early detection and manage later-stage dementia symptoms. Investment in three crucial sectors: diagnostic tools, access disparities, and workforce capacity expansion, along with patient involvement in the design of their care, is essential. Private manufacturers are likely to create an original pricing policy for all existing and potential product beneficiaries.[87] The Lecanemab intervention achieved the threshold for statistical significance in CDR-SB score reduction from baseline at 18 months when compared with placebo in phase 3 trials. Other secondary clinical endpoints were also found to favor lecanemab. The drug selectively targets toxic soluble aggregated Aβ species, showing lower amyloid levels (22.99 centiloids) in treated participants compared to the positivity threshold of 30 centiloids. In amyloid, tau, neurodegeneration, and neuroinflammation markers, lecanemab outperformed placebo, though NfL exhibited slower changes. The trial surpassed the defined target for clinically meaningful effects, with a treatment difference of 0.373 points. An ongoing open-label extension study will further investigate lecanemab's safety and efficacy beyond 18 months.

Lecanemab is a humanized monoclonal antibody targeting soluble amyloid-beta protofibrils, which are more toxic to neurons. In a phase 2b trial with 854 participants, no significant difference in a composite score was found at 12 months compared to placebo. However, at 18 months, lecanemab demonstrated dose- and time-dependent amyloid clearance and less clinical decline on some measures, with a recommended dose of 10 mg/kg intravenously every 2 weeks and a 9.9% incidence.[88] Aducanumab and lecanemab have been approved as anti-amyloid monoclonal antibodies (mAbs) for AD, while donanemab awaits approval; gantenerumab has been discontinued, and remternetug is currently in phase III clinical trials. They belong to a group of new mAbs aimed at enhancing pharmacokinetics, efficacy, and safety, along with increasing subcutaneous injection for a greater number of patients. Trontinemab is in phase II trials.[86] The 2021 FDA approval of aducanumab marked a significant milestone in the quest for a disease-modifying therapy for Alzheimer's, highlighting the complexities of developing treatments targeting amyloid-β peptide.

The amyloid hypothesis gained traction in the 1990s, leading to increased focus on amyloid-β42 as a therapeutic target. Previous immunization trials, including a landmark study carried out in 1999, suggested some advantages in reducing plaque and neurodegeneration in mice. Nevertheless, a 2005 human clinical trial was discontinued due to side effects, which remain a major issue in clinical research. The most recent data indicate that lecanemab can slow cognitive and functional impairment in early symptomatic AD; therefore, it potentially holds promise like

similar monoclonal antibodies to β-amyloid. However, these treatments are associated with different risks and involve considerable changes to routine clinical approaches. Recommendations for neurologists include methods for discussing the benefits of treatment with patients and criteria for selecting patients who will benefit, as well as considerations for surveillance. There is value in having patient-centered care with a special focus on patient and family involvement in the decision-making process, as well as interprofessional teamwork. The article also outlines the current state of knowledge gaps and potential growth areas for development to create an efficient treatment model.

Neurology practices must evolve to maximize patient outcomes with these new disease-modifying therapies.[89] Multiple anti-Aβ therapies developed for AD, including bapineuzumab and solanezumab, have failed Phase III trials, primarily due to ineffectiveness and safety concerns. Poor penetration of the blood-brain barrier has hindered earlier treatments. In contrast, several new therapeutics like aducanumab, lecanemab, and donanemab offer hope for amyloid reduction as well as a reduction in cognitive decline surrounding their central bond.[90] In 2021, the FDA approved an immunotherapy for Alzheimer's, specifically aducanumab, based on its impact on amyloid-β, which generated significant controversy. The European Medicines Agency rejected it, citing insufficient evidence and safety concerns, while Medicare restricted its coverage. Two additional anti-amyloid therapies displayed promising phase 2 results, and five are in phase 3 trials, targeting high amyloid clearance in early-stage Alzheimer's patients. Evidence regarding efficacy, safety, and economic impact is reviewed.[91] Alzheimer and Fischer, however, avoided providing conclusive evidence that amyloid plaques were part of the neurodegenerative process of dementia. This notion that amyloid plaques produce AD started in the 1960s because of their work and current research pointing to amyloid plaques in dementia pathology. The continuing controversy does not detract from the fact that, based on data from preclinical and clinical investigations, as well as brain amyloid imaging, Aβ contributes critically to the early pathogenesis of AD.

Amyloid research postulates that the progressive accumulation of Aβ results in the disruption of neuronal structure and function due to tau protein aggregation.[92] Recent studies indicate that new anti-Aβ antibodies show statistically significant clinical effects in sporadic AD despite previous failures. A systematic review and meta-analysis revealed that these antibodies slightly attenuate clinical worsening and significantly reduce amyloid in PET scans. However, they also elevate the risk of ARIA-E and ARIA-H. Donanemab and lecanemab provided the largest benefits.[93] FDA-approved anti-Aβ monoclonal antibodies (mAbs) improved clinical outcomes and neuroimaging in AD patients, while increasing the likelihood of side effects. Lecanemab demonstrated better efficacy than aducanumab, providing hope for future drug development targeting AD's pathological mechanisms.[94] Discrepancies in the pivotal phase III trials EMERGE and ENGAGE for aducanumab have fueled controversy regarding its FDA approval. Both trials followed the same design to assess the drug's safety and efficacy in early AD, but the implementation of study protocols and premature terminations resulted in inconsistent results. Only the high-dose treatment in EMERGE demonstrated cognitive improvement. Biomarker studies indicated dose-dependent reductions in Aβ plaque and tau deposition, with modest correlations to clinical outcomes.[95]

Amyloid beta (Aβ) pathology is a key target for intervention in disease progression; however, most symptomatic AD trials have failed to demonstrate clinical benefits.

Aβ pathology is an important target for intervention in the progression of the disease, although most symptomatic AD trials failed to demonstrate clinical benefits. Just recently, many therapies demonstrated significant efficiency in addressing Aβ plaques or reducing toxic soluble aggregates; in fact, their action truly assigns these protofibrils a special significance concerning these potential toxicities.[96] Monoclonal antibodies (MABs) against amyloid have ushered in a new era in treating AD. There is already discussion of disease-modifying effects and additional incentives to guide drug development. These groundbreaking therapies require new social and medical care strategies to support their implementation, representing a major advancement in tackling the challenges related to brain health and aging populations.[97] Three key questions should steer the evaluation of Alzheimer's treatment drugs: (1) were trial analyses appropriate and supportive of efficacy claims? (2) Do treatment effects outweigh safety concerns and apply to a representative patient population? (3) Is there evidence of disease course modification with potential long-term benefits? Additional data is necessary for careful interpretation of existing results.[98]

B-secretase (BACE) and γ-secretase inhibitors/modulators

β-secretase (BACE) and γ-secretase inhibitors/modulators are subclasses of the currently targeted treatments for decreasing the generation of amyloid-beta (Aβ), which is considered the main causative agent of AD. The BACE1 inhibitors act by preventing one of the enzymes that cleave APP into Aβ peptides, known as β -secretase. At present, γ-secretase inhibitors/modulators address the enzyme complex that ultimately cleaves APP, thereby reducing the output of Aβ. Preclinical studies suggest that BACE and γ-secretase are ideal therapeutic targets for AD; however, clinical investigations of the respective inhibitors have provided limited cognitive performance benefits and off - target side effects resulting from interference with other crucial γ-secretase substrates like Notch, which plays an important role in cellular differentiation and proliferation. In this regard, modulators of γ-secretase could be a less hazardous approach to CACNG6 since they allow modification without complete deletion of the APP protein and, therefore, the other functions it has in the organism. These strategies, nevertheless, remain under exploration as part of the ongoing efforts for AD treatment and the search for disease-modifying therapies. The enzyme beta-site

amyloid precursor protein cleaving enzyme 1 (BACE1) was identified in 1999 and is critical in the production of amyloid-β (Aβ) monomers, particularly Aβ42, which has been linked to the pathogenic mechanism of AD. The high BACE1 activity in the brains of patients with AD highlights its role in the progression of the disease and makes it a potential target for pharmacological therapy aimed at reducing Aβ. BACE1 also influences synaptic plasticity, as knockout studies reveal complex neurological effects. Although BACE1 has failed in previous clinical trials, the significance of this enzyme remains. Further research is necessary to analyze the function of BACE1 and its interactions with Aβ in both normal and pathological conditions.[99] That is the reason it is so important to develop potential inhibitors of β-secretase in order to alter the disease's course.

New classes of β-secretase inhibitors are being developed due to the enhancement of the three-dimensional structural information of β-secretase with different compounds. Due to the high degree of sequence identity, the selectivity of the peptidomimetic inhibitors remains a problem; however, one can learn from the early peptidic inhibitors of other aspartyl proteases to create quite diverse inhibitors. Furthermore, for a compound to cross the BBB, the inhibitors must be of low molecular size and should not be easily pumped out of the cells by P-glycoprotein. There is a clear trend toward the study of non-peptidic inhibitors , which are quite different from the first peptidomimetics and have better drug-like profiles.[100] Recent advances include β-secretase inhibitors capable of penetrating the BBB to effectively reduce Aβ concentrations in Tg mice; compounds, with some already in Phase I clinical trials, have been shown to decrease human plasma Aβ. Efficacy trials in the following years will be significant and can help to lay the foundation for amyloid reduction in Alzheimer's treatment, as past trials have not provided effective disease modification.

To achieve the same benefits of clinical trials, these new efficacy trials need to address rates of cognitive decline over much longer intervals while enrolling only early-stage AD patients in order to minimize confounding due to unrelated pathologies.[101] Through structure-based design strategies of transition-state analogues, there have been a few promising inhibitors have emerged as instructive, fostering optimism for the therapeutic importance of β-secretase as a beneficial target for Alzheimer's treatment.[102] Blocking the interaction between BACE1 and presenilin-1 (PS1) may effectively suppress Aβ generation without adverse side effects. High-throughput screening revealed that 3-α-akebonoic acid (3AA) disrupts this interaction and reduces Aβ production. Its structural analog, XYT472B, also demonstrates efficacy in reducing Aβ while preserving secretase activities and improving cognitive function in APP/PS1 mice. BACE1 exhibits maximal activity at low pH, aligning with its presence in endosomes, lysosomes, and the trans-Golgi network. γ-Secretase is active in the endoplasmic reticulum, at the cell surface, and within endosomal compartments. The processing of APP via the amyloidogenic pathway necessitates the internalization of APP from the plasma membrane and the localization of both secretases in endosomal environments. The interaction between PS1 and BACE1 likely occurs here, suggesting a complex regulatory environment influenced by certain G-protein-coupled receptors.

Efforts to develop anti-Alzheimer's drugs targeting γ-secretase and BACE1 have largely failed due to side effects, prompting the exploration of protein–protein interactions as therapeutic alternatives, highlighting compounds like 3AA and XYT472B that hinder the PS1/BACE1 interaction.[103] Extensive research indicated that β-secretase, identified as BACE1 (also known as memapsin or Asp2) between 1999 and 2000, exhibits high activity in neural tissue and neurons, with lesser activity in astrocytes. BACE1 predominantly cleaves amyloid precursor protein (APP) at Asp+1, with additional minor cleavage sites at Val-3, Ile-6, and Glu+11. Its enzyme activity is insensitive to pepstatin, and it is classified as a type I membrane protein with two aspartic protease active site motifs. BACE1 is highly expressed in the brain and pancreas, with neuronal expression surpassing that in glial cells. The levels of cleavage products altered significantly upon BACE1 modulation; transfecting BACE1 increased β-secretase activity and Aβ production, while using antisense oligonucleotides decreased activity and enhanced non-amyloidogenic APP processing, supporting its role in APP cleavage and Aβ generation.[104]

Physiological stressors and signaling pathways regulate BACE-1, contributing to elevated BACE-1 protein levels and enzyme activity in AD brains, despite generally unchanged transcript levels. Hypoxia and ischemia are crucial factors in AD risk; they influence amyloidogenic APP processing and elevate BACE-1 mRNA through hypoxia-inducible factor-1α pathways. Oxidative stress also increases BACE-1 expression through the c-junction N-terminal kinase pathway, and it depends on presenilin. BACE-1 can be activated by other factors, including traumatic brain injury and herpes simplex virus 1, together with the ε4 allele of apolipoprotein E4. Therefore, post-transcriptional networks are involved in controlling BACE-1, especially through the 5' UTR that acts as a repressor of translation. Jose et al. highlighted that a membrane - bound organelle, along with alternative splicing, influences translation rates in a tissue specific fashion. During stress conditions such as energy depletion, phosphorylation of the eukaryotic initiation factor-2α (eIF2α) increases to enhance the level of BACE-1. Furthermore, BACE-1 protein is regulated by both lysosomal and proteasomal degradation pathways , as well as lysine acetylation.[105]

Subsequent to the discovery of BACE1, ongoing work has been conducted to identify the bioavailability and resulting use of small molecule inhibitors of BACE1 that are capable of crossing the blood-brain barrier and inhibiting Aβ generation *in vivo*. However, this therapeutics are not yet available for general use for other types of AD. Furthermore, a BACE1 inhibitor has entered the stage of clinical trials, which will likely be beneficial; however, the dosage of this inhibitor must be handled very carefully to minimize or eliminate side effects. An appreciation of BACE1's non-amyloidogenic substrates implicates it in other pathophysiologies such as schizophrenia, raising questions about the development of new therapeutic approaches to AD and related diseases.[106]

Published reports of clinical trials using β-secretase inhibitors have been complicated by side effects and modest therapeutic benefit, and few have been associated with dementia. The role of three BACE inhibitors in amyloid-beta (Aβ) secretion and synaptic transmission was evaluated in a study. All three inhibitors affected synaptic transmission, though at concentrations that sharply reduced Aβ42 release. Lanabecestat maintained the decrease of Aβ40 and Aβ42 throughout the experiments and significantly influenced the reduction of synaptic transmission. However, co-treatment with LY2886721 could affect synaptic transmission only at high concentrations, while BACE inhibitor IV was found to increase synaptic transmission at lower concentrations. Mid-level BACE inhibition may enhance synaptic function through α- cleavage. Further research is needed to explore low-dose combinations and the role of other BACE substrates in treatment efficacy and safety for AD.[107] GSIs (γ-secretase inhibitors) attach to the active site of γ-secretase, limiting its cleavage function, which ultimately decreases the total production of amyloid-beta (Aβ). Many GSMs, including L-685,458, BrA-1-Bt, III-31C, DAPT, and Merck C57, along with GSI-based chemical probes, have demonstrated their value in research conducted on γ-secretase. A GSI photo - affinity probe has shown that these inhibitors bind effectively to the enzyme , thus providing insight into the mechanism of γ-secretase and its implications for therapy for AD.[108] GSIs can greatly contribute to understanding cellular physiology , as it will lead to the modulation of Aβ generation and ultimately to their potential to delicately balance these in the treatment of neurodegenerative disorders associated with Aβ accumulation.[109] GSMs are utilized as a smart and safe method to control γ-secretase activity. γ- Secretase actually binds with these compounds and is modified by GSMs in a way that only Aβ42 levels are impacted. In doing so , much of the Notch cleavage remains unaltered among the treated cell populations.[110]

First-generation GSMs, such as ibuprofen, reduce Aβ42 while increasing Aβ38. Second-generation GSMs, designed for potency and enhanced brain penetration, show varied results in clinical trials: Tarenflurbil failed, while E2212 exhibited a superior safety profile. Recent studies suggest that chronic GSM treatments can effectively reduce amyloid deposition and microgliosis in animal models, indicating potential for AD therapy.[111] Three classes of compounds, BACEi, GSM, and GSI, were tested on fAD patient iPSC-derived neurons. All reduced Aβ42 levels, but only GSI significantly altered transcription and affected Tau protein through Notch pathway inhibition. Similar effects were observed in wild-type mice, highlighting the relevance of patient-derived neurons for studying therapies.[112]

Amyloid aggregation inhibitors and clearance strategies

Small molecule modulators and Aβ disposal include the development of pathways that aim to reduce the toxic contribution brought by Aβ formation into plaques in AD by halting the formation of the Aβ backbone or by promoting its removal from the cerebral cavity. Small molecular targets and peptides targeting Aβ include small molecules that can directly regulate the aggregation of Aβ monomers or oligomers into cytotoxic fibrils and plaques. They hold the promise of preventing the generation of neurotoxic Aβ species that are involved in neuronal dysfunction and inflammation. In contrast, clearance strategies aim to improve the capability to eliminate initially existing Aβ aggregates from the brain. This can be achieved by enhancing microglial phagocytosis with the help of receptors discussed in the text, such as TREM2, or by increasing soluble Aβ clearance through CSF or by affecting BBB related pathways. The actual removal of Aβ plaques is also accomplished with immunotherapies, including monoclonal antibodies as examples. These strategies appear to be promising in preclinical and early phase clinical trials; however, key issues that exist include the inability to achieve efficient and long - lasting clearance without side effects, especially inflammation. There is, therefore, a need for further research on how to standardize these approaches in the treatment of AD.[113] Hippocampal AD and CAA share a common pathological feature of amyloid-β deposition with a decreased ability of the human body to clear the amyloid. Amyloid-β clearance is important, and there are diverse enzymatic and non-enzymatic processes involved. In peripheral organs, modulators include immunomodulation, immune cells, enzymes, and APP. It is therefore advisable to examine both centralized and systemic therapeutic approaches to remove extraneous amyloid-β.[114] The hope of this approach is to enhance the understanding of the molecular processes of the diseases and contribute to additional strategies to prevent amyloid-β production, which is beneficial for sporadic AD and CAA.[115] AD is the most prevalent type of neurodegenerative dementia, characterized by progressive cognitive decline.

Recent papers underscore oligomeric Aβ as the main culprit of synaptopathy, supporting the hypothesis in question.

Present therapies poorly manage the diverse and interacting pathological processes that can involve amyloid-β (Aβ), metal ions, oxidative stress, impaired neurotransmission, neuroinflammation, mitochondrial dysfunction, and neuronal loss. This review systematically presents the correlations between Aβ, metal ions, and other crucial enzymes involved in AD pathology to propose the use of multi-targeting approaches that interfere with both Aβ oligomer formation and other concomitant pathogenic processes to enhance the efficiency of AD treatment.[116] Low-molecular weight compounds that can prevent amyloid formation are divided into two groups by the review: natural and synthetic.

Natural compounds exhibit antioxidant, anti - angiogenic, and anti-inflammatory activity, showing highly effective anti - amyloidogenic activities in a non-specific manner and possessing higher binding constants. Stable hydrogen bonding inhibits aggregation with structural components in polyphenols, tetracyclines, and sterols. New-generation synthetic molecules based on naturally occurring molecules treat distinct amyloidoses, while high-throughput screening and computational approaches enhance inhibitors and antibodies. The

availability of 3D drug/protein structures is a boon to therapeutic design.[117] Peptide inhibitors are being investigated with the aim of inhibiting Aβ aggregation; however, it should be noted that only a few such inhibitors have progressed to clinical trials after promising results in preclinical tests. Among the more notable examples is NAP, which inhibited Aβ aggregation and had protective effects on neuronal cells but failed in a Phase III trial. PPI-1019 (APAN) completed Phase I and II clinical trials for AD, although the outcomes remain unclear. Additional peptides like D3, D-Trp-Aib−OH, and others have demonstrated efficacy in preclinical studies but have not reached the clinical trial stages. Current limitations for these peptides include poor BBB permeability and high cytotoxicity. Research is progressing toward peptide-nanostructure conjugates (PNCs) to address these issues , enhancing therapeutic effects , improving BBB permeability, and creating new opportunities in the treatment of neurodegenerative diseases.[118] The β cascade hypothesis proposed by Younkin in 1992 mainly suggested that Aβ is responsible for the onset of AD; thus, much has been written about Aβ synthesis, aggregation, and clearance pathways. BACE1 and γ-secretase are significant in the cleavage of Aβ, supporting those potential targets for treatment. However, many clinical trials focusing on Aβ have been attempted, and they have failed, which renders the hypothesis invalid. Cortisol analysis reveals amyloid plaques in AD patients but not in non-demented elderly individuals, raising further questions.

Recent papers emphasize oligomeric Aβ as the main culprit of synaptopathy, which supports the hypothesis in question. Several issues remain to be addressed, such as the side effects linked to ab screen targeting of secretases and the challenge of the BBB that hinders drug permeability, influencing their approval and efficacy. More rigorous clinical trials need to be designed.[119] New targets for anti-Aβ therapies have emerged as Aβ aggregation inhibitors, as it has been shown that certain potent compounds, such as Congo red, chrysamine-G, and curcumin, possess two aromatic or inositol moieties that are connected by an appropriate linker. This design enables exposure to Aβ protein residues, thereby improving the binding force. Future work must involve the identified subregions targeted by these inhibitors so that the terminal groups of the inhibitors suit the residue interaction and the linker spans subregions with no steric hindrance. However, inhibitors currently under development, like scyllo-inositol, are limited by their structure, which has only one terminal group that requires targeting a small area - the C-terminus. Specifically, the MTI enantiomer tramiprosate showed efficacy against Aβ but was discontinued for uncertain reasons. While most current inhibitors are singular and primarily target single subregions, inhibitors that can potentially control multiple subregions might do so synergistically. In this case, the ideal inhibitor would be flexible, appropriately sized, and contain multiple interacting groups to achieve increased binding efficiency and specificity. Thus, further dissection of the molecular structure of Aβ, along with knowledge about the aggregation modules required for function, will help achieve this goal. Perhaps an array of different inhibitors could be a strategy that might work in some capacity.[120]

More than twenty-five years after mutations in specific APP genes associated with the early onset of AD were identified; an agent exploiting the amyloid cascade hypothesis is still not available. Elevated levels of Aβ in the form of oligomers, due to overproduction or failure of clearance, increase the risk of disease progression and contribute to cognitive decline. The heterogeneity of AD requires a complex management strategy because we must not only target Aβ but also phospho-tau, inflammation, and synapse loss. These strategies should include diagnosis using existing biomarkers, improved methods for clearing toxic proteins from neurons , and the discovery of neuroprotective drugs.[121] The amyloid hypothesis posits that amyloid is the initiator of neuronal damage and neurofibrillary tangle formation as well as the underlying cause of cell death in AD. Aβ results from β-amyloid and γ-amyloid cleaving enzymes. Current research corroborates that Aβ should be an ideal therapeutic target for CAA and AD. There are Aβ-binding nanocarriers, especially liposomes and PEG-PLA nanoparticles, which are relatively non-toxic and biodegradable. Based on the literature, liposomes containing curcumin derivatives exhibit a strong binding propensity with Aβ fibrils while demonstrating potential to biochemically mitigate Aβ impact through PLGA nanoparticles conjugated with KLVFF peptide. Furthermore, D-FlexCuySe nanoparticles have the potential to facilitate Aβ depolymerization and improve cognitive abilities in experimental models of AD.[113] The effects of vaccination for AD are longer - lasting than those of passive immunotherapy. However, AD immunotherapy was initiated by Dale Schenk, who demonstrated that active immunization with Aβ42 influenced amyloid outcomes in mice and improved their cognition, leading to the development of the Aβ1-42 vaccine known as AN1792.[122] Nonetheless, Phase II trials were halted because T cell - mediated meningoencephalitis developed in 6% of cases. In numerous follow-ups performed, subjects' oral cavities or skateboards were free of plaque for up to 14 years; experts showed 88% plaque elimination upon autopsy, but there were no signs of enhanced cognition. Some initial analyses of the effects of immunization revealed that it reduced amyloid deposits and still had no influence on halting the regression of brain function, but raised safety concerns.

Scientists are working on various vaccines directed at the Aβ N-terminal segment to minimize side effects.

Scientists are working on various vaccines directed at the Aβ N-terminal segment to minimize side effects. Subsequent investigations demonstrated that immunizations using more global Aβ oligomeric epitopes reduced the markers of plaque burden and microhemorrhages in the AD animals, while also promoting cognitive performance without inflammation or neuronal loss.[123] Oxidative stress produces 4-hydroxy-2-nonenal (HNE) through lipid peroxidation, forming adducts with

proteases IDE and NEP, which hinder Aβ clearance in early AD. These HNE-IDE and HNE-NEP adducts degrade via the ubiquitin-proteasome (UPP) and lysosomal pathways, with UPP functioning without ATP hydrolysis. Measures regarding oxidative stress include augmented antioxidants and functionalized nanozymes, but the latter may inhibit natural enzymes at a low cost. Insulin resistance, which may also prevent free fatty acids from being broken down, contributes to increased ROS generation; there are ideas that specific enzymes might be targeted as an initial approach. New advancements involve modulators, including inhibitors and activators of JNK, PTP1B, and glucokinase, as well as activators of FBPase for insulin resistance. New developments in protein design through quantum mechanics/molecular mechanics calculations aim to enhance the accuracy of ligand binding and facilitate the understanding of the impact of HNE on Aβ clearance in early AD treatment approaches.[124]

TAU PATHWAY-TARGETING THERAPIES

Tau pathway-directed agents aim to address neurofibrillary tangles associated with AD, which result from the aggregation of hyperphosphorylated tau proteins. These therapies focus on reducing tau hyperphosphorylation, preventing further formation of tangles, or enhancing cellular clearance of existing tangles. Key strategies include using kinase inhibitors that target tau-associated kinases such as GSK 3β, CK 1, and CDK, alongside small molecules or antibodies that either prevent tau toxicity or inhibit tau oligomer and tangle formation. Additionally, immunotherapeutic approaches are under development, which include enhancing microglial phagocytosis of tau aggregates and passive immunization with monoclonal antibodies. Some compounds have shown promising results in preclinical models; however, clinical trials face challenges due to the complex functioning of tau in disease progression and the delivery of drugs to the brain. Despite these hurdles, tau-targeted therapies are considered a promising avenue for developing modulatory treatments for AD, encompassing inhibitors, immunotherapies, and tau silencing methods.

Tau aggregation inhibitors

Small molecule tau aggregation inhibitors aim to delay neurofibrillary tangle formation in diseases such as Alzheimer's and tauopathies. They bind to free hyperphosphorylated Tau protein, preventing its aggregation into toxic oligomers or insoluble fibrils. NW and NW-derived molecules, including small molecules, peptides, and antibodies, selectively bind to the microtubule-binding domain, preserving the native tau protein conformation and preventing self-assembly. These inhibitors are designed to avert tau aggregation and its associated neuronal dysfunction, synaptic loss, and neurodegeneration. While animal models indicate that tau fibril reduction may restore cognitive function, clinical trials have struggled to demonstrate consistent positive effects. Nonetheless, tau aggregation inhibitors are positioned to be foundational in developing disease-modifying therapies for Alzheimer's and similar conditions.

Tau aggregation inhibitors are intended to prevent or disrupt tau normalization followed by its aggregation; this is usually observed in neurodegenerative diseases. They can bind to tau and alter its shape, thereby affecting tau behavior directly or breaking the aggregated tau resulting from misfolded tau. An inhibitor, HMTM (high-lysine domain-binding tau-aggregated inhibitor), reduces tau pathology and behavioral impairments in genetically modified mouse models. It appears safe for human trials according to HMTM, which demonstrates efficacy with cognitive benefits at 16 mg/day in moderate AD. Curcumin and other inhibitors focus on the disaggregation of tau into individual molecules that should alleviate tau accumulation and its damaging effects on underlying brain cells. Tau or β-amyloid in AD might enter clinical practice based on efficacy and cost, especially in developing countries, as opposed to the expensive β-amyloid theory.[125] Over two decades, the development of Tau aggregation inhibitors has included molecules such as NQTrp-CL, cyanine, and methylene blue, all of which have the potential to reduce Tau aggregation in various ways. Despite their therapeutic potential, this is compromised due to lack of specificity and side effects.[126]

Natural compounds such as flavonoids, alkaloids, resveratrol, and curcumin can perform multifunctional roles, such as inhibiting tau aggregation and promoting the disassembly of existing aggregated tau. These inhibitors thus offer hope for neurodegenerative treatments; however, more studies need to be conducted on their mechanisms, along with safety and efficacy in preclinical and clinical settings.[127] With over 20 types of neuro diseases, this tau protein conforming to others relates to states of tau as it advances a completely new AD world. The VQIINK segment is more capable of aggregating tau than the VQIVYK sequence, leading to its partially obstructing aggregation.[128] Dosing selection is the greatest challenge in developing disease-modifying treatments for AD. For hydromethylthionine mesylate, 16 mg/day has been regarded as the appropriate dose based on pharmacokinetic studies. It turns out that an 8 mg/day dose, initially used as a control, had clinically relevant effects, while higher doses were ineffective.[129]

Tau phosphorylation and dephosphorylation modulators

Tau phosphorylation and dephosphorylation modulators are novel drug candidates aimed at correcting the aberrant regulation of tau protein associated with Alzheimer's disease and other tauopathies. Specific modifications of tau contribute to neurofibrillary tangles, impairing neuronal function. Key enzymes include ERK and CDK5 (tau kinases) and PP1 and PP2A (tau phosphatases). Chronic tau hyperphosphorylation causes tau fibril formation, and thus, modulators are being designed to restore the tau phosphorylation-dephosphorylation balance. Short-term treatments based on selective inhibitors of tau kinases or enhancers of phosphatases intend to reduce the formation of toxic tau oligomers and tangles. There are specific challenges in targeting tau regulation without significant side effects; however, this strategy has not lost its potential as a therapeutic approach in modulating tau-mediated pathobiology in Alzheimer's disease. A tau-targeting chimera named D20 was developed to recruit protein phosphatase 1, leading to the dephosphorylation of tau. This approach effectively reduces tau aggregation, enhancing neuronal plasticity and cognitive

functions in Alzheimer's disease mice.[130] A strategy for designing and optimizing DEPTACs was reported, leading to effective chimeras for dephosphorylating tau. D16, a highly effective DEPTAC, was validated for its functional mechanism in vitro and *in vivo*. Notable advancements include expanding the pools of DEPTAC constituents: five new PPRs (PP1, PP2A, and PP2B) were found to enhance tau dephosphorylation; the 8R cell-penetrating peptide outperformed 4R in membrane translocation; and four new linkers were added to the DEPTACs alongside the previous GSGS linker.[131]

The involvement of calcineurin in this process was examined using the calcineurin inhibitor cyclosporin A.

Hyperphosphorylation of tau contributes to AD. Although kinase-directed small molecule inhibitors have shown some promise in treating diseases, they face challenges in targeting the protein. Thus, the use of PhosTAC offers a better alternative that can specifically induce tau dephosphorylation and improve treatment outcomes.[132] This allows for the regulation of tau phosphorylation in NT2N control cells in a manner similar to that observed in fetal human CNS neurons. This tau from the NT2N cells resembles human fetal tau concerning isoelectric point migration on SDS-PAGE gels before and after alkaline phosphatase dephosphorylation. Interestingly, both sources of tau exhibit phosphorylation at the same residues (Thr181, Ser202, Thr205, Thr231, Ser396, Ser404) as identified by site-specific mAbs.[133] Tau post-translational modifications include phosphorylation and dephosphorylation, depending on the action of protein phosphatase type 2A (PP2A) in the brain. By blocking tau kinases, which promote the phosphorylation of tau, it may be possible to address this issue in disorders such as Alzheimer's disease AD. Since PP2A enzymes that phosphorylate and regulate the activity of tau kinase are downregulated in certain areas of the affected brain, the development of PP2A-overloading compounds is in demand.[134] Noncoding RNA, DNA methylation, and histone modification are involved in the regulation of tau protein phosphorylation. Nevertheless, the specific contribution of epigenetic modifications to tau hyperphosphorylation and NFT formation remains in question and is essential for targeted antitau hyperphosphorylation treatment.[135]

Resveratrol enhances several biological characteristics; it has a neuroprotective effect in Parkinson's disease, Huntington's disease, ALS, and other cerebral ischemia. It also has multiple effects in AD, primarily binding to amyloid-β peptide senile plaques.[136] Increasing intracellular Ca^{2+} levels through NMDA-receptor activation in rat cerebral-cortical slices caused tau dephosphorylation, evidenced by a 40% reduction in 32P incorporation.[137] This effect was blocked by the NMDA antagonist MK801. The involvement of calcineurin in this process was examined using the calcineurin inhibitor cyclosporin A.[138] New assays for measuring phosphorylated tau in cerebrospinal fluid and plasma aid in diagnosis and monitoring. In terms of therapies directed at tau, kinase inhibitors and immunotherapy appear to have value in reducing aggregation in Alzheimer's disease and tauopathies. Currently, there are few disease-modifying drugs that can successfully treat Alzheimer's disease, and tau phosphorylation is one of the goals.

Current approaches, which include the use of kinase inhibitors, phosphatase activators, and p-tau immunotherapy are in clinical trials to prevent tau phosphorylation and enhance the progression of the diseases.[139] Tau hyperphosphorylation at kinase-targeted phospho-sites was either dose-dependently or independently reduced in most inhibitors across different analyses, demonstrating its pharmacodynamics on tau. However, LiCl and SB239063 lacked biochemical analyses to reveal their impact on the cells. Up to 13 phospho-sites were analyzed under conditions of preclinical studies, but the deficiency of sites considered to be less than 16 decreases the abstract results and excludes the kinases.[140] Behavioral deficits in such models are reported, but generalized across AD, and these rodent models do not always exhibit specific, targeted pathologies such as NFTs.[141]

Antisense oligonucleotides (ASOs) and gene-editing approaches targeting tau

ASOs (antisense oligonucleotides) and gene therapeutic techniques targeting tau proteins are new interventions for AD and related neurodegenerative disorders. These are synthetic nucleic acids that either degrade tau mRNA or alter splicing patterns in a way that decreases tau protein production, which may prevent toxic tangle formation. CRISPR-Cas9 is a more selective approach that can delete or correct tau genes in DNA. These innovations may also serve as potential disease-modifying strategies, especially for familial tauopathies associated with gene mutations. Nevertheless, some issues such as brain penetration, toxicity, and long-term safety remain a concern for clinical application.[142] Altogether, these methods are characterized as essential advances in tau-directed strategies.

End-pointed tau and tau-VGG antibodies and vaccines target extracellular tau aggregation but will not prevent intracellular p-tau34. MAPTRx reduces tau production, which may decrease all forms of tau and prevent neuronal dysfunction. Analyzing CSF t-tau and p-tau presents certain difficulties stemming from tau elimination and unequal rates of tau synthesis depending on physiological states.[143] In gene expression modulation and the treatment of tauopathies, antisense oligonucleotides (ASOs), which interact with specific RNA sequences by targeting them, represent a promising form of intervention. Their development has progressed despite challenges, with six tauopathy-specific ASOs, including two in early clinical trials, advancing precision medicine.[144] Administration of ASO targeting Ttbk1 in PS19 tau transgenic mice effectively suppressed Ttbk1 expression while leaving Ttbk2 unaffected. At 8 weeks post-dose, ASO-Ttbk1 significantly reduced levels of phosphorylated tau epitopes linked to Alzheimer's, including pT231, pT181, and pS396. The analysis identified alterations in the microglial phenotype as well as the triggering of the interferon-gamma cascade with only minor consequences.[145] The discovery of an LNA ASO named ASO-001933 marked a major breakthrough due to the potent and selective inhibition of tau. It effectively reduced tau transcript

levels and protein levels in the mouse and non-human primate brains after the administration of an intrathecal dose, demonstrating good drug-likeness and significant treatment potential.[146] Antisense biotechnology makes it flexible to use in studying the biological system.

Splicing modulation of RNA, and changing the percentage protein isoforms can be manipulated by antisense oligonucleotides to block splice sites or regulatory elements.[147] More direct investigation into proteins means without going through downstream pathways, would yield better possibilities for therapeutic benefits in terms of neurodegenerative illnesses. ASOs might effectively reduce or modify protein output effectively.[148] The lack of disease-modifying therapies , due to a failure of more than 2,000 clinical studies on the pathophysiological basis of AD, calls for the development of combination therapies by enabling the successful development of a multi-targeted approach and the advancement of the synthesis of effective antisense oligonucleotides.[149]

EMERGING APPROACHES FOR MODULATING NEUROINFLAMMATION

The emerging therapy for AD aims to modulate the overall impact on neuroinflammation and the sustained activation of microglia and astrocytes, which exert toxic effects on neurons and patients. Microglial activation is a regular, beneficial function of the brain; however, over time, it can become neurotoxic due to the secretion of pro-inflammatory cytokines and other harmful substances. The latest strategies claim to treat M2 activation and enhance the safety of microglia and astrocytes by targeting the immune response, employing small molecules, biologics, and biologically derived agents with either anti-inflammatory or pro-resolution properties. Strategies include promoting the clearance of amyloid-beta and inhibiting factors such as NF-κB and inflammasomes. Additionally, there are initial investigations into the use of the gut- brain axis and the microbiome in managing neuroinflammation through available safe, non-invasive interventions. Their safety and effectiveness in clinical settings are yet to be determined. New therapies focus on improving both tissue healing and functional improvement. Cellular and molecular changes in the spinal cord affect immune function following spinal cord injury SCI. Manipulating immunological pathways during neuroinflammation is crucial for wound healing.[150] Neuroinflammation involves the activation of inflammatory pathways, including immune cell influx and the activation of microglia and astrocytes, along with mediator production. While these processes aid in tissue repair, they can also hinder axonal regeneration and cause neuronal hypersensitivity, ultimately impairing recovery.[151]

Microglia-targeting therapies (e.g., TREM2 agonists)

Microglial modulators, and in particular TREM2 agonists, are a novel treatment for AD and other neurodegenerative diseases. TREM2 is important in the development and function of microglia in relation to inflammation, the response to Aβ plaques, and tissue repair. The TREM2 gene has been shown to be associated with AD and has been involved in neuroinflammation and the progression of this disease. TREM2 signaling enhances the ability of microglia to clear toxic Aβ and to moderate inflammation. Although prior investigations of TREM2 agonism offered neuroprotection in animal models, phase I-III human clinical trials have been initiated to examine the treatment, prevention, and diagnosis of AD safely and efficiently. By inhibiting CNS microglial proliferation and enhancing survival, migration, and phagocytosis, TREM2 plays a crucial role in microglial function. Against AD, it works by eliminating overall neurotoxicity and modulating inflammatory profiles, mediating microglial functions and polarized signaling.[152] Under normal physical conditions, microglia remain quietly on standby, continuously observing the nervous system in search of damage.[153] They are now referred to as investigative or surveillance microglia, always prepared to adapt to changing environmental circumstances, with variable states and functions throughout, rather than assuming an "activated" state.[154] Recent advancements in single-cell technologies, such as scRNA-seq and CyTOF, have revealed various states of microglia in human and murine brains, and these states are relevant to development, aging, and illness. This indicates that microglial cells are heterogeneous and are influenced by intrinsic (e.g., genetic background) and extrinsic factors (such as pathogens and nutrition).[155] Previously, microglia were thought to exist as dormant cells, which can change their functionality according to the cellular environment. The following aspects highlighted here are their variability and interactions with other cells; participation in neurodegenerative diseases; the aspect of reprogramming; the phenomenon of peripheral immunity regulation; and their relation to aging.

AD pathogenesis involves neuroinflammation, whereby microglia serve as immune cells in the central nervous system.

Microglia thus prove to be very dynamic-they remain vigilant and do not come to a halt at their location. Furthermore, they swing along different pathways depending on stress states under healthy and disordered conditions. Some of the notable features include the following: (1) their great diversity; (2) their communication with all the other cells in the brain; and (3) their role as both beneficial and harmful agents in neurodegenerative diseases. Scaling up peripherally, gut microbiota and the microbiota-brain axis affect them, and microglial aging occurs.[156] TREM2 risk variants disrupt signaling through the receptor in neurodegenerative diseases, including FTD & AD, thereby affecting microglial function, phagocytosis, signaling, and its properties as biomarkers.[157] The remarkably early knockdown of Trem2 via antisense oligonucleotides in APP/PS1 mice results in significant reductions in amyloid deposition, particularly when administered during late-stage Aβ pathology. This study shows that Trem2 mRNA and microglial function are influenced by a single injection into the CNS, illustrating how this can inform TREM2-targeted therapeutic strategies.[158] The study investigated the role of TREM2 in microglial cell activation during retinal

degeneration using chemically induced and inherited mouse models. The present study found that the DAM signatory gene expression was significantly upregulated in both animal models. The fact that TREM2 was required for their expression in TREM2 knockout mice clearly illustrates that it is the activation of microglial cells that is TREM2-dependent and plays a crucial role in photoreceptor cell survival during neurodegeneration.[159] Genetic studies on late-onset Alzheimer's disease (LOAD) identified new druggable targets in microglia and CNS-resident macrophages, particularly focusing on TREM2 receptors. Antibody-mediated therapy targeting TREM2 has demonstrated promising preclinical results.[160] Cell signaling is mediated by the TREM2 protein's three regions: the ectodomain binds extracellular ligands, while the intracellular domain interacts with signaling proteins for cellular events. In 2014, Jin et al. identified three alternatively spliced TREM2 transcripts in the human brain using PCR.[161] These isoforms share identical sequences with full-length TREM2 concerning crucial structural components, suggesting that these homologous sequences play significant roles in TREM2 signaling functions.[162] Most researchers believe that sTREM2 has a neuroprotective effect by enhancing microglial clearance, while it can also stimulate microglia to release pro-inflammatory cytokines, negatively affecting neuronal function. Different body fluids exhibit varying levels of sTREM2 across the different stages of AD. Further studies are required to clarify sTREM2's functions and its role in AD pathology to aid in the development of new therapeutic strategies.[163] Disease-associated microglia (DAM) are a specialized microglial subtype linked to aging and neurodegenerative diseases like AD. They demonstrate increased phagocytosis, inhibit the growth of Aβ plaques, and may therefore play a protective role in AD. The study on the Trem2-/- 5XFAD mice indicated two activation stages of DAM: the global, Trem2-independent stage related to ApoE and Tyrobp; and the Trem2-dependent stage regarding phagocytosis and lipid metabolism. The relationship between Trem2, DAM activation, and lipid pathways highlights Trem2's influence on brain lipid metabolism.[164] Dozens of clinical trials targeting amyloid plaques have shown limited success. Controversy surrounds Aducanumab, a newly approved Alzheimer's drug, prompting a focus on microglial immune cells instead. Reduced TREM2 expression and activity impair microglial function, influencing AD progression. Human TREM2 loss-of-function (LOF) variants, such as R47H and H157Y, lead to more rapid dementia progression. In post-mortem AD brains, LOF variants demonstrate impaired microglial clustering. Increased cerebrospinal fluid sTREM2 correlates with slower AD progression, indicating that elevated TREM2 expression occurs as microglia enter a disease-associated state.[165]

AD pathogenesis involves neuroinflammation, whereby microglia serve as immune cells in the central nervous system. Under the influence of external stimuli, microglia differentiate into disease-associated microglia (DAMs) that are involved in processes such as Aβ aggregation and tau phosphorylation. The triggering receptor expressed on myeloid cells 2 (TREM2) is primarily expressed by microglia.[166] TREM2 influences microglial survival and migration, with *in vitro* experiments showing reduced survival rates of microglia in its absence. DAP10 activates AKT, inhibiting GSK3β to promote cell survival via the PI3K-AKT-GSK3β pathway. Focused trials have shown that soluble triggering receptor expressed on myeloid cells 2 (sTREM2) induces the activation of microglia, and subsequently, microglial viability is enhanced through the PI3K/AKT pathway. It is worth noting that, in addition to the elevation of sTREM2 in the CSF in connection with AD development, especially in the early stages, the increase is possibly due to the damage of nerve cells and activation of microglia.[167] Marco Colonna et. al. explored the effects of the antihuman TREM2 monoclonal antibody (hT2AB) and its murinized form (mT2AB) in the 5XFAD model with amyloid pathology.[168] hT2AB binds to TREM2 variants, crosses the blood-brain barrier, and modifies microglial states, indicating distinct signaling pathway activation compared to the control antibody.[169] Tetra-variable domain immunoglobulin (TVD-Ig) enhanced TREM2 activation, improving the EC50 of amyloid-β oligomer (oAβ)-lipid microglial phagocytosis by more than 100-fold. It also increased microglial migration and survival by 100-fold compared to bivalent IgG. The bispecific Ab2 TVD-Ig/αTfR antibody improved brain distribution, significantly enhancing microglia-plaque interactions and amyloid plaque phagocytosis in 5XFAD mice.[170]

Cytokine modulators and anti-inflammatory drugs

Medications used in the management of AD aim to moderate neuroinflammation provoked by chronically active microglial and astrocytic cytokines. Such interventions are cytokine modulators, which work to alter the production or function of cytokines TNF-α, IL-1β, and other cytokines, including IL-6. Delayed union has been managed by NSAIDs and corticosteroids that have demonstrated low efficacy in clinical trials; thus, the turn to the use of biologic agents such as monoclonal antibodies will follow. Novel treatment strategies for the identified molecular targets have been developed as treatments such as NF-κB inhibitors and inflammasome modulators. Challenges remain in drug delivery, toxicity reduction, and effective combination strategies for AD management. Anti-inflammatory therapies using non-steroidal anti-inflammatory drugs (NSAIDs) showed initial promise for AD, particularly after a trial with indomethacin. However, subsequent trials and meta-analyses have not confirmed their efficacy, with follow-up studies on naproxen and celecoxib showing no prevention of dementia or cognitive decline in at-risk older adults.[171] Microglia play a dual role in AD by mediating Aβ-induced neuroinflammation that worsens cognitive decline while also contributing to Aβ clearance, making them a potential therapeutic target. The drug addresses gut dysbiosis and neuroinflammation, expanding the BioModel far beyond the Aβ hypothesis. Current research exploring other possible treatments for AD is centered on neuroprotection, neurotransmitters, genes, mitochondria, and blood vessels. Prolonged neuroinflammation impairs learning and memory through neuroinflammatory mediators and immune cells of the nervous system , affecting neurogenesis and synaptic plasticity.[172] Microglia, phagocytic cells, ingest amyloid β (Aβ)

using various receptors, including CD-14 and TLR4. In AD, the most significant contribution to the accumulation of extracellular Aβ has resulted from impaired microglial function. Moreover, studies involving AD brains indicate that Aβ uptake in microglia near plaques is hindered. Recently developed PET techniques have been utilized to detect activated microglia by employing tracers that target the 18 kDa translocator protein (TSPO).[173] Pharmacological interventions that suppress inflammation to enhance neuropathology in AD are of major importance. Apparently, there is sufficient evidence to show destructive inflammatory processes and suggest the possible utility of anti-inflammatory drugs; nevertheless, confirmed beneficial effects still require substantial trial evidence.[174] Many pathways are implicated in the genesis and progression of AD: amyloid-β deposition, hyperphosphorylated tau protein, and cytokine-induced inflammation.

Elevated microglial activation, cytokines, reactive oxygen species, and NF-κB further drive inflammation. The review emphasizes the potential of natural compounds with anti-inflammatory effects to slow the progression of the disease.[175] The development of AD involves Aβ peptide accumulation, τ protein buildup, pro-inflammatory cytokines, increased microglial activation, and specific signaling pathways, contributing to neuroinflammation and disease progression.[175] Cytokines TNF-α, and IL-6 increase BACE1 activity and NFκB expression, generating Aβ in AD. Aβ interaction with microglia and astrocytes exacerbates neuroinflammation. PPARγ agonists and selective COX-2 inhibitors reduce inflammatory cytokines and improve the phagocytosis of Aβ.[176] Chemokines guide microglial movement and recruit astrocytes in neuroinflammation, influenced by the severity of local inflammation. Key pathways include NFκB, MAPK, and mTOR in microglial activation. Anti-inflammatory agents such as minocycline and iNOS/Cox-2 inhibitors can reduce neuroinflammation and associated pathologies in AD.[176]

Immune system modulation for neuroprotection

Immunomodulation in AD is based on using brain-associated immune cells to restore inflammation and support neuronal integrity. Microglia can also be in an abnormal state and hence contribute to inflammation and neuronal demise. Interventions aimed at improving the immune response or enhancing the removal of extracellular amyloid-beta deposits should not induce toxic inflammation. Analogs currently being evaluated include R-Cell Ab, cytokine-modifying agents, and numerous drugs comprising small molecules and monoclonal antibodies with inclusion of anti-inflammatory signals and activation of neuroprotective signals. Some of the critical issues involve drug penetration to the brain, focusing on inflammation and the timing of intervention with the immune system. Previous studies have shown how anti-Aβ antibodies and T cells respond in the cerebrospinal fluid, suggesting that AD has a certain connection with adaptive immunity. Some of the pathophysiologies of the brain glymphatic and meningeal lymphatic systems could contribute to Aβ and tau accumulation. The metabolism of immune cells also influences AD pathogenesis; specifically, autoimmunity is strongly associated with the disease. Present knowledge regarding the relationship between the immune system and AD is scarce. In this review, immunomodulation in AD and its effects on disease progression are discussed along with possible treatment options.[177] It is integral to modulate dynamically and transform inflammatory immune response for innovative therapy development. Notably, the inflammatory and neurodegenerative effects of TRAIL associate peripheral immune responses and the brain and are potentially involved in a deleterious over-activation of regulatory T cells (Tregs) in AD.[178] The damage signal hypothesis, of course, suggests that endogenous damage signals from cellular distress initiate AD by engaging innate immunity and inflammation. Different risk factors tilt the scale toward neurodegeneration, and the final common pathway involves microglial activation and the release of inflammatory cytokines, including tumor necrosis factor-α and interleukin-6, which cause neuronal changes and tau protein hyperphosphorylation.[179]

Recent studies show that innate immune genes and cells, such as T cells, may provide protection as well as a deleterious effect in AD neuropathogenesis. Genetic consequences, along with the interdependence of adaptive and innate immunity and immune signaling, are the next high-priority research themes in the etiology and pathology of AD. The development of therapeutics for AD has been difficult, with no disease-modifying drugs approved. Investigational drugs in Phase 2 or 3 clinical trials are summarized. The FDA's controversial approval of aducanumab, an immunotherapy developed by Biogen from memory B cells, suggests progress in targeting AD's pathological hallmarks. However, the interim analyses led Biogen to stop two late-stage trials with mild AD that were not showing any real improvement in memory performance.[180]

Micro and astrocytes are activated in AD, and this neuroinflammation amplifies the damage of neurons. The biological pathway involved in the gut-brain axis plays an important role in the brain, and phytochemicals from plants might contribute positively in controlling this pathway to prevent AD.[181] Flavonoids and polyphenols are phytochemicals as antioxidants credited with anti-inflammatory worth, and suggesting features in modulating neural inflammation related to AD.[182] Some tryptophan metabolites affect cytokines associated with innate immunity and prevent the formation of toxic oligomers, possibly in AD. It has been established that tryptophan levels decrease in the normal aging process as well as in AD, leading to impaired cognition. The therapeutic approach includes using the gut microbiome to modulate neuroactive metabolites and synthesizing small - molecule mimetics of tryptophan; however, little is specifically known about these strategies in relation to AD.[183]

THERAPEUTICS INVOLVING STEM CELLS AND REGENERATIVE MEDICINE

Stem cell therapeutics demonstrate the potential of AD treatment through fighting neurodegeneration and supporting brain regeneration. Strategies include the direct transfer of healthy neurons, encouraging the generation of fresh neurons from neural stem cells, and rebuilding neuronal functions in the

brains of affected individuals. MSCs and iPSCs both have the ability to lineage differentiate into neuronal phenotypes promote aspects of synaptic plasticity and synthesis of trophic factors that have anti-inflammatory properties. In addition, there is the general fact that stem cell-derived exosomes offer an opportunity for non-invasive delivery of therapeutic products. One typical question refers to the possibility of graft adaptation to the rest of the neural network in addition to the issues concerning ethical considerations and immune responses. Yet the use of stem cells in AD has potential difficulties before clinical utilization such as neurosurgery and immunosuppression. Tumor formation has been reported in different scientific investigations as issues with the stem cell proliferation control, targeting markers, delivery system and patient variability have been posed.[184]

Cell-based therapies to promote neuronal survival and repair

Cell factor therapies are regenerative medicine as they seek to support neuronal viability and regeneration in AD by introducing stem or progenitor cells into regions that can transdifferentiate into neurons and glial cells. Studied cell types include mesenchymal stem cells, induced pluripotent stem cells, and, of course, neural stem cells, which may increase neurogenesis, synaptic plasticity, and allow for the release of neurotrophic factors. These therapies can also externalize neuroinflammation, placing the brain in a neuroprotective state against amyloid-beta and tau pathological substances. While their potential is evident and many have shown positive results in cell culture and animal models, their clinical use presents challenges in delivering the agents deep into the tissue, and immune responses remain an issue for some of these therapeutic candidates. Yet, they constitute a true disease-modifying treatment for at least some forms of AD.

Neural stem cells are mainly found during early development; thus, they are derived from the nervous system and are capable of self-renewal. Their transplantation holds significant potential for the treatment of neurodegenerative diseases, but it is accompanied by ethical issues and tracking difficulties.[185] According to experts, cutting-edge research on AD refers to the induction of neuronal-glia communication, immune disorders, and risk genes, possibly indicating the use of stem cell models to alleviate pathologies through immunomodulation and neuronal support. Organoids and assembled spheroids are multicellular models used to study AD effects from genetic, chemical, and environmental factors, as well as to evaluate genetic engineering techniques, alongside humanized animal models for improved disease representation.[186]

AD is characterized by the accumulation of amyloid-β and phosphorylated tau, synaptic damage, and neuronal loss, influenced by genetic and environmental factors. Symptomatic treatments remain available according to today's medical practices; however, therapies such as stem cell treatments can self-renew and even differentiate. The brain deposits affect calcium balance and cause the increase in ROS, thereby leading to the death of neurons and inflammation. APP is cleaved by the action of β- and γ-secretase to generate Aβ forms, with Aβ-42 being the form that creates senile plaques in AD patients.[187] Cell technology is designed for an induced pluripotent stem (iPSC) cell that enables a screening platform for potential anti-AD molecules and for surveying mutations implicated in AD. There is a promising future in the pathway of neural stem cell (NSC) transplantation as well as mesenchymal stem cell (MSC) therapy for neurodegenerative diseases, which works through the secretion of growth factors and exosomes that reduce neuroinflammation. This study reviews recent advances and current challenges regarding these stem cell therapies in AD.[188] Mouse-derived neural stem cells (NSCs) show limited impact on cognitive recovery, prompting purpose-specific differentiation, unlike undifferentiated human NSCs. The transplantation of NSCs in Tg2576 mice led to reduced Aβ production and levels of acetylcholinesterase, which manifested as the repair of neurons supported by astrocytes expressing the α7 receptor. A further increase in anti-inflammatory cytokine levels within microglia enhances Aβ clearance and neurogenesis.[189]

Deposits in the brain disrupt calcium balance, stimulate ROS production, and lead to neuronal death and inflammation.

Mesenchymal stem cells (MSCs) and their neurodegenerative potential

MSCs reveal potential as a therapeutic approach to treat AD, being endowed with neurodegenerative effects, immunomodulation, and remarkable bone marrow endothelial cell regeneration and neuronal tissue regeneration. These include mesenchymal stem cells derived from bone marrow and adipose tissue, as well as Wharton's jelly-derived stem cells from the umbilical cord. They also undergo differentiation into neuronal-like cells that secrete neurotrophic factors critical for neuron survival and synaptic plasticity. MSCs may reduce the chronic inflammation linked to AD by modulating microglial and astrocytic behavior. They also release exosomes containing proteins, lipids, and RNAs that influence neurodegenerative processes. Animal studies indicate that MSC-based therapies can improve learning and memory while regulating Aβ deposition and tau phosphorylation, highlighting their potential in regenerative medicine for AD treatment. MSC-based stem cell therapy shows promise in treating Alzheimer's by modulating inflammation, promoting neuronal growth, and enhancing neurotrophin secretion.[190] In AD models, MSC therapy is proposed to reduce Aβ plaques and tau hyperphosphorylation, reverse microglial inflammation, and stimulate anti-inflammatory cytokines. It upregulates neuroprotection, enhances neurogenesis, alters immune responses by increasing protective cytokines while decreasing proinflammatory ones, and improves microglial function, neovascularization, and reduces oxidative stress.[191] Enhanced pro-inflammatory activation of microglia can lead to chronic inflammation and neuronal death. Studies indicate that human mesenchymal stem cells (hMSCs) can modulate neuroinflammation, reducing microglial activation and improving behavior in SOD1 mice. Transplantation of human umbilical cord blood-derived MSCs decreased pro-

inflammatory cytokines and increased anti-inflammatory markers in familial AD models, illustrating a shift from a pro-inflammatory to an anti-inflammatory state.[192]

Intracerebroventricular injection of bone marrow-derived mesenchymal stem cells (BM-MSCs) showed that they could attach to the choroid plexus and secrete exosomes into cerebrospinal fluid. In Alzheimer's model mice, BM-MSC treatment reduced NF-κB levels and increased miR-146a expression, leading to decreased TRAF6 levels in both the mice and astrocytes.[193] Neurodegenerative diseases such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis primarily have therapies that alleviate symptoms rather than address their causes. Investigating mesenchymal stem cells (MSCs) may provide anti-apoptotic, anti-inflammatory, and antioxidative therapeutic options for these conditions.[194] MSCs co-cultured with Aβ-induced neural cells secrete pro-inflammatory cytokines such as IL-10 and TGF-β. They enhance survival-related proteins like mTOR and AMPK in preclinical Alzheimer's models. In SAMP8 mice, UC-MSC administration restores neural cells and cognitive function through HGF, which inhibits hyperphosphorylated tau and improves synaptic plasticity. Systemic UC-MSC injection also enhances cognitive function in Tg2576 mice without altering Aβ levels.[195] Two proteins, RCN3 and FSTL3, have been identified as potential biomarkers for predicting the response of mesenchymal stem cells (MSC) in patients with AD. In addition, four proteins - SCRG1, NPDC1, ApoE, and CysC - are suggested to monitor MSC responses; low baseline levels of RCN3 and FSTL3 in cerebrospinal fluid (CSF) might indicate a better response to MSC therapy. RCN3, a calcium-binding protein, may hinder improvement in AD biomarkers, whereas FSTL3, an antagonistic TGF-β, acts as an anti-inflammatory factor during neurodegeneration. Further validation is needed for these findings and the applicability of RCN3 as a biomarker.[196]

ESCs and iPSCs are important for clinical applications. The human ESC lines were generated from donated embryos, and this practice was surrounded by ethical and political concerns due to the scarcity of available embryos. However, as has been seen above, human ESC research is being translated into practice. The generation of iPSCs from somatic cells through viral vector reprogramming eradicated controversies involving ESCs. Viable like ESCs, iPSCs amplified options for individualized medicine.[197]

Restoration of microglial function and neurogenesis

Microglial activation and regulation of neurogenesis have great potential in the treatment of AD from two primary focal points: neuroinflammation and neuronal degeneration. Microglia, as the main immune cells of the central nervous system, actively participate in AD pathology, leading to chronic inflammation while also enhancing neurodegeneration. This function of microglia involves restoring the balance from pro-inflammatory microglia to neuroprotective phenotypes that clear Aβ plaques and reduce neuronal loss. Furthermore, neurogenesis is also affected in AD; generating new neurons from neural stem cells is defective, especially in areas like the hippocampus, which controls learning and memory. Interventions aimed at optimizing neurogenesis focus on the proliferation and differentiation of neural progenitors (NPs) as well as synaptic integration and the enhancement of cognitive function. Current strategies to promote both microglial health and neurogenesis include growth factors, small molecular compounds, and stem cell therapies. These pathways have been shown in animal models of AD to produce early improvements in otherwise impaired cognition; however, questions remain regarding how these results can be effectively translated into clinical practice , particularly concerning methods of administration and potential long - term side effects. Nevertheless, these strategies offer a future possibility of disease-modifying therapies in AD, with the goal of stopping or delaying the inevitable progression of the disease in the brain.

Microglia play a crucial role in neurological repair and damage management, highlighting the importance of future research on this cell population in neurodegeneration.

The microglial activities under different physiological conditions in animals and brain injuries are complex and exhibit both pro-inflammatory and anti-inflammatory actions. Microglia play an important role in neurological repair and damage management, underlining the need for future research on this cell population in neurodegeneration. Some of the many suggested neuroprotective candidates include HMGB1, AMPK, PPARγ, and GSK3β, with the above medications undergoing various trials. HMGB1, released from injured nerve cells and activated by macrophages, promotes inflammation and is associated with excitotoxicity. In Alzheimer's disease, HMGB1 impedes microglial clearance of Aβ42, exacerbating neurotoxicity.[198] Microglia play a regulatory role in hippocampal neurogenesis during neurodegeneration. Increased microglial proliferation correlates with enhanced neurogenesis, while its inhibition reduces neurogenesis and normalizes neuronal differentiation. TGFβ was identified as a key molecule controlling the microglial pro-neurogenic response in chronic neurodegeneration.[199] AD poses significant challenges due to its complex pathogenesis. Microglia are crucial in preventing neuronal degeneration, with the gene Trem2 linked to dysfunction and increased risk, making microglial restoration a promising therapeutic strategy.[200] Microglia support neurogenic niches through phagocytosis and neuron interactions, releasing growth factors like BDNF and cytokines such as TNF-α. Astrocytes also influence neurogenesis via soluble factors ; however , under pathological conditions, they contribute to inflammation and impede neurogenesis.[201] Microglia serve essential immunocentric and neurobiological roles throughout development and adulthood. Recent efforts focus on creating standardized nomenclature for myeloid activation and polarization, emphasizing the differences between mouse and human microglia in culture conditions and activation requirements.[202] Removal of microglia from the mouse brain minimally influences TBI outcomes; however, inducing their turnover fosters a neuroprotective phenotype that enhances

recovery, relying on IL-6 trans-signaling and promoting adult neurogenesis.

Microglial depletion impairs regeneration within the telencephalon after damage, as it diminishes cell proliferation during neurogenesis. This condition alters the phospho-Stat3 and β -Catenin signaling cascades. Their ectopic activation can overcome the neurogenic defect. Furthermore, silencing microglia prolongs inflammation, leads to increased neutrophil retention, and possibly compromises recovery.[203] Ablation of glial cells interrupts telencephalic regeneration after injury and decreases cell proliferation during neurogenesis, thereby incapacitating the phospho-Stat3 and β-Catenin signals. Ectopic activation of these pathways could counteract neurogenesis defects. However, continued microglial suppression prolongs inflammation, leading to increased neutrophil accumulation, which may hinder recovery.[204] Microglial signatures vary in physiological and injury states, influencing pro- and anti-inflammatory responses. They play crucial roles in neurological repair and damage management, highlighting the need for a deeper understanding of their dynamics in neurodegenerative processes. Microglia can thrive in cell cultures but are sensitive to ischemia and nutrient deprivation. Grafted microglia may survive in host regions but often do not significantly contribute to the local microglial population. Observations indicate that endogenous microglia experience rapid atrophy post-grafting, with only rare survival of embryonic microglia, suggesting they require optimal conditions for survival and may not significantly influence graft survival or differentiation.[205]

NANOTECHNOLOGY AND DRUG DELIVERY TAILORED TO SPECIFIC NEEDS

Nanotechnology is changing the landscape of Alzheimer's disease (AD) treatment through more sophisticated approaches to drug delivery that address challenges , which may include the blood-brain barrier and targeting disease markers specifically. Nanoparticles, such as liposomes and gold nanoparticles, may be utilized to encapsulate various therapeutic agents and transport them to the affected areas of the brain. The conjugation of these nanocarriers provides the capability for "targeting," which can enhance drug delivery systems and their release. While *in vitro* and *in vivo* investigations have demonstrated the potential of these platforms for clinical applications, preclinical research raises concerns related to safety, biodistribution, and toxicity. Nonetheless, in the field of AD, nanotechnology has the potential to create customized approaches to treatment that exceed the capabilities of traditional methods.[206] One primary way in which nanotechnology can significantly improve effectiveness is through the use of theranostics; devices utilized for both diagnosis and treatment, furthering the goal of individualized therapy.[207] New strides in the science of nanomedicine provide evidence that they can now be used for drug delivery to increase the efficiency of existing drugs and also create a new image through selective diagnosis with disease marker molecules for better prognosis management (Figure 5).[208]

Nanocarriers for crossing the BBB

This suggests that the incorporation of drugs into nanocarriers may offer a viable strategy to enhance the penetration of drugs across the BBB in AD, given the poor concentrations typically achieved with standard procedures. Due to their small size and the functionality of their surface coatings, liposomes and polymeric nanoparticles facilitate receptor-mediated transcytosis to areas of the affected brain, such as amyloid-beta plaques and tau tangles. Engineered for controlled drug release, multifunctional nanocarriers also allow real-time monitoring of treatments.[209] Challenges include ensuring safety, biocompatibility, and large-scale production; however, nanocarriers could significantly improve AD therapy. Enhancing therapeutic benefits involves understanding the entire delivery process of nanocarriers in the bloodstream before reaching the brain. This critique emphasizes the need for targeted treatment for CNS sequelae, concentrating on functionalized lipid nanocarriers primarily for brain disorders. This work will highlight common ligands and recent studies on surface-modified lipid nanosystems, clinical translation challenges, and future prospects.[210] Nanocarriers equipped with appropriate ligands could effectively cross the blood-brain barrier to deliver drugs for human neurodegenerative diseases, but exhibit poor targeting efficiency that renders the treatment ineffective.[211] Nanoparticles (NPs) are drug delivery enhancers that can cross the blood-brain barrier with less invasiveness compared to traditionally applied methods. Types of NPs, such as polymeric, magnetic, or carbon NPs, show promise for therapy in AD. Further research should always continue for suitable applications in the clinical use of these metal-based NPs and nanocarrier systems for neurodegenerative diseases.[212] Enhanced targeting plays a major role in the polymerization of modified ligands to improve the permeation of modified liposome-based nanocarriers across the blood-brain barrier.[210] AD may be treated with new nanocarriers that can deliver drugs to the brain, targeting therapies to address disease processes. Recent research has shown that nanoparticles are an effective mechanism for the diagnosis and treatment of AD.[213]

Disease-modifying medications for AD are critically needed, although none is currently available. Clinical trials have frequently failed; however, progress has been made in understanding AD biology. Lecanemab has shown promise in reducing amyloid markers and cognitive decline in early-stage AD, and it was recently approved in the USA, with an ongoing international review.[214] Strategies such as receptor-mediated transport (RMT) and external forces encounter limitations, including safety concerns and immune rejection. Moreover , numerous nanomaterials face challenges with insufficient circulation time and therapeutic efficacy in the brain.[215] Diseases like Alzheimer's have no cure due to the inability of drug molecules to cross the BBB. Nanotechnology plays a crucial role in treating CNS disorders by utilizing drug delivery systems (DDS) such as polymeric nanoparticles, liposomes, dendrimers, micelles, and carbon nanotubes.

Nanocarriers, which can stabilize plasma, enhance solubility, and control drug release, protecting the drug from inevitable

losses and degradation, will be developed for future applications. Increasing our understanding of nanoparticles will enable us to prepare new treatments that can specifically target the brain and reduce the toxicity of drugs to other tissues.[216] Compared to well-established platforms like PET, SPECT, MRI, and CT, there are optical imaging modalities capable of tracing agents *in vivo*. These include bioluminescence imaging, fluorescence molecular tomography, and optoacoustic tomography. Attention will be given to emerging optical techniques and trends in multimodal imaging approaches.[217] Recent discoveries have identified new BBB - targeting agents, including plasma proteins, antibodies, peptides, aptamers, and small molecules. Comparing their effectiveness is complicated due to variables such as ligand density and nanoparticle size. Intermediate affinity antibodies and LDL receptor-targeting peptides, notably angiopep-2 and ApoE, have shown promising results. For instance, angiopep-2 conjugated with paclitaxel is in phase II trials for brain metastases, while an anti-transferrin-receptor conjugate is in trials for Hunter syndrome. Safety concerns regarding nutrient transport due to receptor targeting must also be taken into account.[218]

characteristics of multichannel and multiplex operations, they can produce synergistic effects, which may enhance the drug's efficacy and neuronal protection. However, there are difficulties in the practical application of formulations for controlled release, safety, and large-scale manufacturing; multifunctional nanoparticles offer a potential yet challenging approach to personalized therapy for AD. The three key hallmarks of AD are amyloid-β (Aβ) accumulation and fibril formation, the presence of neurofibrillary tangles caused by aggregated and hyperphosphorylated Tau protein, and neuronal loss. Aβ, formed from the cleavage of amyloid-β precursor protein (APP) by β-secretase and γ-secretase, aggregates to form plaques in the brains of AD patients. The World AD Report 2019 estimates 152 million AD patients by 2050, with annual costs projected to reach two trillion US dollars by 2030. Over 100 AD drugs are in late-stage trials, yet most have proven ineffective. New therapeutic strategies are urgently required.[70]

To improve antibody delivery to the brain, the following approaches have been developed: RVG29, a nAChR and GABAAR receptor-binding peptide. It includes the entrepreneurial concept of functionalizing biodegradable

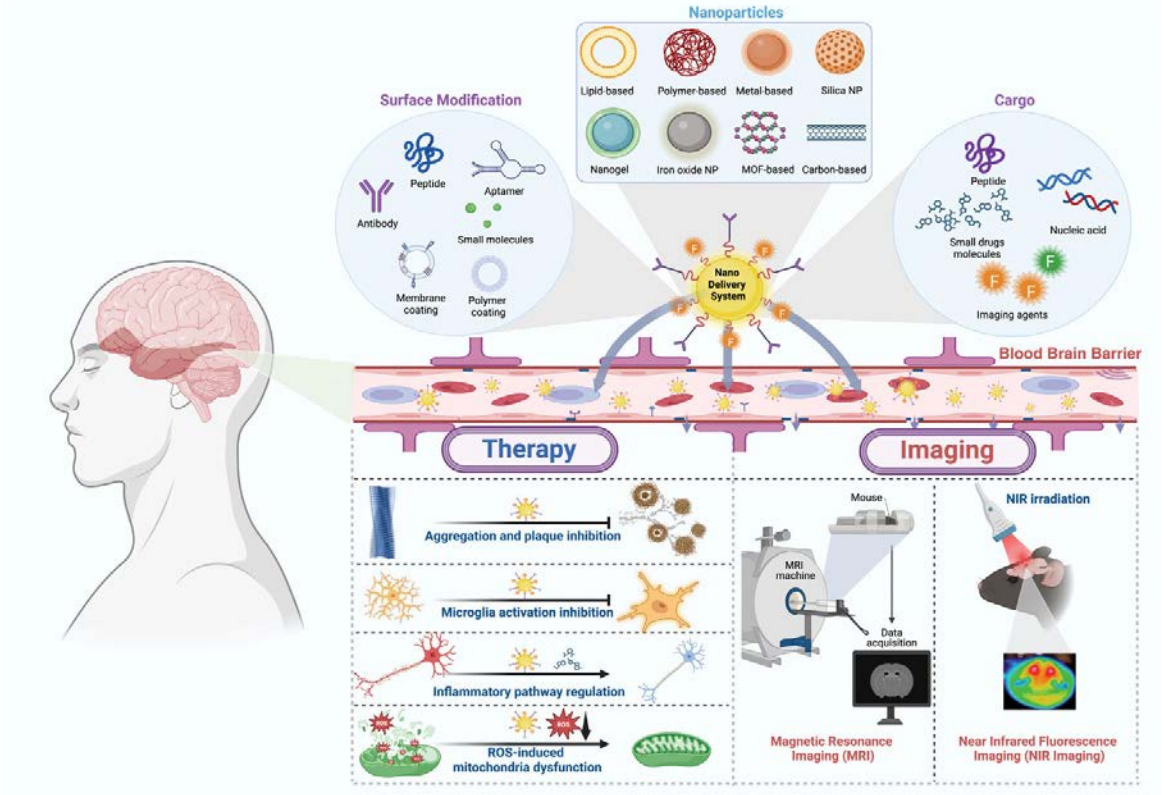

Figure 5. Drug delivery facilitated nanomaterials for Alzheimer disease therapy and diagnosis improvements in passing across the BBB. Reprinted (adapted) with permission from.[215]

Multifunctional nanoparticles for combination therapy

The combined therapy using multifunctional nanoparticles of anti-amyloid beta and neuroprotective molecules explains how the multiple pathways involved in AD could be effectively targeted. These nanoparticles are designed for maximum delivery, with the ability to penetrate the blood-brain barrier and reach the specific regions of the brain affected. Due to the mesoporous silica nanoparticles (bMSNs) loaded with RVG29 and anti-Aβ42 antibodies to prevent Aβ aggregation and neutralize ROS formation. A bifunctional dual-targeted multifunctional nanocomposite, RVG29-bMSNs@Ce-1F12, was designed for the treatment of AD. To capture Aβ42 and prevent its aggregation, the nanocarrier has been incorporated with the RVG29 peptide and anti-Aβ42 antibody 1F12; CeNPs are also

employed to clear excess ROS. This composite has demonstrated a synergistic effect and achieved a reduction in pathological burdens as well as alleviation of cognitive impairment in APP/PS1 mice. Therefore, it shows potential efficacy for AD treatment through the inhibition of Aβ42 and ROS.[219] Neurological disorders that threaten life include amyotrophic lateral sclerosis, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Most people worldwide suffer from these illnesses. However, there is currently no effective treatment to slow the progression of the aforementioned diseases, although nanomedicine has helped to open new horizons in drug delivery. The efficiency of drug delivery and the invasive crossing of the blood-brain barrier, compared to invasive treatment methods, are enhanced with nanoparticles (NPs).

Several types of NPs, including polymeric, magnetic, carbon-based, and inorganic ones, have already been developed for such applications. Promising research on using NPs for Alzheimer's treatment is emerging, though comprehensive studies are necessary for clinical implementation. The review also addresses various nanoformulations targeting neurodegenerative diseases.[212] A novel antioxidation-guided gradient dosing strategy was proposed, applicable for both the prevention and treatment of AD. Currently, research is poorly focused on early preventive measures, primarily concerning itself with preventing severe cases of cognitive impairment. Well-placed adaptable doses of PTCN can effectively combat oxidative stress and improve the pathological processes that rescue cognitive impairment and hippocampal atrophy in APP/PS1 mice. Therefore, traditional biomaterials and high repeatability in the PTCN constructs strongly indicate a future in the development of therapeutic drugs and healthcare products for AD.[220] A multifunctional nanocarrier, CICe@M-K, was developed to inhibit Aβ aggregation and scavenge ROS while efficiently crossing the BBB. It incorporates curcumin and IR780 within mesoporous silica nanomaterials, with surfaces grafted with cerium oxide nanoparticles and a short peptide. Imaging confirmed significant accumulation in the brain.[221]

As of now, the majority of treatment strategies in AD have primarily focused on amyloid beta therapy; however, the lack of proper correlation with cognition or the failure of clinical trials has led to the need for better therapies. The tau pathway has recently been linked to symptoms occurring in AD, thus prompting the development of a methylene blue-loaded nanocomposite to combat tau pathology.[222] A polyoxometalate-based nanozyme with protease-like and SOD-like activities depletes Aβ aggregates, scavenges Aβ-mediated ROS, removes Cu from oligomers, and can cross the blood-brain barrier with minimal toxicity.[223] Levodopa nanoparticles exhibit minimal motor complications in the treatment of Parkinson's disease. Nanotechnology shows potential for managing neurodegenerative diseases, with promising research on Alzheimer's disease needing further investigation.[224] SiRNA nanoparticles with peptide-tagged polyethylene glycol-chitosan deliver siRNA against Ataxin-1, suppressing SCA1 protein in neurodegenerative models. Cerium oxide nanoparticles offer potential treatment options for Alzheimer's and Parkinson's diseases, while galantamine-loaded polymeric nanoparticles are biodegradable. Silica nanoparticles pose a risk of neurotoxicity.[225] A nanostructured GM1-modified reconstituted high-density lipoprotein (GM1-rHDL) was developed for targeting Aβ and aimed at accelerating microglial degradation while improving the efflux of Aβ across the blood-brain barrier. The multifunctional nanostructure, αNAP-GM1-rHDL, provided superior protection against Aβ-induced toxicity and resulted in better behavioral outcomes in AD model mice.[226]

Biocompatible drug delivery systems such as liposomes, nanoparticles, hydrogels, micelles, dendrimers, mesoporous particles, etc., have been developed for targeted therapy. These carriers are particularly useful in chronic diseases like cancer and neurodegenerative disorders. Successful clinical trials have led to the market launch of drugs such as Abraxane® and Caelyx®, while novel agents such as peptides and nucleic acids are emerging as promising nanomedicines.[227] The clinical translation of nanomedicines for all patients is not regarded as a highly viable prospect. Consequently, dosage and toxicology profiles must be standardized and assessed. Short- and long-term side effects, particularly in critical organs, need to be evaluated. In this context, a very favorable benefit-to-risk ratio should be attained, given that side effects may encompass cytotoxicity and immune suppression associated with nanomedicines.

Exosome-based delivery systems for mitochondrial-targeted therapies

Mitochondrial-targeted therapies for AD require a new approach, and an exosome-based delivery system is a promising method to achieve this. Exosomes are small, about nanoscale, and have been shown to penetrate the blood-brain barrier with various therapeutic payloads. These systems are designed to reboot mitochondrial activity, decrease oxidative stress levels, and address energy depletion in neurons by engineering exosomes to package and deliver targeted mitochondrial agents like antioxidants. Animal trials have been positive, but scaling to large numbers and stabilizing the virus remain problematic. In general, exosomes hold potential as a disease-modifying approach in AD and other neurodegenerative diseases.

His genetic tampering enhances exosomes with RVG-peptide for selective targeting at α7-nAChR-intensified levels of neprilysin variant degradation of Aβ. The RVG-EXO with adipose-derived stem cells demonstrated a piggyback action onto the hippocampus; it reduced the expression of IL-1α, TNF-α, and NF-κB, while increasing that of IL-10. The combined use of EXO-RVG and CD10dm operates with higher potency and lower Aβ40 production in N2a cells, facilitating appropriate management of AD.[228] Multi-targeted therapy aims to diagnose and treat AD; however, it is not very effective. Engineering activated neutrophil-derived exosomes (MP@Cur-MExo) enhances mitochondrial function in neurons by targeting Aβ-induced neurotoxicity. The exosomes are modified with mitochondrial and Aβ-targeted ligands and degrade in the AD brain by matrix metalloproteinase-2. MP@Cur-MExo protects neurons from Aβ-induced dysfunction and aggregates in AD areas to enable early diagnosis through bimodal MRI/IVIS

imaging.[229] Cells lacking mitochondrial DNA exhibit resistance to Aβ toxicity, underscoring the significance of a functional electron transport chain (ETC) in mediating Aβ's detrimental effects. Aβ can accumulate in the cytoplasm and enter cells, resulting in neurotoxicity. In AD models, Aβ is localized in mitochondria, impairing Complex I activity, elevating ROS levels, and disrupting Complex IV function. Aβ1–42 specifically induces dysfunction of Complex I, leading to increased ROS and affecting Complex IV through lipid peroxidation. Furthermore, Aβ interacts with Tau protein and mitochondrial ANT, exacerbating mitochondrial dysfunction and energy deficits, affecting mitochondrial dynamics, transport, and bioenergetics.[230] A novel biomimetic drug delivery nanosystem (RVG/TPP NPs@RBCm) has been developed for safe and targeted antioxidant delivery to neuronal mitochondria for treating AD. Its physicochemical properties and the unique functions of the modified outer shell enhance biocompatibility and circulation, enabling the nanosystem to efficiently cross the blood-brain barrier and reach neurons.[231]

Exosomal molecular cargoes need to be released from endosomes to be functional in the recipient cell. Populations of exosomes can fuse directly, thereby allowing the drugs to influence functions such as gene expression and immune response (Figure 6). However, it is not clearly understood how exosome surface proteins selectively bind to receptors on target cells. Some ligands like PD-L1 and TNF have emerged as therapeutic targets in the treatment of cancer, though their efficacies differ with cell type, hampering their application in clinics. Further studies are being conducted to ascertain the nature of exosomes as drug delivery systems.[233] MSC-EVs also possess several benefits in the treatment of AD, such as biocompatibility, low immunogenicity, and the ability to deliver drugs. They can help reduce AD symptoms by delivering therapeutic agents, such as Aβ-degrading enzymes, immunomodulators, and neural protectants, which makes them ideal for use due to the complex etiology of AD pathologies. However, issues like low targeting efficiency, unequal treatment results, and low production yield call for sophisticated engineering approaches to optimize their use as treatments. The inability to scale up EV production at present, largely due to current 2D culture practices, also hinders the advancement of EV-based therapies for clinical use.[234] Clinical research for the cure of AD is significant because no cure-all drug exists. The initial focus on amyloid-beta (such as Aβ) failed to achieve a successful breakthrough in several clinical trials, even prompting companies like Roche to discontinue further A-related studies. Some ongoing trials target neuroinflammation and phosphorylated tau (p-Tau). Recent advancements include studies on gut microbiota and innovations in biotechnology, such as human mesenchymal stem cells and artificial nanocarriers for drug delivery, although most studies remain focused on limited targets.[235]

Gene Therapy and Epigenetic Modification: Innovative Therapeutic Approaches

Both cell and organic therapy, along with epigenetic treatment, are areas considered potential cures for AD due to their multifaceted genetic and molecular aspects. Gene therapy aims to deliver therapeutic genes to the brain to replace abnormal support genes, generate neuroprotective genes, and restore impaired cell functions, utilizing viral and non-viral vectors to transport neurotrophic factors or enzymes that degrade amyloid-beta deposits. Epigenetic modification affects gene expression without altering the DNA molecule, thereby returning neuronal activity to normal and enhancing survival. New strategies, such as CRISPR-Cas9 and RNA-based therapy, have emerged, but

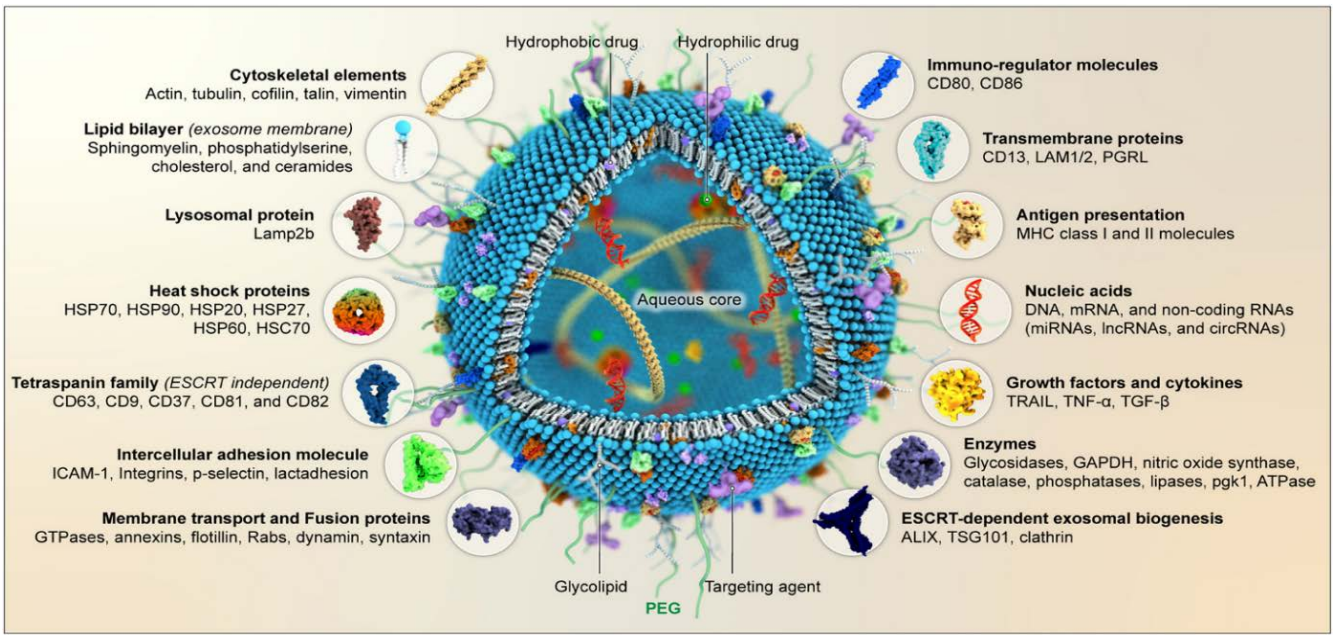

Figure 6. Exosomes are characterized by a specific structure and composition, including various proteins such as CD, HSP, ICAM, TSG101, GAPDH, TGF-β, TNF-α, pgk1, TRAIL, MHC, and Lamp. Reprinted (adapted) with permission from ref[232]

issues like delivery across the blood-brain barrier, as well as specific side effects and safety, remain unresolved. Differentially methylated sites and histone modifications, along with non-coding RNAs—including microRNA-16 and BACE1-AS—are associated with AD. Epigenetic changes provide new insights into AD pathogenesis and present novel treatment options.[236]

Advances in nonviral and viral gene delivery for AD

Newer non-viral and viral vector systems are enhancing gene therapy for AD based on the target molecular pathology. Vectors that efficiently deliver genetic material to the brain include adeno-associated viruses that help introduce therapeutic genes like neurotrophic factors. Peculiarities such as immunogenicity and restricted transgene payload remain unchanged. Liposomes and mRNA therapies exhibit significant benefits, including the ability to evade the immune response and enable targeted delivery; however, the main drawback is lower and short-term gene delivery effectiveness. Both approaches are progressive and could offer the potential for disease-modifying treatment for genetic AD. Innovative, efficient non-viral gene delivery vectors include liposomes, lipid nanoparticles, HPAE, SCKP, PAMAM dendrimers, and PEI. Factors such as size, charge density, DNA condensation, and hydrophobicity influence gene transfection efficiency and vector stability.[237] Minimized DNA vectors present an incredible opportunity for the delivery of short hairpin RNA, miRNA, and biologics, promising diverse applications in controlled delivery and gene expression in the future. They are also ideally suited for the treatment of polygenic diseases through simultaneous administration and are further enhanced by engineering improvements that make them more efficient and cost-effective.[238] The focus of research is on developing vectors for gene delivery, whether viral or non-viral, to treat diseases such as AIDS, cancer, and Alzheimer's. It will also make advances in the future in DNA and RNA technologies toward common clinical applications. DNA viral vectors have been reported to be capable of effective gene delivery, whereas some promising RNA systems naked synthetic mRNA and self-amplifying RNA replicons should offer superior immunogenicity and stability for *in vivo* applications.[239]

Protein engineering represents biotechnological advances for targeted drug delivery systems for anti-cancer agents. Specifically designed distribution-modified systems possess the dual action of enhancing the bioavailability of drugs and improving their anti-tumor activity. The main issues arise from general damage to cells, which occurs by targeting non-specific cells. Future systems must be safe, specific, and biocompatible. Computational models can aid in optimizing formulations and thereby enhance drug-receptor interactions.[240] Cellular uptake and trafficking are fundamental processes for the effective delivery of genes. Improving efficiency will thus involve optimizing routes for administration and modifying vectors with cell-specific ligands. Advances in non-viral systems, as well as specific targeting, will serve to enhance transfection efficiency and therapeutic outcomes with DNA vectors.[241]

Promising in gene therapy, ex vivo genetic engineering, non-viral systems display limitations in delivery modes that affect production, and they incur high costs. Improvements in cell-selective delivery, especially lipid nanoparticles (LNPs), should enhance gene therapy potency as they encapsulate nucleic acids for endosomal escape and facilitate effective cargo release.[242] Gene therapy represents a higher dimension for therapies of various diseases through the modulation of specific genes, with the application of gene vectors enhancing the clinical use of nucleic acids (DNA, mRNA, siRNA, miRNA). However, due to these immunogenic effects, while using viral vectors for therapy, non-viral nanocarriers enable combined gene therapies with treatment combinations such as photothermal therapy and immunotherapy, with safety and toxicity issues well documented.[243] Nucleic acid delivery mediated by lipid-based nanoparticles, such as liposomes and extracellular vehicles, holds great promise in drug development. While LSs are already extensively used in clinical applications, EVs have distinguished themselves in pretreatment for their biocompatibility. Both categories have faced similar issues, namely, immunogenicity and rapid clearance *in vivo*, along with a shortage of comparative studies.[244]

In comparison to viral vectors, non-viral vectors have advantages that include safety, versatility, and ease of preparation. Recent developments include targeted gene carriers for systemic delivery, particularly for tumor therapy. This review describes advancements made in gene delivery over the past two years and discusses future prospects.[245] Nonetheless, conventional viral vector systems have several cost barriers, while non-viral vectors, such as polymers and lipids, may potentially present fewer side effects and immunological reactions. However, there are still many problems concerning gene transfer efficiency, specificity, and safety in these areas.[246] Modification of non-viral vectors with PEG involves a reduction in protein binding and clearance, as well as improvements in accumulation within tumor tissue. Targeting ligands facilitate specific receptor binding. Progress in development includes neutral/anionic liposomes, cationic lipids, and enhanced nucleic acid delivery efficiency through vector convergence.[247] Gene accumulation in the targeted tissue and improved expression efficiency may suffice for successful gene therapy. EPR effects would aid in delivering tumor tissue but would vary from patient to patient, underscoring the need for personalized approaches. Thus, active targeting with ligands would enhance the cellular uptake and release of nucleic acids.[248]

Nucleic acid delivery mediated by lipid-based nanoparticles, such as liposomes (LSs) and extracellular vehicles (EVs), holds great potential in drug development.

Potential of CRISPR/Cas9 and other genome-editing technologies

New techniques, such as CRISPR/Cas9 and other genome-editing technologies, present a novel approach to Alzheimer's disease treatment, as the correction of genetic mutations associated with familial Alzheimer's disease can be achieved

through gene editing. These technology platforms can target specific genes involved in amyloid-beta formation, tau protein hyperphosphorylation, and neuroinflammation, potentially halting or even reversing the course of the disease. Furthermore, the capability of CRISPR/Cas9 allows for the upregulation and downregulation of genes that protect neurons. However, relevant difficulties remain, including delivery methods, the impact on unintended targets and molecules, and the long-term safety profile, which continue to pose challenges for the practical application of Alzheimer's disease treatments.[249] The CRISPR-Cas9 technology, powered by the Cas9 protein and guided by RNA, plays a major role in genome editing, with uses in personalized medicine, gene therapy, and agricultural interventions. Such enhanced delivery, using nanomaterials, along with the ethical standards needed for propagation, transforms the utility of this technology, making interdisciplinary collaboration imperative to safeguard and ensure the competence of safety and efficacy.[249]

These edited genomes, created using genome-editing techniques such as ZFNs, TALENs, and CRISPR-Cas9, significantly improve the understanding of genes associated with disease in precise cellular and animal models. By providing targeted alterations of genes, it is possible to explore gene functions or manipulate cellular behavior to enhance applicability in human diseases or potential future therapeutic interventions for understanding disease process mechanisms.[250] CRISPR and Cas9, as gene editing systems, are promising future treatments for cancer and genetic illnesses. However, the challenge lies in their delivery to target cells. Traditional methods of delivering these therapeutic agents have been exposed to immunological clearance risks. It has been learned that AAV vectors were associated with integration-related disease risks, while nonviral vectors, such as lipid nanoparticles, provided safety but may elicit an immune response. Improvements and exosomes are also considered for safety and efficacy enhancement. Targeting off-target effects is vital for their safe applications.[251]

Neurodegenerative diseases such as Alzheimer’s and Parkinson's are genetic disorders, and CRISPR/Cas9 technology is now emerging as a potential gene-editing approach for treating them. This technology has been shown to be effective in decreasing amyloid beta deposition and tau phosphorylation in models of Alzheimer's disease, especially in familial cases, while its benefits in sporadic cases were limited.[252] Many clinical trials targeting beta-amyloids in Alzheimer's disease have failed, leading to a reassessment of the beta-amyloid hypothesis and new treatment strategies. CRISPR/Cas9 gene-editing systems offer great promise as a novel method for precision, cost-effectiveness, and simplicity. This system is derived from bacterial immune systems, can, therefore, be applied directly for treatment, and improved animal models of neurodegenerative diseases. The guide RNA directs the Cas9 enzyme to the specific DNA sequences needing manipulation, resulting in gene knockout via double-strand breaks and downstream cellular repair mechanisms, thus providing a potential advantage over traditional methods of gene editing.[253] It is known that gene mutations causing AD partly arise from presenilin (PSEN) and amyloid beta precursor protein (APP), as such mutations are crucial for pathophysiology. Clinical trials focusing on genetic conclusions cannot yield fruitful results; however, CRISPR/Cas9 genome editing could be an effective method for correcting such mutations. Delivery-related challenges remain, as systemic routes often exhibit stability and targetability issues, thereby necessitating other effective administration strategies to advance research.[254] CRISPR/Cas9 is a tool for gene editing that is currently being explored for the treatment of AD by correcting affected Aβ metabolism related to both familial and sporadic cases. It has challenges such as delivery to the brain, vector stability, lysosomal degradation, and immunological responses. Smaller Cas9/sgRNA complexes are preferable; however, they are also easily degraded.[255]

Two innovative CRISPR-based therapies for Alzheimer's were presented at the Alzheimer Association International Conference® (AAIC®) 2023. One of them will target the APOE-e4 allele, which is a strong susceptibility gene for the disease, while the other aims to inhibit the production of toxic beta-amyloids in the brain. CRISPR technology is fast-tracking drug target identification and accelerating the drug discovery process, which could lead to next-generation treatments. The diversification of potential therapies gives hope for those affected but, most importantly, affirms the recently approved anti-amyloid drug development that has progressed significantly in the direction of Alzheimer treatment.[256]

Epigenetic drugs targeting histone acetylation and DNA methylation in AD

Histone acetylation and DNA methylation-targeted drugs constitute a new prodrome for the modulation of Alzheimer's disease (AD). Histone acetylation is pro-neurogenic and pro-cognitive, reflecting the fact that HDAC inhibition improves neuronal survival and mitigates cognitive impairments in rodents. The genes involved in neuronal repair are mostly methylated by DNA methylation, and DNMT-targeting epigenetic modulators seek to reverse this. Thus, preclinical studies appear quite feasible in addressing cognitive deficits using drugs that reduce AD-related pathology; however, problem areas include achieving appropriate epigenetic regulation, off-target effects, and long-term safety concerns. These drugs may provide new disease-modulating therapies for neurodegenerative conditions. Regarding learning and memory, their epigenetic components involve irreversible changes such as DNA methylation and modifications to histones, which may serve as therapeutic targets in AD. These reversible changes allow for potential interventions into the pathology of AD. Research is focused on methyl donors and histone deacetylase inhibitors as potential adjuvants for cognitive improvement. These early-life epigenetic changes can have future effects on health, and targeting those mechanisms may provide a possible path for drug development aimed at improving memory or downregulating AD-associated gene expression.[257] Late-onset AD (loAD), prevalent in 95-98% of Alzheimer's patients, causes age-old neurofibrillary tangles and dementia, primarily in the older generation, and is associated with aging. Aging is the greatest risk factor, and the rise in life

expectancy will likely double loAD cases by 2025, with significant economic impact. Genome-wide association studies have identified several genetic variants linked to loAD, with the apolipoprotein E (ApoE) gene being the most prominent among them. ApoE is produced mainly by astrocytes in the brain and has three isoforms: ApoE2, ApoE3, and ApoE4. ApoE4 markedly increases the relative risk of developing loAD, possibly by restoring amyloid beta clearance pathways. Other loAD-linked genes include CLU, ABCA7, and PICALM.[258]

Epigenetics regulates chromatin states through DNA and histone modifications, RNA modifications, and chromatin remodeling. Disruptions can lead to diseases, prompting the development of small molecule drugs that target epigenetic enzymes for therapeutic use, particularly in oncology. Altered epigenetic modifications also affect cognitive functions and are associated with neurodegenerative diseases.[259] Changes occurring in stress responses, notably those affecting the hypothalamic-pituitary-adrenal axis, may amplify the epigenetic effects of chronic stress on Alzheimer's disease. Late-life non-Alzheimer's dementia is associated with chronic psychological stress. In Alzheimer's disease, HDAC2 negatively influences memory, implying that inhibitors may interfere with Aβ deposition and tau hyperphosphorylation, rendering the understanding of neuroprotection quite complex.[260] The epigenetic modifications are nothing but regulators of carcinogenesis. They also serve as possible tumor markers. Each of these types modulates the regulation of specific genes and exhibits an altered pattern in various cancers. They not only regulate the Warburg effect in sugar metabolism but also associate with oxidative stress in carcinogenesis. They are therapeutic targets in combination therapies that bring added effectiveness to cancer treatment.[261]

Epigenetic alterations are reversible changes in histones or DNA that influence gene activity and are associated with diseases such as cancer. Epigenetically targeted therapies are emerging for hematological malignancies and are now being considered in clinical trials against solid tumors. Among the significant targets in epigenetics are DNA methylation, histone modifications, and inhibitors of epigenetic enzymes. For example, a substrate-specific enzyme related to histone methylation has changed, although the expression profiles have always been associated with histone mutations. Some of the major changes observed include those linked to the methylation of histones H3K4me3 and/or H3K27, which relate to cancer progression and poor prognosis. Hematological cancers respond well, but challenges regarding selectivity and solid tumors remain to be addressed, warranting further studies.[262] They are sufficiently capable of affecting epigenetic events, notably DNA methylation and histone acetylation, thus influencing genome-environment interactions associated with certain brain disorders such as Alzheimer's disease. Current literature has focused on developing an epigenetic mechanism targeted by drugs, including histone acetylation, as a therapeutic strategy for Alzheimer's disease. The present drug discovery crisis arises from insufficient mechanistic rigor in the selection and validation of therapeutic targets. Progressing preclinical validation should also involve chemical probes to link therapeutic target-induced biological processes to disease pathogenesis. Developing selective chemical probes and relevant assays is critical for the advancement of new therapies for Alzheimer's disease, requiring substantial investments of time and resources.[263]

Biomarkers in Drug Development: Key Contributions to Advancements

Biomarkers play an important role in the therapeutic management of AD since they aid in diagnosis, assessment of disease progression, and evaluation of treatment outcomes. Other biomarkers, such as amyloid-beta (Aβ) deposits and tau protein pathology, allow for faster clinical trials, as these demonstrate anatomical changes in the brain. Biomarkers include the Aβ42 concentration in cerebrospinal fluid (CSF), structural and functional measurements derived from PET scans and brain MRIs, as well as CSF tau and p-tau levels. Newer blood-based biomarkers are less invasive than CSF sampling; a multi-analyte biosignature integrated with genetic, proteomic, and imaging data has the potential to revolutionize transcript-specific AD treatment plans. Biomarkers play vital roles in diagnosis, target engagement, disease modification, and safety monitoring for AD drug development. The amyloid, tau, and neurodegeneration Research Framework focuses on brain imaging and CSF measures for drug advancement and clinical trials. A Phase 2 study must demonstrate target engagement to be eligible to advance to Phase 3, and such trials must be smaller and shorter in duration than regular Phase 3 studies. Toxicity monitoring includes liver function tests and blood counts, as 30% of drug programs come to a halt due to toxicity. Numerous adverse events, including skin cancers and cognitive impairments observed in drug development, underscore the need to evaluate safety.[264] *In vivo* biomarkers allow for earlier diagnosis of Alzheimer disease (AD) and identification of at-risk individuals. Current guidelines recommend diagnosing AD through clinical symptoms in conjunction with supportive biomarkers. Although these biomarkers serve to differentiate AD from other conditions, clinical assessments remain essential for diagnosis and guiding personalized patient care.[265]

AD manifests itself through several pathoanatomical alterations, such as amyloid-β plaques, tau tangles, neuroinflammation, cerebral small vessel disease, and neurodegeneration. These changes often occur long before any overt clinical symptoms develop. There is significant interest in developing biomarkers that would enable the detection of these changes with a high degree of specificity in at-risk individuals to facilitate earlier diagnosis and the initiation of disease-modifying interventions. Techniques include neuroimaging, fMRI, PET, CSF, and blood tests. Other clinical uses include screening at-risk populations, assisting in the diagnosis of dementia, monitoring therapy, addressing neuropsychiatric symptoms, and planning end-of-life care. Future research will focus on other cohorts and newer biomarkers.[266] Tau pathologies may not only interact but also induce neurodegeneration independently, while their progression is consistently linked to the degree of cognitive impairment. In the development of AD, the accumulation of Aβ

and Tau pathologies results from early immune dysfunction and neuroinflammation. Epidemiological studies suggest that prior infections or even diabetes may serve as precursors to the initiation of AD through such inflammatory pathways. Aβ activates microglia, leading to inflammation and resource limitations for Aβ clearance, subsequently promoting Tau phosphorylation and neurodegeneration. The National Institute on Aging–Alzheimer's Association has already established a diagnostic framework using the A/T/N classification system to evaluate AD biomarkers, which can be further supplemented with additional biomarkers such as neuroinflammation and vascular changes.[267]

Biomarkers hold a very important position in the development of Alzheimer's drugs because they support the creation of an agent and inform toxicity responses throughout preclinical and Phase I trials. They assist in dose finding, patient identification, and outcome measures during Phase II and III trials, including brain imaging.[268] At the time of analysis on 25th January 2022, a total of 143 agents were found to be in 172 clinical trials in AD: 31 agents worked on 47 Phase 3 trials; 82 agents contributed to 94 Phase 2 trials; and 30 agents performed activities found in 31 Phase 1 trials. The agents involved disease-modifying treatments, which accounted for 83.2%, while symptomatic treatments and neuropsychiatric drugs accounted for 9.8% and 6.9%, respectively. Repurposed drugs made up thirty-seven percent of such candidates. Ongoing trials require 50,575 participants, targeting biological procedures and focusing on amyloid therapies, tau treatments, and novel clinical outcome measures. Increased use of biomarkers and strong alliances with patients will eventually lead to significant advancements in AD treatment.[269]

Fluid biomarkers (CSF and blood-based) for early diagnosis and therapy monitoring

Cerebrospinal fluid (CSF) and blood-based fluid biomarkers are ideal for early Alzheimer's disease (AD) diagnosis and for assessing the efficacy of therapies, which can be invasive or impractical with neuroimaging. CSF biomarkers of AD include Aβ42, tau, and p-tau; Aβ42 decreases because it forms plaques, while tau and p-tau increase to form tangles. Existing biomarkers in blood plasma include plasma Aβ, p-tau, NfL, and GFAP. The concept of multiple biomarker panels may ultimately improve diagnostic specificity and facilitate timely treatment; however, issues related to harmonization and proof of concept persist.[270] The accelerated approval of amyloid-targeting monoclonal antibodies, such as Aduhelm and Leqembi, by the FDA was aided by biomarkers like an amyloid PET image. Leqembi also received standard approval due to clinical efficacy, making such imaging techniques more reliable. Amyloid positivity was a requirement for participation in recent trials, including SCarlet RoAD and Expedition 3, to conclusively prove the treatment's efficacy. Improved blood-based biomarker screening will enhance trial productivity, as noted by AHEAD 3-45 and its predecessor, TRAILBLAZER-ALZ 3. Future studies must create and then integrate fluid-biomedical markers to design enhanced clinical trials and explore novel therapies for AD.[271] As for diagnosing Alzheimer's disease (AD) early, an accurate diagnosis is essential for treatment and clinical trials. The cerebrospinal fluid (CSF) biomarkers include amyloid-beta peptide (Aβ1-42), total tau protein (T-tau), and phosphorylated tau (P-tau181), which can be used for differentiation, especially in questionable cases of dementia. A promising marker in CSF is the Aβ1-42/Aβ1-40 ratio for detecting early AD, which correlates well with PET imaging; however, further comparisons between this and other ratios will better define the differential diagnosis between AD and other non-AD dementias.[272]

AD represents a significant public health burden due to neurodegenerative disorders and challenges in clinical diagnosis. Increasingly, biomarkers are being utilized for research and clinical references, although the use of cerebrospinal fluid and positron emission tomography remains limited, as both are costly and invasive. Recent ultra-sensitive assay developments now facilitate the measurement of AD-related proteins in blood, where plasma P-tau emerges as a promising marker in symptomatic and preclinical AD, alongside the ratios of Aβ42/Aβ40. Blood neurofilament light chain (NfL) could serve as a valuable marker of neurodegeneration, though it is not specific to AD. Progress has been made in blood biomarkers; however, there is still much work to be done. Aβ42 levels correlate with cortical plaque load and enhance amyloid PET concordance when analyzed together with Aβ40.[273] Therefore, a multicenter study involving 288 participants validated candidate biomarkers for AD by using targeted proteomic assays. This study discovered 58 potential biomarkers in CSF and identified 12 serum proteins as possible biomarkers. There are the CSF-19 protein panel and the 8-protein serum panel that provide high classification accuracy for mild cognitive impairment. However, more studies on blood-based biomarkers are needed.[274] The Aβ42/Aβ40 ratio is reported as a potential biomarker for AD, despite the limited observations in earlier studies regarding differences in plasma levels between AD patients and controls. A 2016 study utilizing the SIMOA technique showed weak correlations between the CSF levels of Aβ42 and Aβ40 and their plasma counterparts. Recent results from a large cohort using the Elecsys immunoassay now demonstrate that plasma Aβ42 and Aβ40 can reliably predict Aβ status, particularly when combined with APOE status.[275] It is the timely detection of AD that plays a vital role in one of the most effective early interventions. The diagnosis is based on current clinical symptoms and neuroimaging or cerebrospinal fluid biomarkers, which are limited in their availability and invasiveness. Blood-based biomarkers (BBBMs), especially amyloid-β peptides and phosphorylated tau species, show potential for earlier diagnosis and risk management, but they should not be the only diagnostic method. Thus, a complete assessment should include patient history and other tests. Major progress has been made in research on BBBMs, but strict clinical validation remains mandatory.[276]

Imaging biomarkers (PET, MRI) for evaluating therapeutic efficacy

PET and MRI are complementary biomarkers used to assess treatment responders in Alzheimer's disease (AD), including symptomatic, disease modifying, and neuroprotective interventions. PET detects amyloid and tau aggregates, whereas

MRI primarily examines alterations in the brain's structure, particularly in the hippocampus. These techniques provide information on the function of AD and how the efficiency of treatment can be assessed to aid in early diagnosis and tailored approaches. Significant progress has been made in identifying biomarkers of AD using neuroimaging techniques that address changes in structure, function, connections, and amyloid and tau aggregates. The review emphasizes the importance of multimodality for biomarkers in clinical trials, highlighting the necessity of reliability and specificity. It also recommends composite biomarkers that combine diverse information found in large datasets, which will enhance the characterization and treatment potential of AD.[277] Diagnosis of AD mostly depends on symptoms and quicker-detection types of biomarkers, although therapeutic skills are limited. Presently, cerebrospinal fluid biomarkers (CSF), notably those site-specific proteins that have been suggested to link AD molecular mechanisms, are critical. Amyloid-β 42 (Aβ42) and tau are mainstays for diagnosing AD, while blood biomarkers are less effective. Advancements such as amyloid-PET imaging can estimate neuritic plaque density of Aβ among cognitively compromised individuals. The Aβ cascade hypothesis posits that an imbalanced amyloid-beta metabolism may result in AD pathology, which further leads to tau hyperphosphorylation and mitochondrial dysfunction, causing neurodegeneration. The TOMM40 gene is emerging as a potential key marker in mitochondrial involvement in AD. Neuroinflammation, which peaks in the late course of the condition, further exacerbates the disease. Such understanding at the molecular level will guide the discovery of new targeted treatments for neuroprotective purposes.[278]

A convolutional neural network, located in the top layers, classifies the input letters by adopting another neural network in the upper layers through the widely used corrected-based cascaded error method. Issues of plausibility, inconsistency, and disorder associated with these cascaded error methods, which certainly limit the classification ability of the system, are examined to confirm the validity of the claim. These deficiencies are demonstrated with the aid of standard simulation results that were simulated and tested under strict test conditions.[279] AD biomarkers are the most accurate, essential, robust, easy, precise, economical biological samples that can be measured. Foremost, the selected sensitivity was at least 80%, with corresponding specificity according to FDA-prescribed ATN classification correlating to β-amyloid, hyperphosphorylated tau, and neurodegeneration for predicting cognitive impairment. They should complement clinical evaluations.[280] In the year 2011, NIA-AA inscribed imaging biomarkers, including MRI, amyloid PET, and FDG PET, into the guide on the use of biomarkers for pathologic and diagnostic categories of AD.

Longitudinal memory decline was predicted by a linear mixed-effects model.

The International Working Group refined criteria, enhancing Aβ plaques, hypometabolism, and brain atrophy as markers for assessing disease progression and life courses of Alzheimer's disease, while also improving the effectiveness of preclinical work.[281] By classifying individuals by a set of biomarker status levels (Aβ42, T, N), the authors scrutinized CSF and imaging (PET/MR) biomarker data in 282 patients obtained from the ADNI dataset, divided into cognitively normal, subclinical memory concern, MCI, and AD subgroups. Agreement between types of markers was expressed using Cohen's Kappa.[282] Longitudinal memory decline was predicted by a linear mixed-effects model. The strength of correlation among other pairs was weaker; this is particularly surprising when comparing CSF with PET Aβ biomarkers. This indicates that Aβ tracking was different, but not to such an extent that it could not be observed. Increased CSF pTau values compared to PET tau would suggest an earlier event of tau, but recently published longitudinal data question whether CSF is an interesting prognostic factor. At the same time, PET tau seems to be more promising regarding the prediction of a steeper cognitive decline, signifying its higher prognostic potential for the identification of early AD in the clinical trial setting.[283]

Biomarkers, both soluble in bodily fluids and as intracerebral imaging markers, appear relatively early in the development of the Aβ proteinopathy pathway, with soluble Aβ peptides generating the insoluble and fibrillar deposits of packed plaques. All liquid and imaging biomarkers typically display early abnormalities, particularly in Aβ42 assays, before amyloid PET scanning is conducted. Therefore, we begin with the first T biomarkers: p-tau 181, 217, 231, and 262 specifically, with a normal baseline compared to amyloid PET imaging (evacuation) in the brain, indicating a compensatory metabolic reaction within the context of Alzheimer's pathologies, namely, Aβ deposits. These p-tau biomarkers are categorized into T1 (plaque-responsive) and T2 types (mean AD tau aggregates). Core 1 consists of biomarkers that can be detected even in clinically asymptomatic stages of the AD process.[284]

Companion diagnostics for personalized medicine in AD

The use of companion diagnostics in AD further enhances the prospects of creating a more personalized approach to the provision of therapeutic compounds. These tools utilize DNA, genetic profiling, and various protein signatures to assist clinicians in therapy choices. They are generally used to categorize patients according to disease progression and response to treatment. Biomarkers from PET and MRI provide imaging information about disease progression and therapy effects, allowing for adjustments to the treatment plan. LBA integration as CDx aims to drive better patient outcomes and advance the growth of new therapies. Personalized medicine seeks to deliver tailored treatments based on patients' genetic profiles and companion diagnostic tests. However, the high costs of developing therapies for small patient groups hinder access, and without robust support for companion diagnostics, the full potential of personalized medicine may remain unrealized.

The present research stresses the requirement for actionable diagnostic results, and diseases often involve multiple

biomarkers, which complicates response predictions. Improved outcomes are being sought with larger panel tests.[285] Next-generation testing enables broader analysis with smaller samples, yet reimbursement issues may restrict the adoption of advanced genetic testing in clinical practice.[286] In one of the most remarkable papers on markers of pathology and disease sequestration, Hanahan and Weinberg describe the entire process of forming a tumor mass, including the immune system's response. Such citations are numerous and increase as various researchers expand the knowledge base. If the non-cancerous tissue can be disposed of by agents more specific in killing tumor cells than some chemotherapeutic agents are for very small clusters of carcinoma cells, malignant changes would be much easier to cope with scientifically.[287] Currently, there are about 43 approved CDx's by the FDA, which work based on biomarkers such as PD-L1 and p53 in selecting patients for treatment. In the conventional sense, CDx's involve tissue samples preserved on glass slides, but MRI molecular imaging-based CDx's like FerriScan monitor occur in real-time, satisfying the need for personalized medicine in cancer therapy assessment.[288]

Repurposing Existing Drugs for Novel Therapeutic Applications

Introducing neuroactive drugs for the treatment of AD aims to find effective therapies for this multifactorial neurodegenerative disorder based as much as possible on existing FDA-approved medications. This involves assessing other mechanisms involving drugs like anti-inflammatory and neuroprotective molecules relevant to Alzheimer’s and manipulation of amyloid deposition and neuroinflammation. It is known that potential candidates include GLP-1 agonists and NSAIDs. Repurposing has advantages of identified safety profiles and abbreviated development times that offer a rational route for novel Alzheimer’s therapies. A growing number of drug repurposing candidates for Alzheimer’s are now in existence, but none is leading the way. Validation remains inconsistent, and real-world data is underutilized. This adds urgency to the need for further research.[289] A systematic approach has been proposed to use molecular knowledge for evaluating a drug that is in silico repurposing for AD, with a special focus on the NRF2 interactome and regulation. It involved assessing differentially expressed genes in the neighborhood linked to NRF2 and leveraging a computational pipeline to identify drugs inhibiting NRF2 partners, ultimately providing a short list. Using an *in vitro* cell-based screening assay, five candidates were identified that demonstrated activation of NRF2 expression within cellular systems while showing quantifiable changes in NRF2 protein levels and downstream target expressions.[290]

A newer wave of AI encompasses anything that introduces the next generation of AI, such as ChatGPT, which can significantly accelerate the processes of scientific review and summarization. In a study, metformin, simvastatin, and losartan were identified as potential drug candidates for repurposing in dementia known as AD, according to a meta-analysis of large clinical trial datasets that highlight a generally lower AD risk associated with the three drugs.[291] This is a new network-based method for drug repurposing that identifies stage-specific candidate drugs for preventing Alzheimer's disease (AD). It evaluates all these drugs with a score, which is further refined based on connection information networks, and finally, it assesses them more specifically for structural and functional analysis as well as Blood-Brain Barrier permeability. Ten proposed drugs will be validated against each AD stage.[292] In addition, the total amount of trial drug study agents tested for possible disease modification mechanisms was 78 percent (although regarding the Alzheimer drug development of repurposed agents in studies). One can increase the weight of instructional allergy in the drug pipeline architecture of agents, as 20 percent are hematologic-oncologic, 18 percent cardiovascular, 14 percent psychiatric, 12 percent diabetes, and 10 percent neurologic agents supported by Academic Medical Centers, using various intellectual property strategies to increase the marketing value of generic agents.[293] Re-purposing FDA-approved medications will be a speedier and cost-effective method. Developed by Rodman et al., this method involves creating a statistical representation of the severity of AD along with gene mechanisms. Such data were obtained after testing human neural cell cultures against 80 drugs. Through test data on 80 drugs, an ordered list of potential repurposing drug candidates will be generated.[294]

Anti-diabetic drugs (e.g., metformin, GLP-1 receptor agonists)

Anti-diabetic drugs, such as metformin and GLP-1 receptor agonists, are being investigated for their potential neuroprotective effects in Alzheimer's disease (AD). Metformin has shown promise in reducing amyloid plaque formation, improving insulin sensitivity, and modulating neuroinflammation. GLP-1 receptor agonists, including liraglutide and semaglutide, enhance cognitive function and neuronal survival. Both drug classes improve insulin signaling and enhance brain glucose metabolism, suggesting they could effectively address metabolic dysfunctions in AD, making them promising candidates for repurposing in treatment. Recent evidence indicates that Alzheimer's disease and Parkinson's disease are associated with type 2 diabetes, providing a potential repurposing of antidiabetic agents for therapeutic purposes. Two strategies can be envisaged. The first is the targeted inhibition of specific pathophysiologic processes. Particularly promising are the GLP-1 agonists, which suggest improvements in brain function and reductions in inflammation.[295]

The progression of AD involves the accumulation of amyloid plaques made of amyloid-beta peptides, as well as neurofibrillary tangles containing hyperphosphorylated tau proteins. AD also manifests oxidative stress, mitochondrial dysfunction, inflammation, and cellular senescence. About 80 percent of AD patients are insulin-resistant or diabetic and develop Type 2 DM (T2DM), further emphasizing the importance of this public health issue and the need for potential studies on how the two conditions interact.[296] Addressing this from a different angle, there are modifiable risk factors that correlate with cardiovascular risks and lifestyle habits, thus implying that dementia is preventable. Of key socio-medical importance in this context, antidiabetic medication appears to comprise an important component.[297] In rule estimations, doubly robust

estimation combines a propensity score model and outcome regression to estimate the causal effect of exposures on an outcome, such as the dementia risk associated with metformin exposure. This provides unbiased average treatment effects (ATE) when at least one of the models is correctly specified. The method then employs machine learning to identify subgroups and estimate the optimal treatment effects.[298]

correlated among users of antihypertensive drugs in the Taiwan NHIRD. Hypertensive patients showed significantly higher new cases of diagnosed dementia with optimally adjusted hazard risk compared to the controls.[303]

Neuroprotective agents from other therapeutic areas

Neuroprotective agents from various therapeutic areas are being identified for their potential in treating Alzheimer's disease

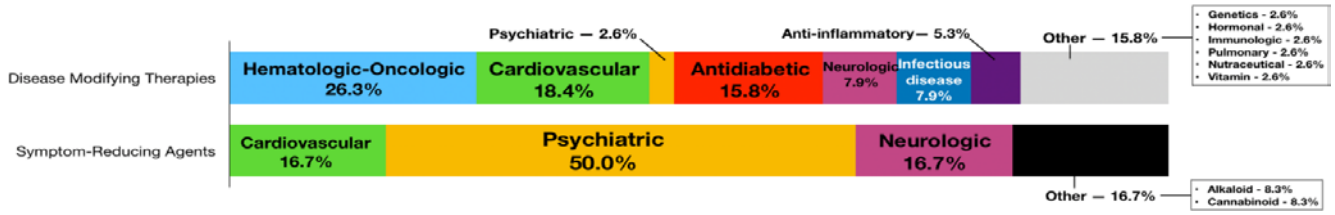

Figure 7. Agents with a repurposed mechanism of action currently undergoing study as a percentage. (ClinicalTrials.gov as of February 27, 2020). Reprinted (adapted) with permission from ref[293]

Anti-hypertensive and cardiovascular drugs

Anti-hypertensive and cardiovascular drugs are being investigated for their potential in managing Alzheimer's disease (AD), as vascular dysfunction contributes significantly to the pathophysiology of AD. Conditions like hypertension and atherosclerosis increase the risk of AD and cognitive decline. Commonly used antihypertensive medications, such as ACE inhibitors and ARBs, exhibit neuroprotective effects by enhancing cerebrovascular function and reducing neuroinflammation and oxidative stress. Statins may also provide benefits by modulating cholesterol metabolism related to amyloid processing.[299] These drugs may serve as adjunctive therapies to address both vascular issues and the progression of AD. Multiple studies have examined the impact of antihypertensives on the pathology of AD. According to data, a greater proportion of normotensive AD cases in the frontal cortex have lower loads of Aβ plaque compared to hypertensive cases, although this finding lacks significant treatment effects. The numbers of neurofibrillary tangles and neuritic plaques are higher in hypertensive cases with AD. Treated cases of AD or mild cognitive impairment exhibit lower densities in silver-stained lesions, suggesting that antihypertensives mainly affect silver-positive neuritic pathologies.[300] Inhibitory effects on acute anxiety symptoms are especially observed in type 2 and 4 Ang-II receptor stimulation through ARB classes, dihydropyridine CCBs, and thiazides. In comparison to inhibitors such as ACE inhibitors and beta-blockers, the effects caused by stimulating agents seem significantly more effective in lowering the risk of dementia. This study involved analyzing a large sample of elderly subjects, which was drawn from over 130,000 subjects in the Netherlands (Figure 7).[301]

Conditional logistic regression was conducted to determine the impact of exposure to antihypertensive drug treatment as a continuous variable, yielding a statistically significant difference in the risk of dementia in the low, intermediate, and high exposure categories—2, 12, and 24 percent, respectively. This trend flowed uniformly across very elderly and frail study participants.[302] A study was conducted over the period from 2000 to 2016, which found a reduced incidence of dementia that

(AD). Drugs such as selective serotonin reuptake inhibitors (SSRIs) and antidepressants exhibit neurogenic and anti-inflammatory effects that may mitigate cognitive decline. Anti-cancer agents, including PARP inhibitors, prevent DNA damage and promote neuronal survival, while anticonvulsants like levetiracetam help reduce excitotoxicity. Repurposing these agents offers a promising strategy for AD treatment, targeting oxidative stress, neuroinflammation, and synaptic dysfunction. By considering pathophysiological processes in AD, which could provide protection to neurons, small molecules that interact with Aβ may be proposed as a neuroprotective approach. Additionally, other molecules will act on stress kinases and caspases, while further protection might be provided through the retention or administration of drugs that counteract the cortical loss of cholinergic neurotransmission and decrease oxidative stress and excitotoxicity.[304] At the beginning of the year 2024, 164 clinical trials of 127 AD drugs were being tested. These include 48 Phase III trials for 32 drugs, 90 Phase II trials for 81 drugs, and 26 Phase I trials for 25 compounds. In terms of trials and drugs, the 2024 pipeline numbers are lower compared to 2023.[305] It reviews various pathogenetic viewpoints of AD and the potential therapeutic effects of plant phytoconstituents in neuroprotective mechanisms and stress-relief treatments. The review encompasses several databases in the literature and focuses on the latest scientific research addressing some promising flavonoids, along with the role of alkaloids in the functioning of the systems mentioned above. The conclusion presents a set of encouraging data demonstrating how bioactive components from plants may serve as valuable product leads in controlling Alzheimer's-like neurodegenerative diseases, among others.[306]

Challenges and Setbacks in AD Drug Development

Formulating effective drugs for AD faces significant challenges due to the disease's complex etiology. Despite extensive research and identified therapeutic targets, clinical trials have not yielded the expected improvements. Key issues include managing pathophysiological processes such as amyloid lesions and inflammation, as well as the difficulty of drug delivery across the BBB. Additionally, the disease's varied clinical, genetic, and environmental subtypes complicate the

analysis of treatment responses. Critics point out that trials often enroll patients at advanced stages, thereby limiting therapeutic potential. Overall, there is an urgent need for specific biomarkers and better predictive models to develop effective personalized therapies. Late-phase clinical trials for AD have failed due to significant shortcomings in medication development methods, particularly in patient sampling. Trials often involve patients at later stages, where neurodegeneration limits treatment effectiveness. Early intervention during the preclinical phase or in mild cognitive impairment (MCI) may improve outcomes. Disappointing results from amyloid-targeted therapies have raised doubts about the amyloid hypothesis, prompting exploration of tau pathophysiology and other factors. Lessons learned emphasize the need for better biomarkers, improved clinical trial designs, and a shift toward a modern approach focused on early intervention and diverse targets. Translating preclinical success to clinical benefits in AD faces significant challenges due to factors such as differences between animal models and humans, including variations in disease pathology and neurodegeneration rates. Many promising molecules perform well in animals but fail in humans, partly because preclinical studies often use models with mild disease, contrasting with the advanced stages seen in elderly patients with comorbidities. The BBB complicates effective drug delivery, and the lack of specific clinical biomarkers hampers accurate assessment of disease progression and treatment efficacy.

Developing better preclinical models and enhancing drug delivery and biomarker identification are crucial for bridging this gap. Ethical and regulatory challenges in Alzheimer's disease clinical trials involve complex issues such as patient consent, trial design, and risk-benefit assessments. A key ethical concern is obtaining informed consent, particularly for those in the early stages of Alzheimer's disease or with mild cognitive impairment, necessitating alternative consent models like proxy consent. Regulatory hurdles in therapy approval arise from a lack of definitive biomarkers and disease variability, complicating efficacy assessments. The reliance on surrogate endpoints raises concerns about their clinical relevance. High costs, lengthy trials, and recruitment difficulties further question ethical justification and equity in benefit distribution, emphasizing the need for enhanced ethical and regulatory practices.

Future Perspectives and Directions in AD Research

Advances in precision medicine, driven by genetic, biomarker, and imaging data, will help to identify patient subgroups for specific treatments. Innovative technologies such as gene therapy, nanomedicine, and AI may enable targeted therapy delivery. Emphasis is shifting toward disease-modifying treatments, combination therapies, enhanced preclinical models, and biomarkers to predict progression. There is also a focus on preventive strategies for the aging population, improving diagnosis, treatment, and prevention. Multimodal therapeutic strategies for Alzheimer's disease combine pharmacological and non-pharmacological interventions to address the disease's complex nature. These strategies include amyloid-targeting agents, tau inhibitors, neuroprotective compounds, lifestyle changes, cognitive training, and physical exercise. By targeting key Alzheimer's disease hallmarks such as amyloid plaques, tau tangles, neuroinflammation, and synaptic dysfunction, these approaches aim to enhance therapeutic efficacy, promote neuroprotection, and personalize treatment using genetic and imaging data, ultimately improving patient outcomes.

The integration of artificial intelligence (AI) and machine learning (ML) in drug discovery is transforming Alzheimer's disease (AD) research by accelerating the identification of therapeutic targets and enhancing drug design and clinical trial outcomes. AI and ML analyze extensive genomic, proteomic, and imaging data to reveal complex patterns of AD pathology and identify biomarkers for diagnosis and treatment response. These technologies improve predictions of molecular interactions and drug properties essential for AD therapies while increasing clinical trial efficiency through better patient recruitment and monitoring. Personalized and precision medicine in AD focuses on tailoring treatments based on individual genetic, molecular, and clinical profiles. This approach identifies biomarkers, such as genetic mutations (e.g., APOE ε4) and neuroimaging characteristics, to classify patients into subgroups based on disease subtype and likelihood of treatment response. It enables earlier diagnosis, individualized therapeutic regimens, and consideration of lifestyle and environmental factors. Advances in genomics, AI, and bioinformatics enhance treatment efficacy and minimize adverse effects, transforming clinical management of AD.

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

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