

Molecular Pathogenesis and Interventional Strategies for Alzheimer's Disease: Promises and Pitfalls

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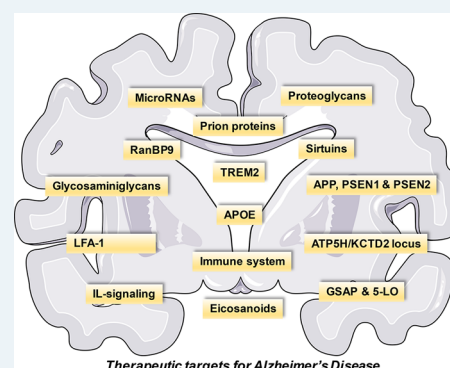
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ABSTRACT: Alzheimer's disease (AD) is a debilitating disorder characterized by age-related dementia, which has no effective treatment to date. β -Amyloid depositions and hyperphosphorylated tau proteins are the main pathological hallmarks, along with oxidative stress, *N*-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity, and low levels of acetylcholine. Current pharmacotherapy for AD only provides symptomatic relief and limited improvement in cognitive functions. Many molecules have been explored that show promising outcomes in AD therapy and can regulate cellular survival through different pathways. To have a vivid approach to strategize the treatment regimen, AD physiopathology should be better explained considering diverse etiological factors in conjunction with biochemical disturbances. This Review attempts to discuss different disease modification approaches and address the novel therapeutic targets of AD that might pave the way for new drug discovery using the well-defined targets for therapy of the disease.

KEYWORDS: proteoglycans, sirtuins, apolipoprotein, immune system, RanBP9, PrP^C



The neurodegenerative disorder Alzheimer's disease (AD) accounts for almost 70% of the cases of dementia. It is primarily diagnosed in older people with symptoms of memory loss and behavioral and cognitive abnormalities.¹ Over 46 million people worldwide have dementia.² Likely, the number will almost double every 20 years, to 81.1 million in 2040. The current total estimated worldwide healthcare cost related to dementia is US\$818 billion, and it is projected that by 2030 it will become a trillion-dollar disease,^{2,3} having an enormous economic impact.

AD is classified on the basis of the age of onset of the condition and whether it is a result of genetic mutation or is developed spontaneously. Early-onset AD is diagnosed before the age of 65 and is mainly caused by genetic mutations that are passed on to a child from a parent.⁴ Fewer than 10% of all AD patients have a familial type of AD.⁵ Mutations may arise in one of three genes—amyloid precursor protein (APP), Presenilin 1 (PSEN1), or PSEN2—which over time leads to amyloid plaque formation.⁴ Several genetic, lifestyle, and environmental factors are said to contribute toward late-onset or sporadic AD.⁴ This accounts for about 90% of the cases and usually occurs after 65 years of age.⁵ A variant of the gene ApoE called E4 allele is reported to increase the risk of developing late-onset AD.⁴ Late-onset AD affects almost half of all people over the age of 85.⁵ AD is an outcome of multiple pathogenic conditions with inherently complex biology and manifesting mainly as a cognitive deficit. While treating the

symptoms, it is of utmost importance to address the cause and find its unknown. Exploring new targets for treating the disease may result in the development of drugs that may impede neurodegeneration. Table 1 summarizes the various disease-modifying strategies along with genetic and immune targets for AD.

■ PATHOPHYSIOLOGY OF AD

Several pathogenic conditions are believed to accelerate the progression of the disease, and in the early stages of the disease these factors cause significant destruction of brain areas like the cortex and hippocampus.¹ Several hypotheses have been put forward for the pathophysiology of AD, including cholinergic hypothesis, amyloid hypothesis, tau hypothesis, and NMDA excitotoxicity theory.

The degeneration of cholinergic pathways and deposition of beta-amyloid ($A\beta$) are hallmarks of the disease. In the cholinergic hypothesis, the acetylcholine (ACh) neurotransmitter level is found to be lower, which is mainly due to increased activity of acetylcholinesterase (AChE) enzyme and

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Table 1. Overview of Novel Targets for AD

target	subcategories	mechanism
disease modifying strategies	proteoglycans (PGs) and glycosaminoglycans (GAGs) sirtuins gamma-secretase activating protein (GSAP) and 5-lipoxygenase (5-LOX) C5 bonding in the beta sheets	The 13–16 amino acid region of beta-amyloid ($A\beta$) is the binding site of PGs and GAGs. PGs and GAGs promote aggregation of $A\beta$ into insoluble amyloid fibrils. Sirtuins act via regulation of serine/threonine Rho kinase ROCK1, known for its role in the inhibition of the non-amyloidogenic α -secretase processing of amyloid precursor protein (APP). Sirtuins are potential modulators of cholesterol biosynthesis. 5-LOX directly and specifically activates caspase-3, which in turn leads to formation of the GSAP-active fragment, potentiating γ -secretase activity. C5 hydrogen bonds especially stabilize the flat β -sheets of the amyloid state.
genetic targets	APOE APP, PSEN1, and PSEN2 ATPSH/KCTD2 TREM2	Apo-E (encoded as APOE) brings about binding and internalization of soluble $A\beta$ by glial cells and disruption of $A\beta$ clearance at the blood–brain barrier (BBB) in an isoform-dependent manner (Apo-E4 > Apo-E3 > Apo-E2). Mutations on <ul style="list-style-type: none"> • Chromosome 21: formation of abnormal APP. • Chromosome 14: abnormal presenilin 1 (PSEN1) formation • Chromosome 1: abnormal presenilin 2 (PSEN2) formation. PSEN1 and PSEN2 are vital components of the γ -secretase complexes responsible for cleavage and release of $A\beta$. APP is broken down into smaller molecules of $A\beta$. Potassium conductance alteration and abolition of ATP to maintain neuronal survival during oxygen deprivation. TREM2 modulates microglial functions, as it facilitates $A\beta(1-42)$ phagocytosis and inhibits $A\beta(1-42)$ -triggered proinflammatory responses. This modulation is dependent on DAP12. TREM2 risk variants impair TREM2 function by decreasing affinity of TREM2 for its natural ligands and affecting its downstream products. Decreased function of TREM2 causes a decrease in phagocytic clearance of amyloid proteins or cellular debris and impairs the survival mechanism, resulting in systemic inflammatory response and neuronal death.
immune system	IL-12/IL-23 signaling inflammatory eicosanoids LEA-1 integrin	The mechanism is unknown, but IL-12 and IL-23 act via a common subunit p40, which can be targeted using small-molecule therapy. Thromboxane A2 (TXA2)-prostanoid (TP) receptor: increase of APP mRNA stability and enhanced APP expression and $A\beta$ production. EP1, EP3, and CysLT1 receptors: additional G α q-linked G protein-coupled receptors, which can modulate APP and $A\beta$ expression. $A\beta$ initiates the transition of LEA-1 from its low-affinity state to its higher-affinity state, thereby enhancing neutrophil adhesion. High-affinity LFA-1 may be critical for neutrophil accumulation and neutrophil-dependent damage during AD. The engagement of LEA-1 triggers neutrophil extracellular trap (NET) formation, potentially damaging the BBB and neural cells. Migrating neutrophils produce IL-17. IL-17 is directly toxic to neurons and the BBB and may recruit more neutrophils.
RanBP9		RanBP9 functions as a scaffold upon which APP, BACE1, and LRP are brought together. It inhibits cell adhesion by accelerating the endocytosis of β 1-integrin complexes. It also promotes apoptosis by activating the actin and mitochondria-associated protein coflin. RanBP9 exerts pro-apoptotic activity by regulating Bcl-2 and Bax protein levels in mitochondria.
prior proteins		Cellular prion protein (PrP ^C) is a mediator of $A\beta$ oligomer-induced synaptic dysfunction.

cholinergic neurodegeneration.⁶ The levels of ACh are reduced in the cortical and hippocampal regions, which are involved in memory function.⁶ The pharmacotherapy targeting this hypothesis is the administration of anti-cholinesterase drugs, like Donepezil, Rivastigmine, and Galantamine. Apart from AChE, butyrylcholinesterase (BuChE) is also targeted to improve cognitive dysfunction. Rivastigmine is an example of a drug that inhibits both AChE and BuChE enzymes.

In the amyloid hypothesis, aggregates of A β (40–42) peptide lead to further activation of inflammatory mediators like TLR4 and JNK, leading to the death of neurons.⁶ The enzymes responsible for the breakdown of APP and β - and γ -secretases lead to the formation of insoluble toxic aggregates called A β fragments.⁷ These fragments are considered to be the primary cause of neurodegeneration in AD.⁶ The enzyme α -secretase acts upon APP under physiological conditions to produce soluble APP α (sAPP α) fragments. These fragments remain within the extracellular space. Other fragments that are formed are the carboxy-terminal 83-amino-acid (C83) fragments, which anchor the plasma membrane.^{8–10} sAPP α is responsible for the modulation of neuronal excitability, increase in neuronal resistance to metabolic and oxidative stresses, and improvement in synaptic plasticity, learning, and memory.⁸ During a neuropathological situation, β -secretase-1 (BACE1) cleaves APP into sAPP β within the extracellular space and a membrane-bound fraction having 99 amino acids (C99). Processing by γ -secretase of the C99 fragment further leads to the formation of either A β (1–40) or A β (1–42) peptides. These peptides are said to be responsible for the generation of senile plaques^{8–12} (Figure 1). While sAPP α is beneficial, A β peptides may cause alteration of energy metabolism, synaptic loss, decreased neuronal plasticity, induction of oxidative stress, and mitochondrial dysfunction and may trigger disturbances in the cellular calcium homeostasis.^{8,9} One of the major goals of AD treatment is to bring about clearance or inhibit the formation of these A β fragments, along with improving patient's quality of life, survival, and function. Numerous A β -targeted therapeutic strategies are being pursued, including inhibition of A β aggregation, modulation of A β production, immunotherapy targeted at A β , and enhancement of A β degradation.¹³ Many drugs targeting A β peptides have failed in clinical trials, and ongoing attempts are being made to address the issue. Many of the genes known to be risk factors of the disease act by increasing the levels of A β peptides and bring about neurodegeneration.

Tau is a protein that stabilizes the microtubule, particularly in the axons.¹⁴ Tau hyperphosphorylation causes neurodegeneration via the formation of neurofibrillary tangles (NFTs), which are insoluble paired helical filaments.⁶ Hyperphosphorylated tau protein and A β plaques are the known hallmarks of AD. Curative therapy of the disease will be called so only if it halts or reverses the disease symptoms in the patient.

Chronic "excitotoxicity" leads to neurodegeneration, which is a result of mild and chronic activation of NMDA receptors. The blockade of the NMDA receptor channel by Mg²⁺ can be removed even by modest depolarization of the plasma membrane,^{6,15} triggering a pathological inflow of Ca²⁺ within the postsynaptic neurons. This Ca²⁺ overload over a prolonged period leads to the loss of synaptic functioning, followed by synaptotoxicity and cell death.⁶ Drugs like Memantine, which are administered at a later stage of AD to antagonize NMDA excitotoxicity, provide only symptomatic relief.¹⁶

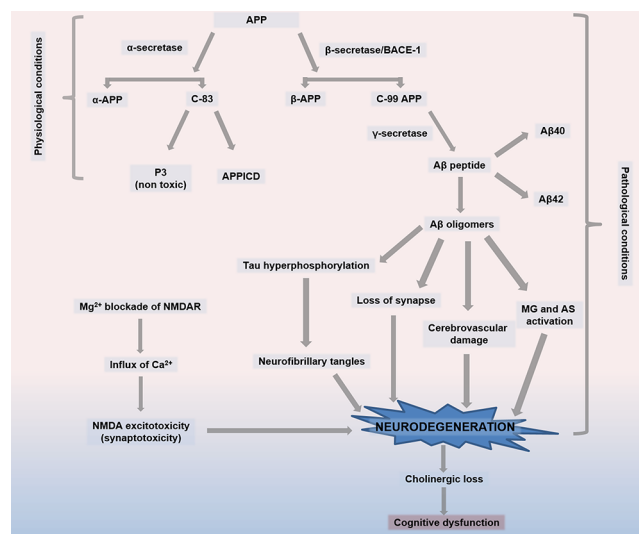


Figure 1. Pathophysiology of Alzheimer's disease. (1) Formation of beta-amyloid (A β) peptides through BACE. Under physiological conditions, APP is acted upon by the enzyme α -secretase to produce sAPP α fragments, which remain in the extracellular space, and carboxy-terminal 83-amino-acid (C83) fragments, which anchor in the plasma membrane. During a neuropathological situation, APP is first preferentially cleaved by BACE, which breaks down APP into sAPP β in the extracellular space and a 99-amino-acid membrane-bound fraction (C99). Further processing of the C99 fragment by γ -secretase results in the formation of either A β (1–40) or A β (1–42) peptides, which are thought to be responsible for senile plaque formation. (2) Tau hyperphosphorylation resulting in neurofibrillary tangle formation, loss of synapse, cerebrovascular damage, and MG-AS activation due to A β oligomers. (3) NMDA excitotoxicity is caused by Mg²⁺ blockade of NMDAR, leading to calcium influx and neuronal death. (4) Cholinergic neuronal loss and cognitive dysfunction. Abbreviations: APP, amyloid precursor protein; sAPP- α , soluble fragment formed from APP after cleavage by α -secretase; sAPP- β , soluble fragment formed from APP after cleavage by β -secretase; BACE, β -secretase 1; C83, carboxy-terminal 83-amino-acid fragment; C99, carboxy-terminal 99-amino-acid fragment; MG, microglia; AS, astrocytes; NMDAR, N-methyl-D-aspartate receptor.

By targeting A β plaques and the intracellular tau-containing neurofibrillary tangles, most therapeutic interventions have been focused on the inhibition of the aggregation of these proteins in the brain. Several other targets have also been dissected to explain AD etiology and physiopathology, but none of the attempts brought a convincing outcome. The present review attempts to address the novel therapeutic targets of AD that might pave the way for drug discovery using a known target and provide a successful pharmacotherapy for the complete treatment of the disease.

■ DISEASE MODIFYING STRATEGIES

Proteoglycans and Glycosaminoglycans. Proteoglycans (PGs) play an integral role in the formation of amyloid proteins,¹⁷ which includes promoting the formation of insoluble amyloid fibrils due to the accumulation of A β .¹⁸ These fibrils contribute to the increased neurotoxicity of A β .¹⁸ Self-aggregating A β peptides *in vitro* freely form amyloid fibrils. But amyloid aggregation and fibril formation are dependent on their interaction with heparin and PGs.^{18–21} Carbohydrate portions of PGs, along with sulfate moiety in glycosaminoglycans (GAGs), are essential for forming amyloid fibrils.^{17,18,22} Colocalization of PGs and A β within the AD brain has been

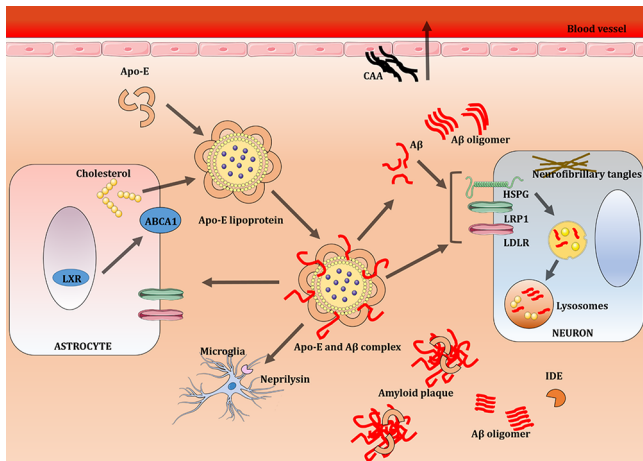


Figure 2. ApoE lipoprotein and $A\beta$ metabolism in the brain. $A\beta$ clearance is brought about by receptor-mediated uptake by glia and neurons, drainage into interstitial fluid, or through the BBB, and also by proteolytic degradation by IDE and neprilysin. Astrocytes and microglia synthesize Apo-E, which is lipidated by the ABCA1 transporter to form lipoprotein particles. This apolipoprotein binds to soluble $A\beta$ and facilitates $A\beta$ uptake through cell-surface receptors like LRP1, LDLR, and HSPG. Apo-E enhances binding and internalization of soluble $A\beta$ by glial cells, disrupts $A\beta$ clearance at the BBB, and influences CAA pathogenesis. Abbreviations: $A\beta$, beta-amyloid; ABCA1, ATP-binding cassette A1; Apo-E, apolipoprotein E; BBB, blood–brain barrier; CAA, cerebral amyloid angiopathy; HSPG, heparan sulfate proteoglycan; IDE, insulin-degrading enzyme; LDLR, low-density lipoprotein receptor; LRP1, low-density lipoprotein receptor-related protein 1; LXR, liver X receptor.⁴⁹ Adapted from Servier Medical Art, licensed under a Creative Commons Attribution 3.0 Unported License (<https://smart.servier.com/>).

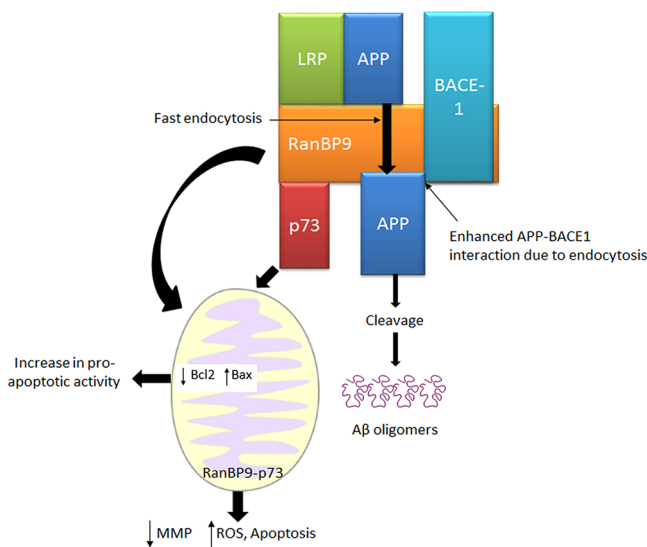


Figure 3. Interactions of RanBP9 with APP, LRP, and BACE1. RanBP9 promotes the endocytosis of APP and considerably increases its BACE1 cleavage to generate $A\beta$ fragments. RanBP9 exerts pro-apoptotic activity by regulating B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X (Bax) protein levels in mitochondria. RanBP9 and p73 together induce abnormal changes in mitochondria (MMP, superoxide levels, apoptotic proteins, and fission) and bring about apoptosis.

reported in NFTs and senile plaques.²³ The $A\beta$ region of 13–16 amino acids (His-His-Gln-Lys) has been identified as a

binding site of PGs and $A\beta$, representing a distinctive site of a target for inhibiting the formation of the amyloid fibril. Mainly the His13 region is critical for interacting with GAGs.^{18,24} Formation of β -plated structures can be inhibited by low-molecular-weight heparins (LMWHs), e.g., enoxaparin and dalteparin, and also reverse amyloidosis.^{25,26} At the same time, it also decreases amyloidosis associated with inflammation.²⁵ Anionic disulfides are said to inhibit $A\beta$ fibril aggregation. These compounds also cross the blood–brain barrier (BBB) and have been shown to have a protective effect against $A\beta$ associated cytotoxicity.²⁷ Heparin oligosaccharides (e.g., neuroparin, CSPG-DS) have inhibitory effects on the assembly of proteoglycans and demonstrate anti-inflammatory activity.^{28,29} Other drugs interfering with the interaction of GAGs with amyloidogenic proteins include eprodinate and its structural analog, tramiprosate.³⁰ The drugs prevent the polymerization of amyloid fibrils and their deposition in the tissues.³⁰

Sirtuins. The sirtuins are a group of enzymes controlling a range of diverse and fundamental cellular functionings.³¹ Modulation of sirtuin activities results in the activation of many cellular processes, namely, anti-inflammatory, anti-apoptotic, and anti-stress responses. Sirtuins, at the same time regulate the aggregation of proteins, which are intricately involved in the progression of neurodegenerative disorders.³² Recently, in various models of neurodegeneration, sirtuins were seen to act as disease modifiers.³³ SIRT1 overexpression or its pharmacological activation using NAD^+ promotes the activity of α -secretase and mitigates $A\beta$ peptide generation, as seen *in vitro* in Tg2576 embryonic mouse neurons.³⁴ This process takes place via the regulation of serine/threonine Rho-kinase ROCK1, a protein that is well known for its role in inhibiting the non-amyloidogenic α -secretase processing of APP.³⁴

SIRT1 is recognized to regulate the process of cellular cholesterol biosynthesis, demonstrating its role in neuro-protection.³⁴ Culture-based models of AD tauopathy and ALS have shown that activation of SIRT1 by resveratrol, a well-proven antioxidant, promoted survival of neurons.³⁵ Resveratrol has been shown to reduce hippocampal neurodegeneration and prevented learning impairments in an inducible transgenic mouse model of AD tauopathy.³⁶ This process correlates with the decreased acetylation of PGC-1 α and p53, which are the known SIRT1 substrates.³³ Injecting lentivirus expressing SIRT1 within the hippocampal region of transgenic mice provided substantial protection against neurodegeneration.³³ Such studies suggest the positive therapeutic benefits of SIRT1 activators for tauopathies. To date, resveratrol is the only SIRT1 activator for which a double-blind, randomized, and placebo control trial has been carried out.³⁷ Although resveratrol did not provide any significant beneficial outcome as compared to the placebo, it was, however, found to be well tolerated even at very high doses, cross the BBB, and alter the pattern of AD biomarkers.³⁸

Gamma-Secretase Activating Protein and 5-Lipoxigenase. $A\beta$ formation is catalyzed by γ -secretase.³⁹ A novel γ -secretase activating protein (GSAP) was recently discovered, which has been shown to significantly and selectively increase the synthesis of $A\beta$ through mechanisms involving interactions between APP carboxy-terminal fragment (APP-CTF) and γ -secretase.⁴⁰ Recombinant GSAP stimulates $A\beta$ production *in vitro*.⁴¹ In AD mouse model, knockdown of GSAP reduced the levels of $A\beta$ and plaque development.⁴¹ Thus, GSAP can serve

Table 2. List of Stem Cells under Clinical Trials for AD^a

NCT number	type of stem cells	phase	status	place
NCT02600130	HMSCs	1	recruiting	United States
NCT02833792	HMSCs	2	recruiting	United States
NCT02672306	HUCMSCs	1 and 2	active, not recruiting	China
NCT01547689	HUCMSCs	1 and 2	unknown	China
NCT02054208	HUCMSCs	1 and 2	recruiting	Korea
NCT01297218	HUCMSCs	1	completed	Korea
NCT00927108	PSCs	2	unknown	Thailand
NCT03117738	HAMSCs	1 and 2	active, not recruiting	United States
NCT03172117	HUCMSCs	1 and 2	recruiting	Korea
NCT02912169	ADSVFCs	1 and 2	withdrawn	United States
NCT02899091	HPMSCs	1 and 2	unknown	Korea
NCT03724136	BMSCs	N/A	recruiting	United States/United Arab Emirates
NCT01696591	HUCMSCs	N/A	unknown	Korea

^aAbbreviations: HMSCs, human mesenchymal stem cells; HUCMSCs, human umbilical cord mesenchymal stem cells; PSCs, progenitor stem cells; HAMSCs, human adipose mesenchymal stem cells; ADSVFCs, adipose-derived stromal vascular fraction cells; HPMSCs, human placental mesenchymal stem cells; BMSCs, bone marrow stem cells; N/A, not available. Data from clinicaltrials.gov.

Table 3. List of Selected Anti-AD Drugs in Active Clinical Trials^a

NCT number	drug	mechanism	phase
NCT01966666	TPI-287	microtubule stabilization	I
NCT02991235	JNJ-63733657	tau elimination	I
NCT03698695	THN 201	N/A	I
NCT02947893	Nilotinib	A β inhibition and tau stabilization	II
NCT03352557	BIIB092	tau stabilization	II
NCT03790982	AD-35	inhibits neuroinflammation	II
NCT02292238	Benfotiamine	slows decline in brain glucose metabolism	II
NCT02615002	Piromelatine	melatonin and serotonin receptor agonist	II
NCT01767311	BAN-2401	A β inhibition	II
NCT03417986	Thiethylperazine (TEP)	A β inhibition	II
NCT03289143	RO7105705	N/A	II
NCT02880956	ABBV-8E12	tau stabilization	II
NCT03367403	LY3002813	A β inhibition	II
NCT01409915	Sagramostim	immunostimulator	II
NCT02756858	ANAVEX2-73	muscarinic receptor agonist	II
NCT01843075	Liraglutide	slows decline in brain glucose metabolism and prevents A β accumulation	II
NCT03518073	LY3303560	tau stabilization	II
NCT01998841	Crenezumab	A β inhibition	II
NCT02788513	BI 425809	modulates NMDA receptor function	II
NCT03131453	CNP520	BACE1 inhibitor	II and III
NCT03036280	Elenbecostat	BACE inhibitor	III
NCT02484547	Aducanumab	A β inhibition	III
NCT01872598	Masitinib	reduces neuroinflammation	III
NCT02051608	Gantenerumab	eliminates A β	III
NCT02008357	Solanezumab	inhibits A β aggregation	III

^aAs of September 2019. Data from clinicaltrials.gov.

as a therapeutic agent with an A β -lowering potential to inhibit γ -secretase.³⁹ Imatinib, an anti-cancer agent, is a direct target of

GSAP, prevents activation of γ -secretase activity, and was found to reduce A β production.⁴² However, a recent study has shown uncertainty in the role of GSAP and imatinib in regulating A β generation and γ -secretase activity.⁴³

The role of the enzyme 5-lipoxygenase (SLO) in the pathogenesis of AD was recently highlighted by demonstrating its association in A β formation and its deposition.^{44–46} Within the GSAP precursor protein sequence, a caspase-3 processing domain was identified, and a piece of experimental evidence for its involvement in the formation of active fragments 16 kDa GSAP along with A β peptides biogenesis was shown.⁴⁷ Further, SLO was discovered to be an endogenous GSAP formation regulator.⁴⁵ It is shown to act by specifically and directly activating caspase-3, which was confirmed using transgenic mouse models of AD where SLO activity was regulated pharmacologically or genetically.^{46,48} These results provide support toward SLO as a viable therapeutic target for AD lowering A β without having the concomitant toxic effect of the classical γ -secretase inhibitors.⁴¹

Approaches to Lower the Impact of Genetic Abnormalities for AD. Majority of the strategies available for AD treatment target either the A β pathway or the symptomatic pathology. In the view of rising failure in several clinical trials for drugs targeting A β , today, there is a vital necessity to identify new targets and develop other novel therapeutic strategies aimed toward the treatment of AD.⁴⁹ Another approach seen in the picture is called the genome-wide association study (GWAS).⁵⁰ This approach is being used to identify regions of interest within the genome, which increases the susceptibility of an individual toward the development of late-onset AD.⁵¹ Thirty-three such regions were reported by 2015.⁵⁰ Recent approaches that include the use of gene and cell therapies in preclinical and clinical studies have shown encouraging results.

APOE. The cause of late-onset AD is not much clear as it does not run in families, although some cases have been reported in several families. It is probably related to variations in one or more genes, as well as lifestyle and environmental factors.⁴ Apolipoprotein E (Apo-E) is an essential carrier of cholesterol⁵² and, along with repairing tissue injury, supports the transport of lipids in the brain.⁴⁹ The gene APOE, which expresses apolipoprotein, has been studied as a risk factor extensively for the disorder.⁴ An individual's chances of

Table 4. List of AD Drugs Failed in Clinical Trials

treatment strategy	drug	mechanism of action	reason for failure	ref
<i>Disease Modifying Treatment</i>				
Monoclonal Antibodies	gantenerumab	binds to and neutralizes A β	lack of clinical efficacy and futility	186
	solanezumab	binds to and neutralizes A β	no change found in levels of AD proteins and no significant improvement in cognitive outcome	187
	crenezumab	converts A β from insoluble to a soluble form	no clinical benefits noticed along with primary and secondary end points on ADAS-Cog/12 and CDR-SB score were not improved compared to placebo	188
	bapineuzumab	binds to and neutralizes A β	lack of clinical efficacy as ADAS-Cog/11 and CDR-SB score were not improved in comparison to placebo	185
	aducanumab	binds to and neutralizes A β	inability to meet primary end point	189
gamma-secretase inhibitors	semagacestat	inhibits γ -secretase	lack of clinical efficacy and worsening of cognitive abilities	190
	avagacestat	inhibits γ -secretase	narrow therapeutic window	191
BACE inhibitors	elenbecestat	inhibits enzyme BACE	unfavorable risk–benefit ratio	192
	verubecestat	inhibits enzyme BACE	lack of clinical efficacy	193
	atabecestat	inhibits enzyme BACE	elevated liver enzymes in participants	194
	umibecestat	inhibits enzyme BACE	worsening of cognitive abilities	195
<i>Symptomatic Treatment</i>				
neurochemical modulators	idalopiridine	increases Ach release by inhibiting SHT ₆ receptors	lack of clinical efficacy	196
	encenicline	nicotinic Ach receptor agonists	GI toxicity	198
	dimebon	H1 receptor antagonist	lack of clinical efficacy	197

developing late-onset AD increases with the presence of the apolipoprotein E (*APOE*) gene on chromosome 19 AD.⁴⁹

There are several forms of *APOE*. *APOE2* isoform is relatively rare and, in certain instances, provide some sort of defense against the disease.⁵⁰ The most common allele, *APOE3*, has a neutral role in the disease progression—neither, reducing, nor elevating the risk. The *APOE4* allele raises the risk for AD and is also responsible for the early onset of the condition. An individual can have zero, one, or two *APOE4* alleles, amplifying the risk of developing AD with an increasing number of alleles.⁵⁰

Synthesis of Apo-E primarily takes place in the microglia and astrocytes. It is followed by the lipidation of the protein by ATP-binding cassette A1 (*ABCA1*) transporter, which helps in the formation of lipoprotein particles.⁵³ Soluble A β then binds to the lipidated Apo-E facilitating the uptake of A β through several cell-surface receptors, which includes low-density lipoprotein receptors (*LDLR*), low-density lipoprotein receptor-related protein 1 (*LRP1*), and the *HSPG*.^{53,54} Apo-E brings about binding followed by soluble A β internalization by glial cells and disturbance in the clearance of A β at the BBB takes place in an isoform-dependent manner (Apo-E4 > Apo-E3 > Apo-E2).⁵⁵ This impacts cerebral amyloid angiopathy (*CAA*) pathogenesis, whose risk is elevated by the presence of *APOE4* allele.⁵⁵ This also influences the age-related cognitive decline.⁵⁶ For delivering lipids, binding of Apo-E lipoproteins takes place with various cell-surface receptors^{53,54,57} and also to hydrophobic A β peptide.⁵⁸ A β peptide aggregation and its clearance in the brain are differentially regulated by the different Apo-E isoforms. Each isoform performs a distinct function for modulating the brain lipid transport, glucose metabolism, neuronal signaling, neuroinflammation, and mitochondrial function.⁴⁹ AD pathogenesis is known to involve Apo-E4, which takes place via an A β -independent mechanism involving cholesterol homeostasis, synaptic plasticity, neuroinflammation, and neurovascular functions⁴⁹ (Figure 2).

Genin et al.⁵⁹ used the Rochester (USA) incidence data to calculate AD Lifetime Risks (*LTR*) from 10 132 controls and 7351 cases from Caucasian ancestry. The AD *LTR* without reference to *APOE* genotype was 14% in females, while 11% in males at the age of 85. *LTR* for *APOE44* female carriers increased up to 60%, 30% for *APOE34* female carriers, 51% for *APOE44* male carriers, and 23% for *APOE34* male carriers for the same age.⁵⁹ These results urge a shift of category to “major gene” from the “risk factor” of the *APOE* gene.⁵⁹

Numerous approaches have been sought out for targeting *APOE*. The use of A β antibodies that target the Apo-E binding site is one of the approaches.⁶⁰ A β 12–28p antibody reduced A β accumulation, microgliosis, and tau phosphorylation both *in vitro* and *in vivo*.⁶¹ CPO_A β 17–21p, an A β 12–28p derivative, also decreased A β load and its mediated neurotoxicity. This peptoid antibody treatment reduced glial activation and improved both spatial and short-term memory in mice.⁶² The use of a direct Apo-E antibody has also been suggested. One of the Apo-E antibody, HJ6.3, targets all forms of Apo-E to prevent its binding with A β . HJ6.3 is, however, reported to have safety issues that limit its use.⁶³ Recently, anti-human Apo-E4 (*HAE4*) was developed and found to be more specific than HJ6.3. Both of these antibodies worked by a microglia inflammation pathway to reduce the A β plaque load.⁶⁴

Small molecules that change the structure of Apo-E are also under consideration. Apo-E small-molecule structural correctors (*SMSC*) changed the structure of Apo-E4 into Apo-E3-like structure, reducing the detrimental effects of Apo-E4 on the pathology.⁶⁵ PH-002 is one such molecule, which in *in vitro* studies showed positive results. This molecule reduced A β production and decreased tau phosphorylation.^{65,66} Overexpressing Apo-E2 is another approach, as this allele is said to be the protective allele against AD. The use of gene therapy to express Apo-E2 has shown benefits in preclinical studies.⁶⁷ Lentiviral mediated Apo-E2 delivery to the hippocampus was

able to increase the Apo-E load. However, lentiviral-mediated delivery is less efficient due to its local distribution.⁶⁸ Adeno-associated virus (AAV) based Apo-E2 delivery was found to be more effective. Hippocampal injection of AAVrh.10hAPOE2 in mice exhibited increased Apo-E2 load along with a reduction in insoluble A β 42 and A β 40.⁶⁹ AAVrh.10hAPOE2 may be closest to being in clinical therapy because of its efficacy, wider distribution, and safety.⁶⁰

Agonists or antagonists of the nuclear receptors peroxisome proliferator-activated receptor- γ (PPAR γ) and liver X receptors (LXRs), which form complexes with Retinoid X receptors (RXRs) and control the expression of Apo-E,^{70,71} act as potential candidates for modulating Apo-E.⁴⁹ LXRs, apart from Apo-E, also modulate ABCA1, promoting cholesterol efflux.⁷² A β fibrils deposition requires Apo-E, as demonstrated in mouse amyloid models.⁷³ Interrupting the interaction between A β and Apo-E using A β -mimicking peptides could decrease A β aggregation and deposition, and this approach may be an effective therapy for treating AD.

APP, PSEN1, and PSEN2. Early-onset familial AD accounts for a tiny proportion (<1%) of all AD cases and typically develops before 65 years of age.⁴⁹ It may be a result of several different single-gene mutations occurring on chromosomes numbers 1, 14, and 21. These mutations lead to the formation of abnormal proteins.⁵⁰ Mutations on chromosome 21 are responsible for the formation of abnormal APP. Chromosome 14 mutation results in abnormal formation of presenilin 1, and a mutation on chromosome 1 leads to the formation of abnormal presenilin 2.⁵⁰ PSEN1 and PSEN2 are responsible for the cleavage and release of A β .⁴⁹

APP is broken down into visible clumps of A β .⁶ These clumps or plaques of A β are a hallmark of AD.⁹ Jonsson et al. explored the association between the variants of APP with AD. The most significant association was found with single nucleotide polymorphism (SNP) rs63750847. Allele A of SNP rs63750847-A results in the substitution of alanine by threonine at 673 positions in APP (A673T) and was found to be relatively common in the elderly control group than in the AD group. It is, therefore, protective against AD.⁷⁴ The amino acid is close to the site where the enzyme BACE1 ordinarily cleaves APP into smaller A β moieties—and the alteration is enough to reduce the efficacy of the enzyme.⁷⁴ Behavioral improvement and reduction in A β pathology were observed in the AD mouse model following lentiviral delivery of siRNA, which mediated knockdown of BACE1.⁷⁵ Pharmaceutical companies have been working on developing “BACE inhibitors” for more than a decade, and several are now under clinical trials. The drug that would mimic the effects of the mutation would have the potential both to prevent AD and to slow down cognitive decline. Downregulating APP using siRNA in APP overexpressing mice by herpes simplex virus (HSV) short hairpin (shRNA) has been shown to reduce A β pathology.⁷⁶ Gene therapy mediated by AAV acts on the proteolytic fragments of APP, which in APP/dE9 mice may have demonstrated neurotrophic effects and improved working memory.⁷⁷

ATP5H/KCTD2 Locus. Destefano and Gonza⁷⁸ recognized a novel association within the H⁺ transporting, ATP synthase, and mitochondrial F₀ (ATP5H)/potassium channel tetramerization domain-containing protein 2 (KCTD2) locus in AD.⁷⁸ KCTD2 gene is responsible for functions like DNA transcription,⁷⁹ voltage-dependent potassium channel function, and GABA_B receptor hetero-multimeric composition,⁸⁰ regu-

lating proteasome physiology⁸¹ and degradation of ubiquitinated proteins. This gene is also a member of the KCTD family. The ATP5H gene regulates cell energy production via respiration and is embedded in the third intron of the KCTD2 gene.⁸² Mitochondrial ATP synthase brings about ATP synthesis and comprises of two multi-subunit complexes, namely, membrane-spanning component, F₀, which contains the proton channel and the soluble catalytic core, F₁. ATP5H gene encodes the “d” subunit of a total of nine subunits of the F₀ complex.⁸³ The oxidative stress hypothesis for AD is well known, and AD cases express a significantly low level of the ATP5H gene encoding subunits.^{84,85} KCTD2, ATP5H, or both are attractive candidates for AD risk⁸⁶ and therapeutic targets. The ATP5H/KCTD2 expression can be used as a diagnostic tool for late-onset AD. AD is usually diagnosed when the disease progresses to the late stage. Thus, early diagnosis and prophylaxis are of utmost importance. Since mitochondria have a significant role to play in AD, future therapy targeting mitochondrial components like ATP Synthase subunits may have a novel approach in the treatment for AD.⁸⁷

TREM2. The membrane protein triggering receptor expressed on myeloid cells 2 (TREM2) is expressed in the peripheral tissues by myeloid cells.⁸⁸ It is encoded by the TREM2 gene and forms a receptor-signaling complex with the tyrosine kinase-binding protein (TYROBP) or DAP12, which triggers in macrophages and dendritic cells the activation of immune responses.⁸⁹ In microglia, TREM2 brings about signaling in two ways. One of the signaling pathways regulates phagocytosis⁹⁰ while the other pathway suppresses cytokine production and secretion.⁹¹ This may support survival by prompting the secretion of tumor necrosis factor (TNF) through tumor necrosis factor receptor 2 (TNFR2) at levels that potentiate survival and repair pathways.⁹²

Heterozygous rare variants of the TREM2 gene predispose to AD.⁹² TREM2 risk variants impair TREM2 function, which can be by decreasing the affinity of TREM2 to its natural ligands and affecting its downstream products. Reduced function of TREM-2 causes a decrease in phagocytic clearance of amyloid proteins or cellular debris and impairs the survival mechanism, resulting in a systemic inflammatory response and neuronal death.⁹³

Jiang et al.⁹⁴ demonstrated that during the disease progression, there was an upregulation in the levels of TREM2 in the microglia, which was credited to the increased levels of A β (1–42) in the brain. TREM2 was also shown to regulate the functions of microglia by inhibiting A β (1–42) triggered pro-inflammatory responses and facilitating A β (1–42) phagocytosis in primary cultured microglia where TREM2 was knocked down or overexpressed. Overexpression of TREM2 in brains of APP^{swe}/PS1^{dE9} mice markedly ameliorated the AD-related neuropathologies, accompanied by an improved spatial cognitive function.⁹⁴ This suggests that TREM2 upregulation serves as a compensatory response to A β (1–42), and by regulating the microglial function, it protects against AD progression.⁹⁴ TREM2 expression rises in parallel with a rise in A β levels in the cortex. Other components of the cascade, such as DAP12, are not dysregulated. Thus, for controlling microglial responses, TREM2 acts as a gateway.⁹²

Antibodies that stimulate the signaling of TREM2 are under development. TREM2 activating antibodies *in vitro* have been shown to activate ERK, and Ca²⁺ signaling in human dendritic cells.⁹⁵ Overexpressing TREM2 has been shown to reduce

inflammation and promote phagocytosis.⁹⁶ Lentiviral mediated increase in TREM2 expression in mice brain attenuated neuropathologic and cognitive alterations.⁹⁷ However, the use of lentiviral mediated approaches is not favored in humans due to the risk of developing oncogenic transformation.⁹⁵

Other Possible Targets. Along with the familial AD variants in *APP* and *PSEN1/2*, coding variants in *BINI*, *ABCA7*, *ADAM10*, *EPHA1*, *CD2AP*, *CLU*, *CRI*, *MS4A4A/MS4A6A*, *SORL1*, *PICALM*, and *PLD3* are associated with late-onset AD. They have been identified by targeted sequencing or exome sequencing.⁹⁸

Targeting Immune System. The immune system plays a major role in the pathogenesis of AD, and this has been supported by findings from studies that reported reduced incidence of the pathology in transgenic AD mice following pharmacological and genetic manipulation of the IL-12/IL-23 signaling pathway.^{99,100} Modulation of the immune system is widely accepted to influence the progression of the disease, although there is a lack of clarity on whether the process of inflammation is spontaneous or is an associated event of the pathology. Epidemiological studies propose that non-steroidal anti-inflammatory drugs (NSAIDs) can diminish the risk of AD, but numerous prospective placebo-controlled trials evaluating the efficacy of NSAIDs in AD have been futile in demonstrating the same.^{101,102}

Following $A\beta$ release and deposition, certain hallmarks of inflammation have been reported in AD-like proinflammatory cytokines upregulation (tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β)) and generation of reactive oxygen species.¹⁰³ In AD mice models, it was observed that inhibition of IL-1 β or IL-6 signaling did not alter the plaque load while deletion of CD40 ligand (CD40L) or neutralization of prostaglandin E2 resulted in a slight reduction in $A\beta$ plaque load.⁹⁹ Expression patterns of inflammatory mediators in AD are not well defined.¹⁰⁴ Microglia, which is closely associated with $A\beta$ plaques, and other glial cells are described to be the primary source of proinflammatory cytokines in many CNS diseases, including AD.¹⁰³ TNF- α , in particular, has been reported to have a significant influence on neurodegeneration in mice.^{105,106}

Modulating IL-Signaling. AD pathology is marked by an increase of proinflammatory cytokines as a response to $A\beta$. p40 is an IL-12/IL-23 signaling molecule and a shared subunit of IL-12 and IL-23. Increased production of this subunit p40 by microglia was found in the *APPPS1* AD mouse model. The knockout of p40 resulted in decreased cerebral amyloid load. Likewise, a decrease in cerebral amyloid load was observed in *APPPS1* mice following the peripheral administration of a neutralizing p40-specific antibody. When p40 antibodies were delivered intracerebroventricularly (icv), there was a significant reduction in the level of soluble $A\beta$ species and a reversal of deficits in cognition in aged *APPPS1* mice. In cerebrospinal fluid of AD patients amount of p40 was found to be increased, suggesting that inhibiting the IL-12/IL-23 pathway by targeting the p40 subunit may attenuate AD pathology and its associated cognitive deficits.¹⁰⁷ However, the real challenge lies in delivering mAb-based therapy across the BBB. Alternatively, small-molecule inhibitors of IL-12/IL-23 can be easily made to get across the BBB.¹⁰⁸

Anti-inflammatory cytokines may have beneficial effects in AD, as demonstrated by various studies. Delivery of IL-2 via AAV viral vector into the brains of APP/PS1dE9 mice reportedly enhanced memory function increased synaptic

plasticity and restored spine density.¹⁰⁹ AVV-mediated enhanced IL-10 gene expression in APP/PS1 mice hippocampus led to amelioration of cognitive dysfunction, reduced astrogliosis, and promoted neurogenesis.¹¹⁰ However, a similar study carried out in TgCRND8 mice and Tg2576 mice demonstrated opposite effects. The study reported an increase in the plaque load, reduction in synaptic proteins, and memory impairment.¹¹¹ Delivery of IL-4 AVV viral vector in AAP/PS1 mice hippocampus and frontal cortex at 3 months of age was able to reduce the accumulation of soluble and insoluble $A\beta$ following 3 months of treatment.¹¹² In a different study, IL-4 gene delivery in the hippocampus of AAP/PS1 mice showed increased neurogenesis, improved cognitive function, and suppressed $A\beta$ pathology.⁷⁵

Inflammatory Eicosanoids. Increased microglial activation observed in areas surrounding the senile plaques in the AD brain results in inflammation.¹¹³ The thromboxane A2 (TXA2)-prostanoid (TP) receptor located on the neurons is activated, leading to increased stability of APP mRNA, which increased $A\beta$ production and APP expression.^{114,115} Activated microglia produce TXA2 leading to an increase in its concentration in the AD brain.¹¹⁶

An important finding is the identification of the CysLT1, EP1, and EP3 receptors as supplementary $G\alpha$ q-linked G protein-coupled receptors which regulate $A\beta$ and APP expression. The knockout of these receptors leads to a substantial decrease in plaque load in APP transgenic mice.^{117–119} A similar knockout of PGE2 EP4 receptor or its pharmacological inhibition also reduces plaque burden as observed in an APP transgenic mice model.¹²⁰ The increase of APP and $A\beta$ in AD due to eicosanoids may be potentially prevented by the inhibiting eicosanoid production. However, the approach has yielded varying results. In culture systems, an approximate 2-fold rise in APP levels was seen following multiple $G\alpha$ q-linked eicosanoid receptor activations, which is coherent with enhanced levels of APP in AD patient brains, as well as in plaque-bearing 5XFAD mice an increase in APP expression was observed. Limiting the synthesis of eicosanoids in aged 5XFAD mice brings about a reduction in the total levels of APP and decreases the APP fragments with COOH-terminal. This further drives researchers to scrutinize the involvement of these inflammatory mediators along with their receptors toward $A\beta$ plaque pathology.¹²¹

Lymphocyte Function-Associated Antigen 1 (LFA-1 Integrin). Neutrophils are involved in the destruction of tissue during inflammation and also known to be the first in line for defense against inbound pathogens.^{122–124} $A\beta$ induces adhesion of neutrophils, which is dependent on LFA1, by initiating the shift of LFA-1 to its higher-affinity state from its low-affinity state, thereby increasing adhesion of neutrophil. The high-affinity LFA-1 act as a stop signal for the arrested neutrophils, which are present in the parenchyma, which suggests that high-affinity LFA-1 might be crucial for neutrophil-dependent damage and its accumulation during AD.

LFA-1 integrin blockade or neutrophil depletion at the inception AD had a significant ability to decrease memory deficits and other neuropathological hallmarks in AD-like mouse models.¹²⁵ This clearly demonstrates the role of neutrophils in the induction of cognitive dysfunction. Also, at the earlier stages of the disease, brief suspension of neutrophils had an effective long-term effect on older animals, which shows that neutrophils are indeed vital for the

development of a chronic disorder. Antibodies blocking LFA-1 in mice displayed evidence of memory restoration in behavioral tests as compared to control animals.¹²⁶ Also, mice that lack LFA-1 integrin showed improved cognition with lesser neuropathological changes as compared to the wild-type mice.¹²⁶

Silva¹²⁷ showed that blocking LFA-1 integrin leads to neutrophil depletion and inhibition of neutrophil trafficking, which dramatically reduces the activation of microglia. These two processes may create a series of feedback loops that sustain and amplify their activation.^{127,128} This hints toward the probable existence of a neutrophil–microglia crosstalk, which formerly has not been hypothesized in AD. The engagement of LFA-1 triggers neutrophil extracellular traps (NET) formation, potentially damaging the neural cells and BBB. Furthermore, confocal microscopy confirmed the presence of NETs within the parenchyma and cortical vessels of patients with AD.¹²⁹ Evidence from AD patients and AD animal models suggests that targeting NET components, such as DNase I, protein arginine deiminases (PAD), and NADPH oxidase, may help to limit tissue damage.^{130,131} Migrating neutrophils produce IL-17, which is directly toxic to neurons and BBB and may further recruit more neutrophils.^{124,132} Therefore, targeting the neutrophil-dependent inflammatory mechanisms may hinder early pathogenesis and, during the disease progression, may protect AD patients from cerebral injury.¹²⁹

RanBP9. The scaffolding protein RanBP9 interacts with LRP, APP, and BACE1 via its cytoplasmic tails. It acts as a scaffold on top of which APP, BACE1, and LRP are brought together.¹³³ Such interactions of RanBP9 with APP and BACE1 promote APP endocytosis, considerably increases BACE1 cleavage, and generate A β fragments in cultured cells and *in vivo*.^{134,135} Also, in the brains of AD patients, there is found to be a robust increase of a 60 kDa proteolytic fragment of RanBP9, which strongly accelerates A β generation through BACE1 processing of APP.¹³⁶ Moreover, RanBP9 inhibits adhesion of cells by potentiating the β 1-integrin complex endocytosis.¹³⁷ By activating the actin and mitochondria-associated protein cofilin, it promotes apoptosis.¹³⁸ A 4-fold increase in RanBP9 proteins was found in mutant APP transgenic mice, and a significant increase in neurodegeneration, gliosis, spatial memory deficits, and increased synapse loss in RanBP9 transgenic mice was observed.¹³⁸ Woo et al. showed that APP-PS1 mice exhibited a 3.5-fold increase in the levels of RanBP9, and in the same mice, they demonstrated that reduction in RanBP9 protected neuronal cells against synaptic damage, cofilin-actin pathology, A β accumulation, and gliosis.¹³⁹ A study showed that in DNA damage-induced apoptosis, RanBP9 exerted pro-apoptotic activity. It acts by regulating B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X (Bax) protein levels within the mitochondria.¹⁴⁰ Physical association with the C-terminal of p73a (a tumor suppressor protein) with RanBP9 has also been reported. This interaction modulates the exogenously expressed p73a levels along with the nuclear translocation of RanBP9.¹⁴¹ Apoptosis can also be induced via nuclear and non-nuclear pathways by p73a.¹⁴² Liu et al.¹⁴³ found that RanBP9 and p73 together induce abnormal changes within the mitochondria and initiate apoptosis that depends on their supportive actions. This demonstrates the critical role of the RanBP9/p73 pathway in maintaining apoptosis in mitochondria mediated manner during neurodegeneration (Figure 3).

Prion Proteins (PrP). AD progression has been shown to correlate with soluble non-fibrillar forms of A β protein.¹⁴⁴ These soluble A β oligomers (A β o) block the long-term potentiation (LTP) in the hippocampus at nanomolar concentrations¹⁴⁵ and impair rodent spatial memory.¹⁴⁶ Antibodies targeting PrP prevent A β -oligomer binding to cellular prion proteins (PrP^C) and ameliorate synaptic plasticity in hippocampal slices.¹⁴⁷ PrP^C have demonstrated a high affinity for A β o. PrP^C are highly expressed in the mammalian nervous system and are a glycosyl-phosphatidylinositol-anchored protein located on the plasma membrane.¹⁴⁸ Interaction of PrP^C with A β o in AD models has been shown to mediate synaptic loss, aberrant signaling pathway, and decline in cognition.¹⁴⁹ This binding has been shown to recruit metabotropic glutamate receptors type 5 (mGluR5) and abnormally activate Fyn kinase to impair synaptic function.¹⁵⁰ Salazar et al. studied the effects of PrP^C ablation in advanced AD stages in APP/PS1 mice and demonstrated an increase in protein synthesis, which restored neuronal activity. This improvement in neuronal activity was attributed to blockage of increased phosphorylation of eEF2.¹⁴⁹ A study by Li and Gotz showed that PrP^C deletion had a positive influence on tau hyperphosphorylation as a somatodendritic accumulation of Tau has been linked with Fyn.¹⁵¹ Thus, the A β o-PrP^C-mGluR5 interaction can be a potential therapeutic target for AD as it plays a significant role in A β o-induced synaptic dysfunction. It was found that the constitutive knockout of PrP^C expression in a mouse model of AD upturned several pathological phenotypes¹⁵² as did the peripheral treatment with an anti-PrP antibody on the same mouse model.¹⁵³ *In vitro* data of LTP recordings from PrP null mice and congenic wild-type mice have confirmed the PrP^C dependence of the inhibitory effect of A β -derived diffusible ligands (ADDLs) on synaptic plasticity.¹⁴⁴

However, the ability of a few A β preparations to integrate with PrP and form a complex is questioned by one report.¹⁵⁴ Different synthetic A β preparations lack characterization and have heterogeneity.¹⁵⁵ This makes it imperative to state the active PrP-independent, PrP-dependent, and benign species to draw a parallel to their presence with the pathogenesis of AD and its progression. Electron microscopy has revealed that the PrP-binding protofibrils have a triple-helical structure and are called A β nanotubes. These are present within the human brain and play a significant role in disease progression. This has been demonstrated by a study that shows that PrP expression is requisite for protofibril synaptotoxicity, and similar results have been demonstrated by water-soluble AD brain extracts blocking LTP in a PrP-dependent manner. Thus, using humanized anti-PrP monoclonal antibodies to target PrP–A β nanotube interactions with maybe an essential therapy for AD.¹⁵⁶

Multi-Target-Directed Ligand (MTDL). At present, another area of emerging therapeutics is multi target-directed ligand (MTDL) strategy, which emphasizes targeting multiple enzymes like AChE, BChE, BACE1, and MAO.¹⁵⁷ In the future, MTDLs may offer enhanced efficacy against AChE and A β plaque formation or offer metal-complexing, neuroprotective, antioxidant, inhibition of glutamate-induced excitotoxicity, voltage-dependent calcium channel antagonistic activity, cannabinoid CB1 receptor antagonism, histamine H3 receptor antagonism, and BACE1 inhibition to nip the evil of disease with multiple arms.¹⁵⁸ This will eventually lead to the

mitigation of AD symptoms resulting in better therapeutic outcomes and enhanced patient compliance.

MicroRNAs (miRNAs). miRNAs within the neurons play a vital role in regulating synaptic plasticity, neuronal differentiation, neurite outgrowth, and maintaining dendritic spine morphology.¹⁵⁹ Dysfunction of miRNAs is now well recognized in AD, and their use as a therapy for AD is increasing widely. Antagonists or mimics of miRNAs are known to modulate the expression of various genes involved in the pathogenesis of AD.¹⁶⁰ miRNAs can be modulated in two ways: first, by inhibiting the functioning of miRNA by using a single-stranded complementary antisense oligonucleotide (ASO), and second, by increasing its expression by administration of compounds that stimulate its secretion or using a double-stranded synthetic oligonucleotide miRNA which mimics the function of the endogenous miRNA.¹⁵⁹ Studies from cell cultures and animal models have shown that increased expression of specific miRNAs may inhibit A β and tau accumulation by acting at different molecular levels.¹⁶⁰

Higaki et al. identified miRNAs from human neuroblastoma cells and murine primary neurons, miR200b and miR200c, to be downregulators of A β secretion by regulating the mTOR pathway.¹⁶¹ Overexpression of miR-124 in SHSY5Y cells suppressed the expression of BACE1, while its inhibition increased BACE1 levels.¹⁶² Transfecting SHSY5Y cells with miR-15b inhibits BACE1, thereby reducing A β .^{162,163} miR-302/367 has been shown to convert astrocytes into neurons, replacing the dead ones in animal models of AD.¹⁶⁴ Neuronal stem cells transfected with miR-9 increased differentiation of stem cells into neurons.¹⁶⁵ Improvement in synaptic and cognitive activity was observed in AD mouse models following upregulation of miR-188.¹⁶⁶ miR-214 and miR-let-7f-5p have been shown to inhibit autophagy and decrease caspase-mediated apoptosis.^{167,168} Inhibition of miR-128 in cell cultures decreased A β cytotoxicity by inactivating NF κ B pathway.¹⁶⁹

Several pharmacological compounds can be utilized for modulating miRNA expression. NSAIDs and simvastatin are known to modulate miRNA expression and may prevent the progression of AD.^{170,171} Natural compounds such as resveratrol and osthole act as potential neuroprotective effects by modulating miRNAs levels.^{165,172} Recent studies have demonstrated that exosomes may be utilized as therapeutic agents in AD patients to deliver miRNAs or siRNAs.¹⁷³

The use of miRNAs for therapy of AD patients looks enticing and will be expanded in the coming years with the help of computerized gene analyses. However, limitations in its clinical applicability persist due to difficulty in delivering to the target site and determination of the specificity of miRNA silencing.¹⁷⁴ Also, altering the miRNA levels may have an undesirable effect in AD patients as every single miRNA is known to have a plethora of effects on the biological functioning of the body.¹⁷⁵

Stem Cells. Stem cell therapy for AD is being explored widely. Both preclinical, as well as clinical trial results, have supported the use of stem cells as a therapy for AD. Stem cells are an attractive means of replenishing neurons that are damaged or lost by means of neurogenesis.¹⁷⁶ They provide a ray of hope for developing disease-modifying therapies for AD. The ability of stem cells to proliferate, differentiate, self-renew, and reprogram themselves into different lineages make them excellent candidates for AD therapy.¹⁷⁷ Different types of stem cells have been identified, which are useful for AD therapy.

These include pluripotent stem cells (induced pluripotent and embryonic stem cells (ESCs)) and adult stem cells (mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), neural stem cells (NSCs), and olfactory ensheathing cells (OECs)).^{176,178} Table 2 lists the ongoing clinical trials with stem cells for AD. Although promising, however, scientists are trying to address answers to some technical difficulties that arise with the use of stem cells.¹⁷⁶ The time point for stem cell intervention is still under debate as many trials have failed following intervention failure at the appropriate time.¹⁷⁶ Gender-based differences exist and have to be taken into account for establishing a proper therapeutic regimen with stem cells.¹⁷⁹

Clinical Trials. Results from clinical trials have been disappointing and have pushed scientists toward intervening the disease in its earlier stages. However, this poses a challenge as determining therapeutic benefits in subjects who are healthy in the early stages of the disease becomes difficult.^{180,181} Keeping in view, a new draft was issued by the U.S. FDA in 2018 for conducting clinical trials in AD. The taxonomy of AD was expanded to contain four stages, namely, Stage 1: Preclinical; Stage 2: Preclinical/Prodromal; Stage 3: Prodromal; Stage 4: Dementia.¹⁸⁰ Candidates under clinical trial for AD treatment majorly include therapies that are targeted against A β , those which decrease A β production, and those which reduce the formation of neurofibrillary tangles.¹⁸² Other candidates include 5-HT₆ and H₃ receptor antagonists, AGE inhibitors, glucagon-like peptide-1 (GLP-1) agonists, calcium channel inhibitors, and TNF- α inhibitors. Table 3 shows a selected list of AD drugs under clinical trials.¹⁸³ As of 2019, nine trials for eight interventions targeting the amyloid pathway are in phase III, and over 30 interventions targeting the non-amyloid pathway are in phase III.^{180,184} The number of drugs in clinical trials targeting the amyloid pathway lowered in 2019, as compared to 2018 and 2017. Also, trials now have moved toward the earlier prodromal or preclinical stages of the disease.¹⁸⁴

Over the past few years, several promising candidates for AD have failed in clinical trials. Table 4 provides a list of selected drugs that have recently failed in clinical trials. The majority of the candidates who failed were the monoclonal antibodies (MAbs). MAbs such as bapineuzumab, gantenerumab, crenezumab, and solanezumab act by binding to A β and neutralizing it, but these agents failed to become a promising therapy due to several reasons.^{185–189} Bapineuzumab trial was prematurely terminated due to lack of clinical efficacies between ApoE4 carriers and non-carriers. In both the cases, fluctuation of Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog/11) and Disability Assessment for Dementia (DAD) total score at 78 weeks was the primary efficacy endpoint which was not improved when compared with placebo.¹⁸⁵ Similarly, gantenerumab clinical trial was stopped earlier due to futility. Groupwise comparison of primary Clinical Dementia Rating-Sum of Boxes (CDR-SB) looked at changes from baseline between placebo and two different doses of drug that were considered as the endpoints. The exploratory analysis for biomarkers suggests a necessary study with higher dose.¹⁸⁶ Solanezumab clinical trial failed, as no significant improvement in cognitive outcome was observed with respect to placebo.¹⁸⁷ Crenezumab study at phase 2 failed because the primary and secondary endpoints on ADAS-Cog/12 and CDR-SB score were not met.¹⁸⁸ Two γ -secretase inhibitors, avagacestat and semagacestat, failed mainly due to

lack of any clinical efficacy and reports of worsening of cognitive abilities. The narrow therapeutic window of these agents also deterred their use.^{190,191} Similar results were reported in the case of BACE inhibitors elenbecestat, atabecestat, verubecestat, and umibecestat.^{192–195} Neurochemical modulators such as idalopiridine and dimebon, which act by increasing Ach release and inhibiting H1 receptor, respectively, failed due to their lack of efficacy.^{196,197} Encenicline specifically failed as it caused gastrointestinal toxicity.¹⁹⁸ Experts point toward various reasons for the failure of drugs, one of which being targeting the wrong pathological substrates.¹⁹⁹ The question still remains whether one should target the plaque or the monomeric, oligomeric or the protofibrillar form of A β . Second, the methodology pursued from trial to trial. It is essential to look out for discrepancies that might exist between trials. Third, the timing of the treatment is of importance, and scientists are pushing toward starting treatment at the early stages of the disease. However, the patients selected for clinical trials may sometimes be far too advanced in terms of pathology, and the therapy might be late for such patients.¹⁹⁹

CONCLUSION

Currently, there are no curable treatments available for AD, but several symptomatic treatment regimens like the use of anti-cholinesterase drugs, NMDA antagonists, etc. are available. These treatments available result in a temporary improvement in cognitive functions. Targeting neuronal degeneration directly or its underlying cause would offer a better cure for AD. The targets and intervention strategies discussed above can be explored for the development of new chemical entities using several drug discovery techniques. This may find a lead that may further prove useful in the treatment of the disease and pave the way for novel drug regimens for AD.

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Notes

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