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Review

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Unraveling the tau puzzle: a review of mechanistic targets and therapeutic interventions to prevent tau pathology in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a prevalent neurodegenerative disease characterized by irreversible neural degeneration and cognitive decline. The prion-like propagation of the β-amyloid (Aβ) and tau proteins leads to the formation of protein plaques and, subsequently, neuronal dysfunction, contributing significantly to AD pathogenesis. Although effective AD treatments remain elusive, targeting tau protein aggregation has emerged as a promising therapeutic approach. However, recent anti-tau antibody trials have shown limited success in improving cognition, underscoring the need for a more advanced, multifaceted approach to address multiple mechanisms of tau pathology. This review examines the role of tau protein in the context of AD, with a particular

focus on potential therapeutic interventions. Emphasis is placed on the modulation of tau protein expression, tau post-translational modifications and aggregation, receptor-mediated uptake and extracellular release pathways, neural inflammatory response pathways, intercellular organelle exchange, mitochondrial function, microtubule stability, and nuclear factor expression as critical intervention points. Despite the challenges faced in ongoing anti-tau clinical efforts, a comprehensive strategy targeting multiple pathways involved in tau pathology, by using either combinations of existing drugs or novel multitarget drugs, holds promise. By gaining a deeper understanding of the complex mechanisms underlying tau pathology, researchers can develop innovative therapeutic strategies to combat AD.

Keywords: Alzheimer's disease, neurodegenerative disease, tau protein, protein aggregation, therapeutic approach, tau pathology

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease globally, afflicting nearly 50 million individuals and causing predominantly irreversible neural degeneration[1,2]. The disease is characterized by progressive memory loss, deterioration in coordination and spatial awareness, intellectual decline, and eventually complete loss of autonomy, often leading to death[1,3]. One distinguishing pathological characteristic of AD is the atypical neural buildup of two proteins: β-amyloid (Aβ) and tau. The formation of fibrillar amyloid contributes to protein plaques that disrupt neuronal signaling. The accumulation of tau into tangled fibrillar aggregates in neurons leads to neuronal and glial cell degeneration, ultimately leading to cognitive impairment[4,5]. Therapies targeting these two proteins may hold potential in the clinical context of AD.

Currently, no treatments are available for the disease, although certain therapeutics have demonstrated efficacy in slowing its progression in preclinical models and some patients[6]. A thorough understanding of the established mechanisms of AD pathology, specifically, tau protein aggregation and Aβ plaque formation, can provide valuable insight into possible therapeutic targets[7,8]. However, efforts to target Aβ aggregation mechanisms have yielded limited success in restoring cognitive function in AD patients over the past few decades[6]. Although implicated in neuronal toxicity, Aβ is not directly associated with symptomatic cognitive decline in AD, which may explain the limited efficacy of anti-Aβ treatments in mitigating symptoms[6,9]. An analysis of the correlation between Aβ, tau, and cognition concluded that Aβ levels are a poor indicator of memory decline[10].

In contrast, tau tangle formation and accumulation are closely associated with neuroinflammation, memory loss, and other symptoms of neurodegeneration[11-14]. Among a multitude of other effects, misfolded tau contributes to neural excitatory toxicity through extrasynaptic N-methyl-D-aspartate (NMDA) receptor activation and facilitates the migration of pro-inflammatory agents and immune cells to afflicted regions through blood-brain-barrier (BBB) transmigration, thereby exacerbating tau-related damage[15,16]. With recent advances in biomarker detection, tau has also emerged as a highly accurate predictor of cognitive decline in patients, strengthening the case for a tau-centric approach to AD treatment[12-14,17]. In short, targeting tau pathology, which is strongly linked to cognitive decline in AD patients and is localized in the brain regions most affected by the disease, holds promise for effective treatment[6,9].

Unfortunately, anti-tau antibody trials have continued to fall short as well. Drugs targeting tau aggregation and accumulation, such as Genentech and AC Immune's semorinemab, have failed to alleviate symptoms in early clinical trials[9,18]. Despite these translational challenges, a tau-based approach still has potential. Tau pathology arises from a complex interplay of mechanisms, including phosphorylation, protein transport,

and immune modulation[19]. A viable approach may involve combining two or more drugs or exploring novel multitarget drugs to employ a multifaceted strategy[19]. For example, utilizing a regimen that targets tau hyperphosphorylation, removes aggregated tau from the extracellular matrix (ECM), and inhibits intercellular vesicle transport of tau could provide a more substantial neuroprotective effect than individual drugs do[4,9,19,20].

This review will primarily focus on the pathogenic mechanisms of tau pathology and potential therapeutics that could target these pathways, as well as the limitations of current anti-tau clinical campaigns. To this end, the paper is intended for a wide range of audiences. For those new to the field, it provides a broad overview of the mechanisms of tau pathology and its importance to AD while emphasizing the potential of novel drug strategies. For pharmacologists and clinicians, the review discusses existing drug options and possible therapy combinations for use in the clinic. For researchers, the review offers a new perspective on the state of the field and possible future directions. Although the intricacies of tau pathology go beyond the scope of a single review, this paper describes some of the most promising prospects.

METHODS

We performed a comprehensive literature review of studies elucidating the mechanisms involved in neuronal tau propagation and describing potential therapeutics to counter such mechanisms. To begin, we queried the PubMed and Google Scholar databases for general terms, including "tau role in Alzheimer's", "tau uptake", "tau propagation", "tau aggregation", "tau therapies", and "tauopathy mechanisms". Based on the results of this preliminary search, we categorized major pathologic tau mechanisms into several groups: (1) genetic expression; (2) post-translational modifications; (3) extracellular uptake and release; (4) immune modulation; (5) intercellular organelle exchange; (6) mitochondrial modulation; (7) microtubule modulation; and (8) nuclear factor modulation. We queried specific receptors and mechanisms that appeared most frequently in this broad overview to explain mechanism significance in detail. Many studies of specific receptor targets or mechanisms in the propagation of tau reference existing drugs or potential therapeutics, which informed our subsequent queries into therapeutic possibilities and concepts.

TAU ROLE AND PROPAGATION

Tau, a microtubule-associated protein, is vital to the central nervous system (CNS) and the peripheral nervous system (PNS)[1,4]. It primarily operates in neuronal axons, which drive the formation and stabilization of microtubules (MTs), promote axonal outgrowth and transport, and inform cell morphogenesis and neural plasticity[1,7]. In its native form, tau is an unfolded, soluble protein characterized in part by a high number of lysine residues that contribute to tau binding capabilities[8,21]. Alternative splicing of the human gene coding for tau results in six different tau isoforms responsible for these various roles in the CNS. Phosphorylation of tau isoforms results in tau oligomerization and the formation of neurofibrillary tangles (NFTs), otherwise known as protein assemblies or aggregations[8]. This fibrillar form compromises normal tau function, leading to synaptic and neuropathological dysfunction[7]. Tau NFTs are heavily linked with cognitive impairment and the onset of AD[1,7,8]. These tau NFTs spread through cell-to-cell transmission in a prion-like manner, leading to neurodegeneration[4,7]. The mechanisms behind tau aggregation and cell-cell transmission should be targeted to hinder this propagation.

Cell-to-cell transmission of aggregate tau is central to AD pathogenesis[7]. This transmission occurs largely through receptor-mediated endocytosis and, specifically, macropinocytosis[7,8]. Specific factors, often stimulated by the tau protein itself, facilitate the receptor-mediated uptake of tau from the ECM into cells[21]. Tau transmission also occurs due to direct vesicular transport through tunneling nanotubes or exosomes, or via astrocytic, microglial, mitochondrial, and reactive oxidative species (ROS)-driven

mechanisms[22-26]. The mechanisms of tau hyperphosphorylation and post-translational modifications are also central to the tau pathology in AD, driving the structural breakdown that leads to aggregation and dysfunction[22,23]. These mechanisms likewise provide a wide range of cellular target options to reduce tau pathology. Targeting these aggregation, transmission, and post-translational modification mechanisms could be crucial to slowing tau propagation and restoring cognitive function[22,23,27].

Both monomeric tau and tau aggregates are players in the tau pathology characterizing AD[7,21]. Research has shown that elevated levels of monomeric tau in the ECM can trigger the formation of fibrillar seeds, which are aggregation-prone soluble conformations of tau that drive the assembly of tau aggregates[21]. Therapeutic receptor targets may have binding tendencies for either monomeric or oligomeric tau, a distinction that can inform therapeutic specificity[9,18,21]. This nuanced nature of the tau protein structure itself could explain the failure of anti-tau drugs thus far[9,18]. While certain drugs, like semorinemab, have targeted the general full-length form of tau, irrespective of post-translational modifications or phosphorylation state, drugs that focus on a more specific, phosphorylated epitope on the tau protein might yield greater success in reducing tau pathology[18,28]. It also may be the case that explicitly targeting the aggregate form of the protein rather than the monomeric form yields higher success rates and that use of anti-tau drugs in the early stages of disease onset yields dramatically more effective results[18]. Dosage level is also crucial in ensuring a drug's passage across the BBB while avoiding toxicity[18,29,30]. CNS therapeutic intervention involves many factors, especially considering the nature of tau and the various mechanisms and receptors that can be targeted[9]. Although the limited success of anti-tau drugs raises concerns, there remains justification for the ongoing pursuit of pathogenic tau as a target for AD treatments.

TARGETS AND INTERVENTIONS

The propagation of tau through the central nervous system involves numerous mechanisms [Figure 1][8,22,31-57]. These can be generalized into several primary categories, including the post-translational modifications of tau that drive aggregation, uptake of tau from the extracellular space into the cell, and conversely, the release of tau into the ECM, immune cell modulation, intercellular organelle exchange, mitochondrial dysfunction, microtubule instability, and nuclear modulation, among others[7,22-26,58]. Gene expression of tau also presents an intriguing target to modulate tau production and deter aggregation at its root[31,32,59]. This section will highlight several specific targets or mechanisms in each category and elaborate on potential therapeutic mechanisms to combat tau pathology.

Gene expression modulation

Microtubule-associated protein tau gene

Splicing of the tau gene, Microtubule-associated protein tau (MAPT), results in six isoforms of the protein with varied structure and function[8]. The isoforms with four microtubule-binding regions (MTBRs) tend to be more fibrillogenic than those with three MTBRs[8]. In a healthy brain, these two isoforms exist in relatively equal proportions[8]. However, missense mutations in MAPT resulting in the exclusion of certain exons and splicing variations lead to altered isoform proportions that can contribute to greater levels of the tau protein, especially in its 4-MTBR form, which drives more significant tau pathology[8]. The duplication of the mutated 17q21.31 region on chromosome 17 can increase MAPT gene dosage and drive early-onset, Aβ-negative dementia with symptoms resembling AD[60]. This reaffirms pathogenic tau as a central cause of AD.

With these mutations and the genetic underpinnings of tau production already reasonably well understood, therapies can directly target the MAPT gene to reduce tau production. One possible therapy utilizes Antisense Oligonucleotides (ASOs), short strands of oligonucleotides that bind to complementary mRNA

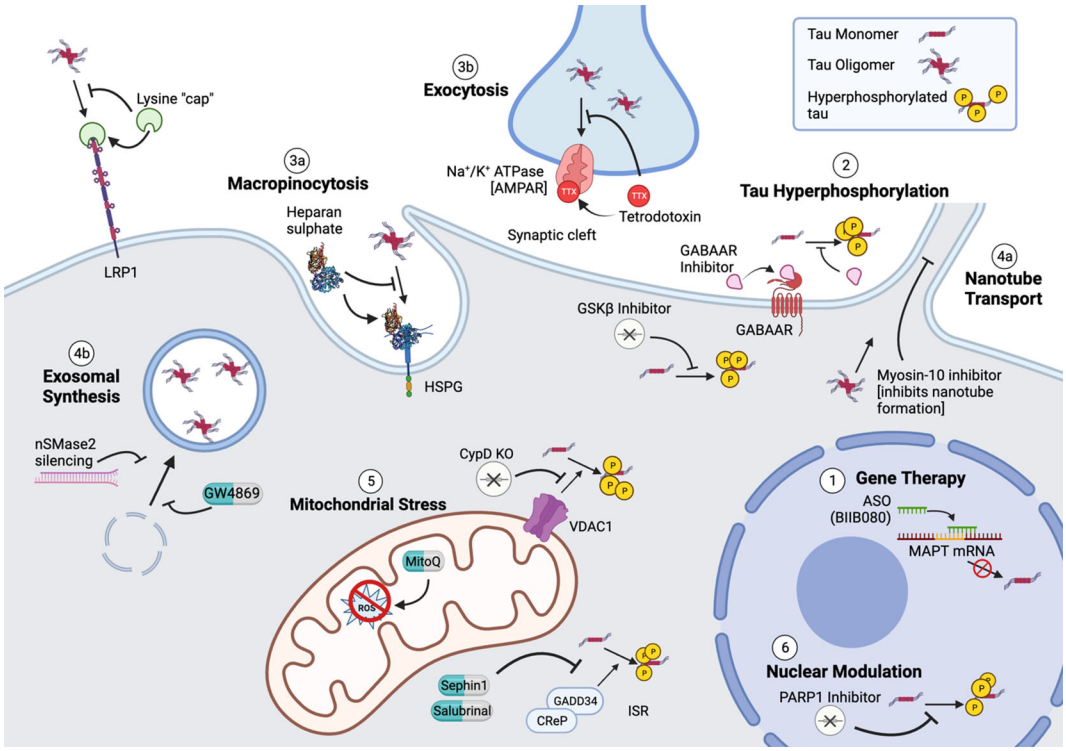


Figure 1. Overview of therapeutic mechanisms targeting neuronal tau production, aggregation, and propagation. (1) Antisense oligonucleotide (BIIB080) binding to MAPT mRNA can inhibit tau expression[8,31-34]; (2) Inhibition of the GABAAR complex or GSK-3β genetic KO inhibits tau hyperphosphorylation[22,35-40]; (3a) Genetic modification of low-density LRP1 to block lysine functional group and heparan sulfate competitive binding of HSPGs inhibit tau macropinocytosis[41-45]; (3b) Tetrodotoxin blockade of Na^+/K^+ ATPase or AMPARs inhibits tau exocytosis from synaptic terminals[46,47]; (4a) Myosin-10 blockers can inhibit nanotube formation, inhibiting tau intercellular spread[48-51]; (4b) Drug GW4869 and nSMase2 silencing of ceramide inhibits exosomal synthesis, thus inhibiting tau intercellular spread[52]; (5) CypD KO-driven inhibition of the VDAC1 pathway and sephin1/salubrinal inhibition of the ISR inhibit tau monomer hyperphosphorylation[53,54]. MitoQ reduces ROS[55,56]; (6) PARP1 KO inhibits tau hyperphosphorylation in the nucleus[57]. Created with BioRender.com. AMPAR: AMPA receptors; ASO: antisense oligonucleotide; CReP: constitutive repressor of eIF2α phosphorylation; CypD: cyclophilin D; GABAAR: γ-aminobutyric acid sub-type A receptor; GADD34: growth arrest and DNA damage-inducible protein 34; GSK-3β: glycogen synthase kinase-3β; HSPGs: heparan sulfate proteoglycans; ISR: integrated stress response; KO: knockout; LRP1: lipoprotein receptor-related protein 1; MAPT: microtubule-associated protein tau; PARP1: poly (ADP-ribose) polymerase-1; ROS: reactive oxidative species; TTX: tetrodotoxin; VDAC1: voltage-dependent anion channel protein 1.

strands[8]. By binding with high specificity to mRNA sequences, ASOs can mediate the degradation and translation of the sequences from the MAPT tau gene to partially limit tau production[8,31] [Figure 2]. ASOs can either modulate splicing to reduce the 4-MTBR isoform while maintaining tau levels or indiscriminately reduce MAPT tau expression[61,62]. The genetic reduction of endogenous tau has been shown to reverse tau pathology and neurodegeneration[8,31]. Studies have also shown the efficacy of MAPT ASOs in crossing the BBB and reducing MAPT mRNA expression in the cortex and hippocampus in primates by up to 75%[8]. With proven models of ASO MAPT reduction and a link between reducing MAPT expression and AD pathology, ASOs present an encouraging option to modulate tau pathology[8,31,33]. Biogen's BIIB080 is the first MAPT mRNA-targeting ASO to be tested in clinical trials[32,34]. Results from this early, small trial (n = 46) demonstrate ASO effectiveness in reducing total tau levels and phosphorylated tau levels by up to 50% in patients with mild AD while being well tolerated, warranting larger-scale trials and evaluations of the effect on neuronal loss[32]. Small interfering RNAs (siRNAs) have also exhibited success in suppressing MAPT tau expression in the hippocampus while avoiding cytotoxic effects in mouse models, presenting another gene therapy tool in the campaign against tau pathology[59,62].

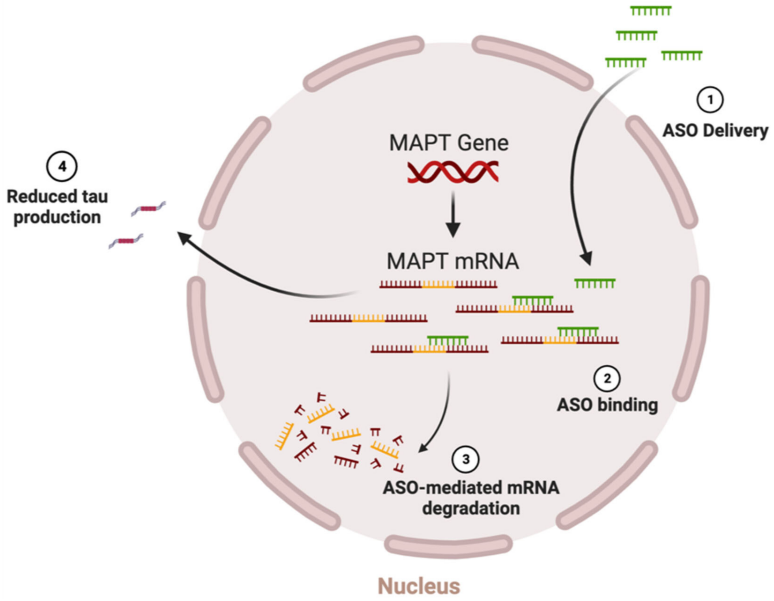


Figure 2. Schematic of ASO-mediated degradation of MAPT mRNA in the nucleus. ASO sequences bind to specific sequences along MAPT mRNA in the nucleus to facilitate the degradation of this mRNA and reduce the production of tau[8,31-34]. Created with BioRender.com. ASO: Antisense oligonucleotide; MAPT: microtubule-associated protein tau.

It must be noted that given the tau protein's diverse role in neurobiology, ASO-driven tau reduction can result in detrimental downstream consequences[63-67]. Lithium has been demonstrated to lower tau protein and mRNA levels in neurons *in vitro*[63]. However, evidence suggests that by knocking out tau in such models, iron was allowed to accumulate to toxic levels[63]. These elevated iron levels in the substantia nigra are characteristic of Parkinson's Disease (PD) itself, thus implicating systemic tau reduction in the onset of PD[63]. Another study confirmed that a drop in soluble tau levels due to the transition to an aggregated, diseased tau phenotype resulted in a decline in the amyloid precursor protein (APP)-mediated efflux of iron, driving the toxicity characteristic of tauopathies[64]. Similar effects may occur during the intentional mediation of tau protein expression in gene therapy, though dosage with iron chelators can help facilitate this hindered effect[64]. Other studies indicate that mouse models of reduced tau, usually via genetic KO, have exhibited motor deterioration and PNS deterioration in aged subjects, impaired neuronal migration leading to inhibited cortical development and mitochondrial abnormalities in young brains, and impaired dendritic maturation of granule neurons and adaptation to external stimuli[65-67]. Tau likely plays an instrumental role in the developing brain, so any gene therapy efforts must proceed cautiously, either by specifically targeting 4-MTBR isoform to target insoluble tau or by using added active molecules like an iron chelator to modulate effect. Potential remains, but much still needs to be understood about MAPT KO.

The MAPT gene is not the only potential target for genetic modulation to counter AD. Researchers are working to identify other specific genes whose inhibition reduces tau levels and to identify novel tau regulators that could serve as therapeutic targets for treating AD and other tauopathies. Downregulating expression of ubiquitin-specific peptidase 7 (USP7), ring finger protein 130 (RNF130), or ring finger protein 149 (RNF149) in adult mice with tauopathy increased carboxyl terminus of heat-shock cognate 70-interacting protein (CHIP) levels and reduced tau levels, in addition to reducing tau-mediated damage and neuroinflammation and improving memory[68].

Post-translational modifications and aggregation

Hyperphosphorylation

γ-Aminobutyric acid sub-type A receptors (GABAARs) refer to a class of receptors embedded in neuronal cell membranes that are involved in tau phosphorylation and thus participate in tau aggregation and transmission [Figure 3A][22,27,34-40,69-81]. Excessive phosphorylation of tau, known as hyperphosphorylation, contributes to tau NFT formation[4,27,81]. Phosphorylation of tau at serine and threonine residues along the tau protein is highly correlated with these processes, indicating that GABAARs could have potential as a therapeutic target[23,82-84]. Tau plays a vital role in assembling and stabilizing MTs in the brain and facilitating the transportation of vesicles and organelles when functional[23,85]. However, with increasing phosphorylation to the point of hyperphosphorylation, tau loses its ability to bind MTs and descends into its aggregated, insoluble form[4,85].

In particular, the Ser-202 and Thr-205 residues of the tau AT8 epitope demonstrated marked increases in phosphorylation following neuron treatment with GABAAR agonists[22]. A protein-fragment complementation assay was used to assess tau interactions in living cells throughout the experiment, offering a novel glimpse into the real-time activity of phosphorylation pathways[22]. Increased phosphorylation was shown to be associated most prominently with a reduction in tau-protein phosphatase 2A functionality[22,86]. Conversely, using bicuculline, a competitive GABAAR antagonist, was shown to reduce tau hyperphosphorylation *in vitro*, potentially warranting *in vivo* testing and additional GABAAR antagonist testing[22].

Research has also indicated a correlation between anesthetics and the neuropathogenesis of AD[81,87]. GABAARs are often the main activation target of such anesthetics, reaffirming a connection between GABAAR activation and the hyperphosphorylation of tau contributing to AD[87]. This tau hyperphosphorylation promotes "GABAergic" neurotransmission that drives further activation of GABAARs, thus exacerbating tau hyperphosphorylation in the CNS[87]. Anesthetics are known to activate GABAARs and induce tau hyperphosphorylation, thus potentially playing a role in the development and spread of tau pathology[81,87]. GABAAR modulator drugs can reduce the activity of GABAARs, allowing for modulation of tau hyperphosphorylation and, thus, regulation of tau aggregation[35].

It is well established that the hyperphosphorylation of tau leads to NFT formation[4,27,81]. This phosphorylation can be performed by several kinases, thus providing more potential targets for reducing tau aggregation[36]. Glycogen synthase kinase-3 (GSK-3) is a kinase expressed as an α or β isoform found in nearly all cells[36]. GSK-3 regulates several major cellular processes, including cell differentiation and metabolism[36]. It is well known to contribute significantly to the development of neurological disorders, including PD and AD[88,89]. As discussed in the context of GABAARs, high activity of tau-related kinases can lead to tau hyperphosphorylation, leading to tau aggregation and fibrillar disarray[36,89]. GSK-3β plays a key role in tau phosphorylation, as indicated by the GSK-3β-phosphorylated sites in tau NFTs[89]. Blanket inhibitors of GSK-3, affecting all isoforms, have proven effective in reducing neural death and AD symptom progression but pose the risk of disrupting a range of kinase activity and leading to adverse health effects[36]. Application in translational mice models indicated a slowdown in AD progression but an uptick in inflammation and behavioral aberrations[36].

A more recent study discusses an alternative, more finely-tuned therapy for targeting GSK-3β, the primary antagonistic isoform of GSK-3[36]. Isoform-selective reduction follows a similar approach to any other receptor-KO therapy, but can reduce only specific conformations of GSK-3[36]. The study involved a GSK-3β KO in mice models[36]. With full KO, adverse liver effects occurred[36,37]. With only a partial KO,

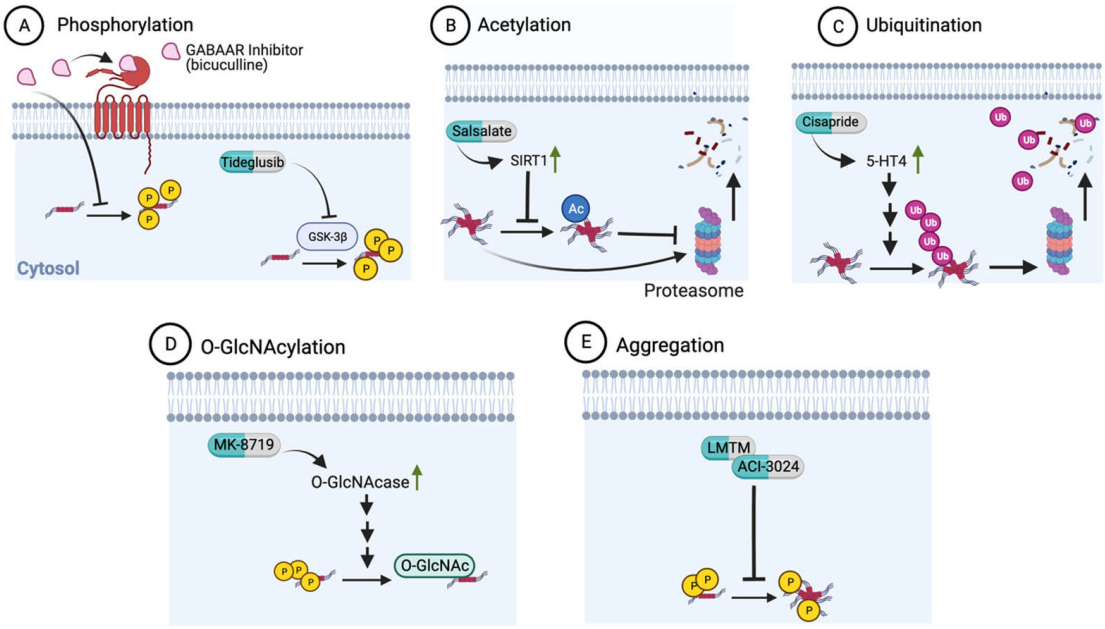


Figure 3. Therapeutic pathways to mediate post-translational tau modifications. (A) GABAAR inhibitors bind to GABAAR to inhibit the hyperphosphorylation of tau[22,35]. GSK-3β inhibitors likewise block tau hyperphosphorylation[36-40]; (B) Acetylated tau exhibits lower levels of proteasomal degradation. SIRT1 promoters inhibit the acetylation of tau to enable pathologic tau degradation in the proteasome[69-71]; (C) Upregulation of 5-HT4 promotes the ubiquitination of tau to promote proteasomal breakdown on pathologic tau[69,72-74]; (D) Upregulation of O-GlcNAcase promotes the O-GlcNAcylation of tau to lower levels of tau hyperphosphorylation[34,75-77]; (E) The drugs LMTM and ACI-3024 inhibit tau aggregation[34,78-80]. Created with BioRender.com. GABAAR: γ-aminobutyric acid sub-type A receptor; GSK-3β: glycogen synthase kinase-3β; LMTM: leuco-methylthioninium bis(hydromethanesulphonate); SIRT1: Deacetylase protein sirtuin 1.

approximately 45%, health was maintained while reducing pathogenic tau levels[37]. Partial KO of GSK-3β thus offers a promising means of reducing the tau aggregation and NFT formation that drives the pathology of AD[36,37].

Several blanket inhibitors have already undergone advanced testing. Sodium valproate, a GSK-3 inhibitor, has previously failed to yield notable symptomatic improvement in progressive supranuclear palsy, a primary tauopathy[38]. Another known GSK-3 inhibitor, tideglusib, demonstrated acceptable safety but failed to yield cognitive benefits in a phase II trial[39,40]. Tideglusib, however, has been shown to attenuate hypoxic-ischemic brain injury in translational mouse models and exhibit a neuroprotective effect, so further testing with increased duration and in earlier patient stages of AD may be warranted[39,40,75]. Conversely, enhancement of the expression of protein phosphatase-2A and protein phosphatase-2B has been demonstrated to dephosphorylate abnormally phosphorylated tau[76]. Likewise, results indicate a protective role of peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1 (PIN1) in tauopathies[90]. PIN1 binds to cyclin-dependent kinase 5 (Cdk5), a major player in the hyperphosphorylation of tau at proline-directed sites, and facilitates dephosphorylation of these tau sites[90]. Positive modulation of key phosphatases and inhibitors of certain kinases can play a role in decreasing pathogenic tau presence[76,91].

Acetylation and ubiquitination

Tau acetylation has recently been implicated as a major post-translational modification in AD[69,71,92]. This effect is two-fold: acetylation of the lysine residues of tau causes tau to disengage from MTs in addition to driving tau aggregation, both of which contribute to pathogenic tau propagation[71]. The KO of the

deacetylase protein sirtuin 1 (SIRT1) led to greater tau acetylation and, subsequently, more pathogenic tau[69]. Meanwhile, inhibition of the histone acetyltransferase p300 promoted the deacetylation of tau and reduced pathologic tau quantities[69] [Figure 3B]. Post-translational acetylation plays a direct role in tau pathology. Another study by the same group demonstrated the efficacy of salsalate, a derivative of salicylate with anti-inflammatory properties, in inhibiting tau acetylation by blocking the activity of p300 acetyltransferase and the acetylation of the K174 residue[70]. Salsalate treatment also prevented memory deterioration and hippocampal damage in mice, though it has thus far failed to induce such cognitive recovery in humans[70,93]. Acetylation plays a prominent enough role in tauopathies to warrant continued investigation into acetylase and deacetylase mediators.

The acetylation process may also block ubiquitin-proteasome-mediated tau degradation, enabling tau build-up[69]. In the ubiquitin-proteasome system, ubiquitin binds to the lysine residues of a protein, marking it for degradation and facilitating the transport of the protein to cytosolic proteasomes, where degradation occurs[92]. Ubiquitination and acetylation thus compete to bind to lysine residues; when acetylation occurs at lysine residues, ubiquitin cannot bind to these same residues. Thus, proteasomal degradation of tau decreases[69,92]. Modulation of tau acetylation, especially at specific lysine residues, is an intriguing prospect for reducing tau pathology[71]. Acetyltransferase and deacetylase proteins are involved in these processes and thus are the logical targets[71].

The tripartite motif containing-21 (TRIM21) is a cytosolic IgG receptor and E3 ligase that plays a central role in sensing cellular intruders and marking for degradation with ubiquitin[94,95]. TRIM21 activation enabled effective tau immunotherapies in preclinical models by leading to the inactivation and degradation of tau assemblies via proteasomal degradation, halting continued tau propagation[94]. 5-Hydroxytryptamine receptor 4 (5-HT4) receptor agonists, targeting the 5-HT4 receptor found in neural synapses, led to increased proteasome activity, reduced pathologic tau, and improved cognitive function[72] [Figure 3C]. Promoting the ubiquitination-proteasomal degradation pathway via proteasome promoters TRIM21 and 5-HT4 can facilitate the degradation of pathologic tau to alleviate tau burden[69,92]. Vilazodone and cisapride are known promotors of the TRIM21 and 5-HT4 pathways[73,74]. As such, they are worth further investigation as mediators of tau pathology in AD. Given its synaptic location, 5-HT4 is potentially implicated in neuron-to-neuron transmission or exosomal release of tau aggregates, which will be discussed in detail shortly[72].

O-GlcNAcylation

O-GLcNAcylation is the glycosylation of proteins with an O-linked N-acetylglucosamine (O-GlcNAc) group and presents another post-translational modification of tau that could offer therapeutic potential[83,96]. Just as the processes of acetylation and ubiquitination compete by their similar binding to lysine residues, the process of O-GlcNAcylation competes with the phosphorylation of serine and threonine residues[82-84]. O-GlcNAcylation negatively regulated tau phosphorylation *in vivo* and was found in lower frequencies in pathologic AD brain tissue, reaffirming its protective role[83]. O-GlcNAcase cleaves O-GlcNAc groups off of tau. As such, O-GlcNAcase inhibitors can promote O-GlcNAcylation and reduce tau hyperphosphorylation and aggregation. In preclinical trials, the O-GlcNAcase inhibitor MK-8719 demonstrated effectiveness in elevating O-protein levels in brain tissue in a dose-dependent manner[75] [Figure 3D]. MK-8719, in addition to O-GlcNAcase inhibitors ASN120290 and LY3372689, was well tolerated in Phase 1 patient trials, warranting continued testing in patients[34,75-77].

Tau aggregation

Tau aggregation is central to all tauopathies and is related to most mechanisms involving tau propagation in AD[1,78,80]. The topic of aggregation arises repeatedly throughout this review regarding numerous mechanisms, and it should be noted that tau aggregation itself also presents a direct therapeutic target rather than treatments intended to blockade the transmission of pathogenic aggregate tau[78,79]. Therapeutic mechanisms to combat tau aggregates involve the activation of proteasomes to facilitate tau breakdown or gene therapies to modulate protein overexpression and aggregation, among many other means of targeting aggregates or post-translational modifications that facilitate aggregation[72,94,97].

A clear link has been demonstrated between AD pathology and an elevated cluster of differentiation 40 and 80 (CD40/80) expression levels[97]. Overexpression of the two genes led to tau aggregation, with co-expression leading to a more marked increase[97]. Gene therapies targeting CD40 and CD48, thus, could likely mediate tau aggregation, though at the risk of other dramatic effects given the importance of the two genes in the immune system[97].

Leuco-methylthioninium bis(hydromethanesulphonate) (LMTM), a selective inhibitor of tau aggregation, also known as methylene blue, yielded somewhat positive, though underwhelming, results in limiting AD progression[78,79] [Figure 3E]. Multiple phase 3 trials have employed an LMTM monotherapy in patients with mild AD[78,79]. Earlier trials demonstrated mixed results, while more recent data from 2018 suggests a consistent decline in brain atrophy rate in monotherapy-treated patients. Further testing is needed, but this drug and ACI-3024, another tau aggregation inhibitor, have therapeutic potential[34,79]. Methylene blue has succeeded in inhibiting tau fibril formation but has failed to reduce tau oligomers, the critical drivers of AD, in clinical trials[80]. This option remains on the table, given some degree of success.

Receptor-mediated uptake and extracellular release

LRP1

LRP1 is intertwined with the spread of the tau protein and AD progression[41,43]. Just as some receptor proteins activate signaling pathways, LRP1 appears to be closely involved with phagocytosis and an endosomal/exosomal model of tau protein spread, irrespective of aggregated or monomeric form [Figure 4][41,43-47,98-100]. The gene that informs the creation of this protein, low-density lipoprotein receptor (LDLR), is a potential target for mediating tau protein propagation[43]. Clustered, regularly interspaced short palindromic repeats (CRISPR) interference technology was used to create a panel of LDLR variants and then selectively assess the ability of these variants to uptake tau via endocytosis[43]. In neuroglioma cells with a knocked-out *LRP1* gene, tau uptake into the cell was significantly decreased in both an aggregated and soluble form[44]. Specificity of LRP1 to the endocytosis of tau as opposed to other proteins that undergo endocytosis was confirmed after demonstrating that the uptake of the glycoprotein transferrin was generally unaffected by manipulation of LRP1[43]. Further downregulation of the *LRP1* gene in mouse models reduced the rate of tau propagation between neurons[43]. In subsequent experimentation, neurons with an LRP1 knockdown were confirmed via immunofluorescence measurements to have a diminished spread of tau protein[43]. Interspreading of a given tau protein was quantified by identifying tau in the presence of another protein, green fluorescence protein (GFP), which would be produced alongside tau within the cells and thus would accompany any tau transduced between cells[43]. GFP was found accompanying tau to only a limited extent among the cell population when LRP1 knockdown was performed[43].

A salt bridge knockdown experiment capping lysine residues on the LRP1 protein demonstrated the importance of lysine residues to LRP1 binding activity by preventing tau uptake in cells when performed[43]. Intriguingly, the four different ligand-binding domains in LRP1 were found to play varied roles in the

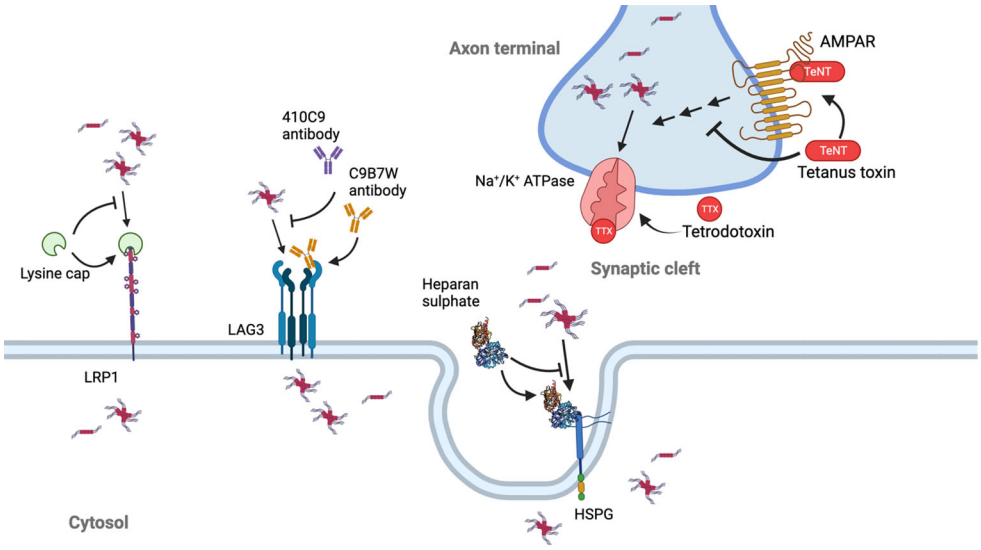


Figure 4. Therapeutic options to mediate intracellular uptake and extracellular release. (1) Competitive binding agents inhibit intracellular uptake via LAG3 and HSPGs[41,45,98-100]. Lysine modification on LRP1 inhibits LRP1-driven intracellular uptake[43]; (2) Tetrodotoxin and Tetanus toxin impair the extracellular release of pathologic tau from axon terminals by blocking the Na^+/K^+ ATPase and AMPAR pathways, respectively[46,47]. Created with BioRender.com. AMPAR: AMPA receptors; HSPG: heparan sulfate proteoglycan; LAG3: lymphocyte-activation gene 3; LRP1: lipoprotein receptor-related protein 1; TTX: tetrodotoxin.

internalization of tau, with subdomains two and four somewhat restoring tau uptake when involved in binding[43]. Inhibition of LRP1 tau-binding function would thus need to involve targeting specific subdomains[41-43]. Ultimately, however, experimentation has demonstrated that some tau aggregate internalization persists after LRP1 KO, raising doubts about LRP1 KO efficacy in slowing tau fibrillar pathology[43].

Lipoprotein receptors, including LRP1, tend to interact closely with HSPGs as well as apolipoprotein E (APOE4), both of which are other central targets for AD[41,101]. LRP1 operates synergistically with both HSPGs and APOE4 to mediate Aβ propagation in AD. These relationships may help to elucidate similar mechanisms of neuronal tau spread. Such a correlation was discovered between LRP1 involvement in tau binding and HSPGs on a given cell[58]. LRP1 levels were found to be upregulated in response to monomeric tau treatment in HSPG-deficient cells but downregulated in cells with normal HSPG presence[58]. Therapeutic blockade could help mediate tau spread.

HSPGs

Both PD and AD can be likened to prion-like diseases. The role of HSPGs reiterates these parallels[102,103]. HSPGs are glycoproteins with covalently added heparan sulfate groups that exist in either the ECM between cells or on cell surfaces[104] [Figure 4]. Surface-level HSPGs are known to facilitate endocytosis of a range of macromolecules. Existing studies highlight the role of HSPGs in binding to prion-like protein aggregates and transmitting pathology into the cell[105,106]. Specifically, the process by which HSPGs bind to trans-activator of transcription (TAT), a cell-penetrating peptide associated with HIV, and facilitates the macropinocytosis of the peptide has been elucidated. This mechanism inspired investigation into a similar analysis of fibrillar proteomic seeding of tau and α-synuclein (α-syn), a protein associated with PD[45].

Tau fibrils have also been shown to have extensive colocalization with TAT; thus, the HSPGs that bind to TAT could mediate the uptake of tau aggregates[45,107,108]. After cell treatment with RD-488 tau fibrils,

immunostaining for HSPGs found a strong HSPG presence around RD-488 sites, indicating HSPG-tau binding[45]. Furthermore, in assessing RD-488 binding to the cell surface, one study found that tau binding to the cell surface was significantly reduced when HSPG propensity for binding was inhibited, either by sodium chlorate to prevent sulfation or by the competitive inhibitor heparin[45]. Evidently, HSPGs play an important role in tau binding to the cell surface.

The same study went on to demonstrate that genetic knockdown of exostosin 1 (Ext1), an HSPG synthetic enzyme, and treatment with heparin, heparinase III, or chlorate, all of which block HSPG binding, effectively blocks tau uptake and diminishes the aggregation of recombinant tau fibrils within the cell[45]. Such treatment functioned as an effective intervention to inhibit the propagation of aggregated tau and could potentially slow AD onset[45]. Likewise, a positive correlation was observed between HSPG activity and monomeric tau[58]. This study observed a dual modulation by HSPGs and LRP1 on the cell surface by which the two receptor proteins play distinct roles in the uptake of monomeric tau, which induces interleukin-6 (IL-6) and interleukin-1β (IL-1β) inflammatory factor activation[58]. This research offers potential for the therapeutic modulation of tau uptake and tau-induced neuroinflammation before fibrillization[109].

Lymphocyte-activation gene 3

HSPGs and LRP1 mediate the internalization of tau in both its aggregate and monomeric form, with low specificity for pathogenic tau fibrils[41-45,58,110,111] [Figure 4]. In contrast, a recent study indicated that Lymphocyte-activation gene 3 (*LAG3*), a major cell surface receptor, is specifically involved in tau fibrillar uptake but not monomeric tau uptake. LAG3 KO thus may present a more effective modality for inhibiting pathogenic tau propagation in AD[110]. Of note, all three of these tau receptors - HSPGs, LRP1, and LAG3 - can also bind to α-syn and mediate α-syn pathology, indicating some shared molecular pathways of cell-to-cell transmission of pathogenic tau and α-syn[45,98,112-114].

Similar to LRP1, LAG3 possesses four subdomains - D1, D2, D3, and D4. Studies indicate that tau fibrils bind preferentially to the D1 subdomain, with only genetic deletion of D1 inhibiting LAG3-tau binding significantly[110]. LAG3 was observed to play a direct role in the uptake of pathogenic tau in cortical neurons[110]. A microfluidic device simulating cell-to-cell transmission also illustrated greater tau transmission between LAG3-positive neurons[110]. LAG3 antibodies 410C9 and C9B7W were demonstrated to inhibit both pathogenic tau uptake and pathogenic tau transmission, presenting another option in targeting receptor-mediated internalization of tau[98-100].

Na/K-ATPase complex and AMPARs

Pathogenic tau can be released into the extracellular space following neuronal death and exhibit toxicity for surrounding cells[85,115]. Stimulation of neuronal activity can promote the endogenous secretion of tau by cortical neurons and, subsequently, the release of pathological tau via a calcium-dependent pathway modulated by phosphorylation[86,116].

As discussed, fibrillar tau in the ECM has been shown to interact directly or indirectly with numerous neural cell membrane receptors[7,22,35] [Figure 4]. One such receptor is the AMPAR, a tetrameric membranal complex that plays a significant role in excitatory synaptic transmission through the CNS[46]. Another is the closely related Na/K-ATPase (NKA) complex, which, aside from its purpose as an ion-pumping ATPase, also mediates protein assembly and signal transduction across membranes similar to AMPARs[105].

AMPARs are ion channel-coupled glutamate receptors that facilitate the ion flow driving neurotransmission when activated[106]. Each AMPAR consists of four subunits: GluA1, GluA2, GluA3, and GluA4[106]. Similarly,

each NKA complex has four different subunits involved in ion binding and transmembrane ion transport; α 1 and α3 are most active in neurons[47]. Studies suggest fibrillar tau can impact AMPAR activity and mediate tau endocytosis, even without direct tau-AMPAR binding[1,47]. Shrivastava *et al.* demonstrated that an increase in exogenous tau clustering triggers an increase in GluA2[1]. The same study indicated that higher levels of exogenous tau trigger a decrease in α3-NKA. *In vitro*, tau exposure and immunostaining allowed for quantification of the colocalization of tau with subunits of AMPARs and NKAs[1]. *In vivo* results obtained after tau injection into the brain and immunostaining of brain sections indicated similar results, with apparent colocalization of tau with AMPARs and α3-NKA[1].

AMPARs facilitate tau release and thus present a possibility for a therapeutic target[47]. Pooler *et al.* demonstrated the efficacy of tetanus toxin and tetrodotoxin in inhibiting AMPA-mediated tau release; the botulinum-B neurotoxin has exhibited similar properties[46,117]. With effective blockage of the AMPA release pathway while maintaining viable neurons, anti-AMPA toxins have potential as AD therapeutics[46]. However, tetrodotoxin and tetanus toxin are highly potent and dangerous agents unless their activity is strictly regulated[46,118]. Acting via nerve transmission blockage, they can inhibit the neuronal spread of aggregate tau and provide pain relief in cases of debilitating disease, but remain risky options due to their broad-spectrum activity[46,118].

Similar to the discussed blockers of neurotransmitter activity, cholinesterase inhibitors (ChEIs) have traditionally been heavily utilized in alleviating the symptomatic expression of AD[119]. ChEIs enhance the activity of acetylcholine transmission and cholinergic function to protect neural function in the forebrain[119]. However, data on the efficacy of ChEI treatment on tau pathology is conflicting[91,119,120]. Post-mortem study and *in vivo* results indicate a possible link between ChEI dosage and tau phosphorylation, while a recent positron emission tomography assessment *in vivo* depicted significant effects of ChEIs in reducing tau pathology[91,119,120]. Given the diverse set of pathological hallmarks across the continuum of AD, it is possible that ChEIs offer some neuroprotection while also being involved in the mechanism of tau phosphorylation. Using a ChEI in conjunction with anti-tau therapeutics may enable enhanced therapeutic efficacy.

Immune response modulation

Triggering receptor expressed on myeloid cells 2

Triggering receptor expressed on myeloid cells 2 (TREM2) is a cell surface receptor found on microglia in the brain[121]. Located on human chromosome 6p21, the *TREM2* gene codes for a relatively large glycoprotein that binds to tyrosine kinase in a signaling pathway [Figure 5A][25,121-133]. Recent studies point to a potentially positive role of microglia in protecting against tau spread and, subsequently, AD onset[134,135]. Variants conferring a loss of TREM2 function were found to increase AD risk by up to four-fold, rather significant in the context of single-gene modulation[135]. It is highly likely that TREM2 plays a direct role in preventing Aβ plaque and indirectly modulates tau aggregate spread[135,136]. Progression of Aβ plaque accumulation creates a more favorable environment for tau aggregation; thus, any loss of the mediating effect of TREM2 could foster further tau spread, NFT formation, and AD onset[134]. Experimental setup involved the injection of tau aggregates from human AD brain tissue into mice with either an active *TREM2* gene or those with a *TREM2 KO* gene[135]. Subsequent brain extraction and immunofluorescence indicated significantly more NP tau aggregation and Aβ around plaques in the TREM2 KO groups[135].

Additional research supports the tie between TREM2 genetic variance and the risk of developing AD, strongly indicating a microglial role in the disease[122]. As suspected, anti-inflammatory molecules induce effective TREM2 functionality, generating a neuroprotective effect.[122,123] Another study noted that enhanced

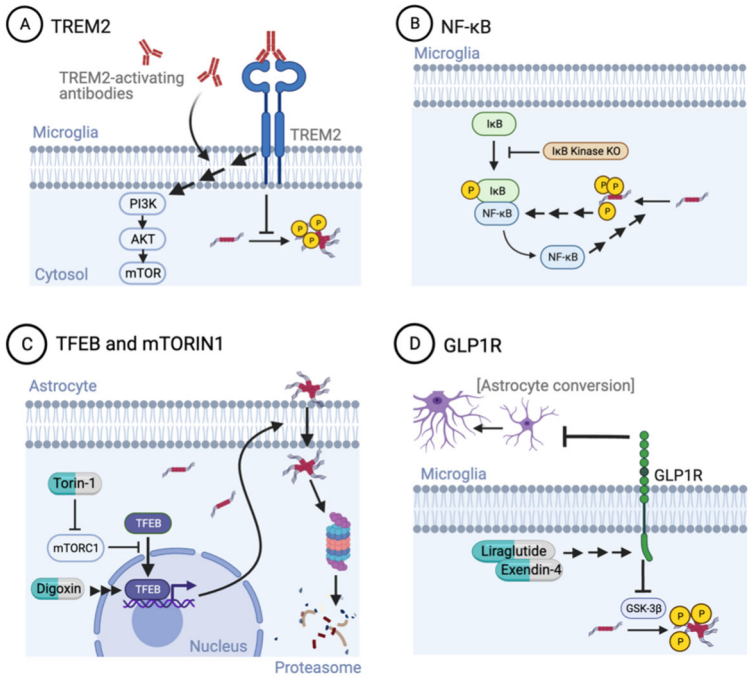


Figure 5. Immunomodulatory therapeutics to inhibit pathologic tau propagation. (A) Activating TREM2 with specific antibodies inhibits the hyperphosphorylation and aggregation of tau while also activating the PI3K/AKT/mTOR pathway to promote microglia survival[122-125]; (B) KO of IκB Kinase inhibits the phosphorylation of IκB, thus preventing the NF-κB-driven hyperphosphorylation of tau[126-128]; (C) Torin-1 and Digoxin promote the nuclear internalization and expression of TFEB in astrocytes, which promotes cellular uptake of tau and the proteasomal destruction of pathologic tau[25,129,130]; (D) GLP1R agonists drive the inhibition of GSK-3β to prevent hyperphosphorylation and subsequent tau aggregation, while inhibiting the conversion of astrocytes to their proinflammatory phenotype[131-133]. Created with BioRender.com. AKT: Protein kinase B; GLP1R: glucagon-like peptide-1 receptor; GSK-3β: glycogen synthase kinase-3β; KO: knockout; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: phosphoinositide 3-kinase; TFEB: Transcription factor EB; TREM2: Triggering receptor expressed on myeloid cells 2.

TREM2 expression and activity led to reduced hyperphosphorylation and aggregation of the tau protein, attenuated neuroinflammation, and enhanced microglia survival rate through the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway[124]. Other studies present inconclusive results on the effects of TREM2 activation on tau, though TREM2-activating antibodies have proved effective in reducing neuroinflammation and bestowing a generally neuroprotective effect in models of AD[125]. Further research is necessary to elucidate this pathway, but TREM2 activation does present a viable therapeutic modality, primarily consisting of antibody stimulation of TREM2 in the early stages of tau pathology and neurodegeneration[122,123].

Nuclear factor kappa-light-chain-enhancer of activated B cells

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a major transcription factor involved in several microglial immune response mechanisms pertaining to inflammation, microglial activation, and oxidative stress[24,126]. Further translational research indicated that activation of microglia NF-κB stimulates tau spread and seeding in mice brains, while inhibition of NF-κB diminishes such seeding[127,128,137] [Figure 5B]. NF-κB is well associated with AD via Aβ activation and production; analysis found NF-κB signaling among the most aberrant pathways in AD brains[127]. Confirmation of a connection between NF-κB and tau seeding also makes NF-κB an even more promising target in slowing the onset of AD. Maphis *et al.* demonstrated that reactive microglia facilitate the hyperphosphorylation of tau, which acts as the first step in NFT formation[24]. Therefore, the activation of microglia by NF-κB is linked with the spread of tau pathology; RNA-seq transcriptomic analysis of microglia in mouse models when treated with

tau confirmed NF-κB to be one of the most altered immune pathways[24]. A buildup of tau protein triggers this pathway, leading to further tau proliferation in a similar manner to a positive feedback loop.

NF-κB activity can be precisely mediated via genetic inhibition of IκB kinase, which promotes NF-κB while also degrading an inhibitor of NF-κB[126-128]. In this way, therapeutics can target NF-κB activity to limit tau seeding. An *in vivo* study indicated that inactivation of NF-κB led to the slowdown of microgliosis in the hippocampus and cortex and a reversal of the cellular tau pathology. Behavior studies indicated the restoration of cognitive deficits and learning ability in mouse models of tauopathy subject to NF-κB inactivation[126]. IκB kinase, therefore, has promise as a therapeutic target against AD progression[126,138]. As previously mentioned, elevated *CD48* and *CD40* gene expression has been implicated in tau and AD pathology[97]. Upregulation and co-regulation of the two genes enriched the NF-κB pathway to promote tau pathology, further establishing the two genes as candidates for gene therapies and therapeutic modulation to mitigate AD symptoms[97].

Transcription factor EB and mammalian target of rapamycin 1

Astrocytes are a subset of glial cells in the CNS that play a role in synaptogenesis and synaptic transmission[139-141]. Aβ accumulation has been highly implicated in astrocytic activation[131,142,143]. Though they may induce some neuroprotective effects, astrocytes have been shown primarily to trigger cytokine and chemokine release to contribute to damaging neuroinflammation[144,145]. Increased astrocyte activation was found in the retina of AD mouse models, as was increased microglia activity, both of which contribute to inflammation[143]. The pro-inflammatory marker IL-1β colocalizes with microglia but not astrocytes; however, the microglia-induced activation of IL-1β is upstream of the upregulation of the astrocytic complement factor C3, contributing to neurotoxicity[143,146]. Furthermore, the expression of osteopontin, an ECM protein, was found to be upregulated in AD retinas, which can contribute to systemic degeneration[143]. This Aβ model may be instructive in the immune modulation of *in vivo* tau models.

Transcription factor EB (TFEB), expressed primarily in the CNS, modulates lysosomal synthesis and activity in response to tau pathology[25,147] [Figure 5C]. In primary astrocytes, exogenous expression of TFEB has been proven to increase pathologic tau uptake and stimulate the autophagy-lysosomal pathway, facilitating fibrillar tau breakdown while leaving monomeric tau intact[129,147]. *In vivo*, inducing TFEB expression in the CNS with drugs such as digoxin leads to decreased fibrillar tau in the hippocampus[129,130]. Tau pathology also induced increased nuclear localization of TFEB in mice, particularly in astrocytes[25]. The protein complex mammalian target of rapamycin 1 (mTORC1) confines TFEB to the cytoplasm via phosphorylation[25]. Inhibition of mTORC1 with torin-1 prevents this phosphorylation, allowing for TFEB nuclear entry and transcriptional activation[25]. In models of slower onset tau pathology, such as in PS19 mice, mice injected with TFEB factors experienced a reduction in total tau levels; astroglial overexpression of TFEB ostensibly mitigates tau pathology in such models[25,148,149].

Glucagon-like peptide-1 receptor

Glucagon-like peptide-1 receptor (GLP1R) is a hormone critical to modulating glucose and insulin levels that has garnered increasing attention in the study of neurodegenerative disorders[150,151]. GLP1R agonists have achieved neuroprotective effects in models of AD[150-152] [Figure 5D]. One such agonist, NLY01, was shown to protect against the loss of dopaminergic neurons while also prolonging the life and reducing behavioral deficits in a transgenic mouse model of neurodegeneration induced by α-synucleinopathy, which could prove instructive regarding neuroprotection in tau-induced neurodegeneration models[153]. The direct treatment of NLY01 in human dopaminergic neuronal cultures cannot protect the neurons against α-syn induced apoptosis[153]. However, when treated with α-syn, microglia release cytokines such as interleukin-1α

(IL-1α), tumor necrosis factor alpha (TNFα), IL-1β, and IL-6, converting astrocytes into their toxic A1 phenotype, a process that *can* be inhibited by NLY01 treatment[153,154]. The treatment also prevents α-syn induced cell death in mouse primary cortical cultures[132]. NLY01 was found to reduce ionized calcium-binding adaptor molecule 1 (IBA1) immunoreactivity and microglial density induced by α-syn[153]. NLY01 depletes GLP1R in transmembrane protein 119 (TMEM119)-positive microglia, thus preventing α-syn induced cell death[153].

A similar mechanism applies to tauopathies[132,153]. The GLP1R agonist liraglutide has been found to reduce neurotoxicity, especially in tauopathies[132]. Exendin-4, another GLP1R agonist, has proven helpful in preventing hyperphosphorylation of tau in the hippocampus while also resulting in a decline of GSK-3β activity four weeks after intervention[133]. Astrocyte-related immunomodulation ties into post-translational phosphorylation of tau, emblematic of the complexities of tau propagative processes. Park *et al.* similarly demonstrated that NLY01, which had already been proven to have therapeutic effects in α-syn models of PD, activates GLP1R to deter the Aβ-driven activation of microglia and the subsequent reactive astrocyte conversion in AD mouse models[131]. NLY01 treatment has neuroprotective effects, slowing neuronal loss and improving spatial cognition and memory. GLP1R agonists thus represent viable options for general mitigation of AD symptoms and neurodegeneration[132,153].

Intercellular organelle exchange

Tunneling nanotubes

Receptor-mediated endocytosis as a mechanism of tau NFT transmission is one central area of study in AD; tunneling nanotubes (TNTs) are an alternative means of tau propagation[26,48] [Figure 6A]. TNTs are membranous actin-based structures that cross between cells, typically on a long-distance scale[26,48]. They participate in the transport of many different cellular structures and molecules, including the retrovirus HIV-1, in a wide range of cells and promote the propagation of several neurodegenerative diseases[26,155]. Recent studies have implicated TNTs in the propagation of aggregate tau between cells[48,156]. Furthermore, Tardivel *et al.* indicated that exogenous tau presence may trigger TNT activity and thus facilitate intercellular tau transport between neurons[48]. Mediation of TNT-related factors, therefore, may allow for the reduction of pathologic tau transmission[48].

Researchers first established a strong relationship between tubulin-negative, actin-positive TNTs and soluble tau protein[48]. Confocal microscopy analysis of tagged actin allowed for easy detection of TNT structures[48]. Subsequent tau tracking via an immunoreactive tau epitope revealed clear localization between tau and TNT actin structures. To test the effect of modulation of exogenous tau on TNT formation, neuron TNT formation was measured via actin tagging in either basal conditions or when exposed to tau NFT medium[48]. TNT formation was elevated across catecholaminergic-a-differentiated (CAD) cells and primary neurons when extracellular tau NFT was present; specificity to tau NFTs was demonstrated by the failure of other amyloid fibrils to modulate TNT formation[48]. Immunostaining indicated the presence of tau protein in structural TNTs as well[48]. With the relationship between soluble tau and TNTs established, additional experimentation based on fluorescent tagging was performed to demonstrate the movement of tau fibrils through TNTs from a donor cell to a recipient cell[48]. Fibrils were found moving through TNTs at a rate in line with actin-facilitated transport, confirming the TNT role in tau fibrillar propagation.

Experimental modulation of TNT formation, thus, presents a slew of possibilities for AD therapeutic targets. Modulating the proteins that contribute to TNT formation - F-actin, α-tubulin, β-tubulin - could be effective[26]. However, altering these proteins could lead to unintended consequences due to their ubiquitous roles in cellular structure[26]. Myosin-10, in conjunction with exogenous tau, appeared to facilitate TNT

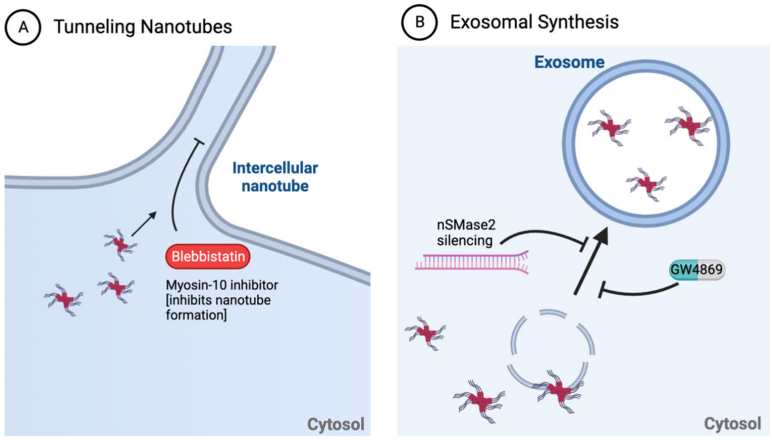


Figure 6. Therapeutic modulators of tau propagation through intercellular organelle systems. (A) Myosin-10 blockers inhibit nanotube formation to inhibit intercellular propagation of pathologic tau[48-51]; (B) nSMase2 silencing and delivery of GW4869 inhibit exosome formation to prevent the exosomal release of tau[52]. Created with BioRender.com.

formation[48,49]. If this correlation is proven, drugs targeting myosin-10, such as blebbistatin (though not specific to myosin-10), may yet prove to be effective therapeutics[50]. Usage of tolytoxin, a cyanobacterial macrolide that targets actin, has demonstrated efficacy in reducing TNT proliferation in α-syn cell lines; further exploration in preclinical tau models is warranted to test safety and side effects[51]. Research into TNT's role in AD pathogenesis and the delineation of specific targets in this mechanism are ongoing.

Exosomes

Similar to TNTs, exosomes, small membranous vesicles that move through the ECM, represent a method of intercellular communication and transport of material between cells[1,157]. Exosomes are secreted from donor cells into the ECM, then uptaken by recipient cells, where they can deposit their contents[158,159]. In such a way, cellular material and molecules can be transmitted from cell to cell[158-160]. Given the prion-like nature of AD, it is logical for such a process to play a role in tau propagation: studies have confirmed that exosomes play a role in cell-to-cell fibrillar tau transmission and seeding[160,161] [Figure 6B].

In order to establish that exosomes released by neurons carry the tau protein, exosomes were first isolated from mature cortical neurons[157]. Enzyme-linked immunosorbent assay (ELISA) was performed on the isolated exosomes to quantify tau presence and confirmed that tau is found in relatively high concentrations in the exosomes[157]. Colocalization of tau with Alix and Flotillin, exosomal markers, further indicated that tau is transported out of cells via exosomes[157,158,162]. Observations suggest that neural activity may stimulate the secretion of tau from cells in exosomes[157]. Chemical stimulation of mature neurons was performed by depolarizing these neurons with concentrated potassium chloride. Alix levels in exosomal matter increased significantly in these stimulated neurons, as did tau levels[157]. Exosomal secretion increased, and thus, so did tau secretion[157]. A direct model of cell-to-cell transmission through exosomes was created with a microfluidic device, with first and second-order neuron chambers established and strictly controlled flow and specific dendritic positioning, allowing for precise results[157]. Fluorescent tagging of tau allowed researchers to track its movement[157]. When sonification disrupted exosomal processes, tau transmission from first to second-order was decreased, supporting the theory that exosomes are instrumental in transporting pathologic tau[157]. Examination of tau aggregates through thioflavin-S staining indicated that tau aggregates are spread through exosomes and that exosomal aggregation-prone tau can induce full-form tau aggregates[157].

Targeting this trans-synaptic transmission thus could take the form of anti-extracellular tau antibodies or inhibition of exosomal synthesis[52,158]. This would prevent further seeding and propagation of pathologic tau. Ceramide is a lipid critical to the formation of exosomes: inhibition of sphingomyelinase-2 (nSMase2) - a modulator of ceramide synthesis - will thus inhibit exosomal synthesis[52]. This can be performed via genetic silencing with siRNA or inhibition of the nSMase2 protein with the drug GW4869[52]. Both methods were successful in reducing exosomal tau secretion and cell-cell tau transduction *in vitro* as well as *in vivo* with mouse models[52].

Mitochondrial dysfunction

Voltage-dependent anion channel proteins

Voltage-dependent anion channel proteins (VDACs) modulate the transmission of metabolites across the mitochondrial membrane[163-165] [Figure 7]. Three isoforms exist - VDAC1, VDAC2, and VDAC3 - with VDAC1 representing the most commonly expressed and operating in the brain, heart, liver, and muscles[165,166]. These channel proteins play many roles pertaining to cell growth and survival as well as basic mitochondrial functionality[167]. In patients with AD, VDAC1 levels have been observed to increase as the pathogenesis of AD progresses[166].

Immunoprecipitation analysis was performed to elucidate the link between VDAC1 and phosphorylated tau[166]. Immunoblotting using the phosphorylated tau antibody on AD patient brain lysates indicated a relationship between VDAC1 and phosphorylated tau[166]. These findings were confirmed when the double-labeling of VDAC1 and phosphorylated tau from cortex tissue indicated colocalization between the two proteins[166]. Elevated levels of VDAC1 in AD progression indicate that the VDAC1 mitochondrial transport and cell metabolite mechanisms may play a role in neurodegenerative pathogenesis[166]. Tau protein is responsible for these VDAC1 failings[166].

VDAC1 binds to the mitochondrial inner-membrane protein Cyclophilin D (CypD) to form the mitochondrial permeability transition complex[166]. CypD levels are also elevated as AD progresses, indicating mitochondrial pore dysfunction and the deterioration of membrane integrity[166,168,169]. CypD, therefore, presents a possible therapeutic target. Genetic KO of CypD in mouse models successfully prevented mitochondrial dysfunction and slowed AD progression[53] [Figure 7]. CypD KO thus may prove to be a viable strategy to stop fibrillar tau from driving AD pathogenesis[53].

Integrated stress response

The integrated stress response (ISR) is a cellular pathway that regulates protein formation[54,170]. When under high-stress conditions, mitochondrial dysfunction can occur, thus triggering increased ROS output and modulation of protein synthesis[171]. ISR inhibition is one such result of these conditions[171]. Mitochondrial stress has been demonstrated to induce the phosphorylation of the translation factor eIF2α in the short term, leading to activation of the ISR and thus reducing protein synthesis[172]. Such a mechanism plays a protective role in the short term by reducing mitochondrial degeneration but drives the onset of widespread neurodegeneration in the long term[171,172]. The same study confirmed that the reduction of eukaryotic translation initiation factor 2α (eIF2α) phosphorylation and, thus, inhibition of the ISR induces tau aggregation in the long term[172]. Mitochondrial dysfunction leads to tau hyperphosphorylation in these conditions, which is a leading cause of NFT formation and AD pathogenesis[172]. This was confirmed by elevated tau hyperphosphorylation and aggregation in neurons deficient in prohibitin-2 (PHB2), a mitochondrial structural protein characteristic of mitochondrial dysfunction[172].

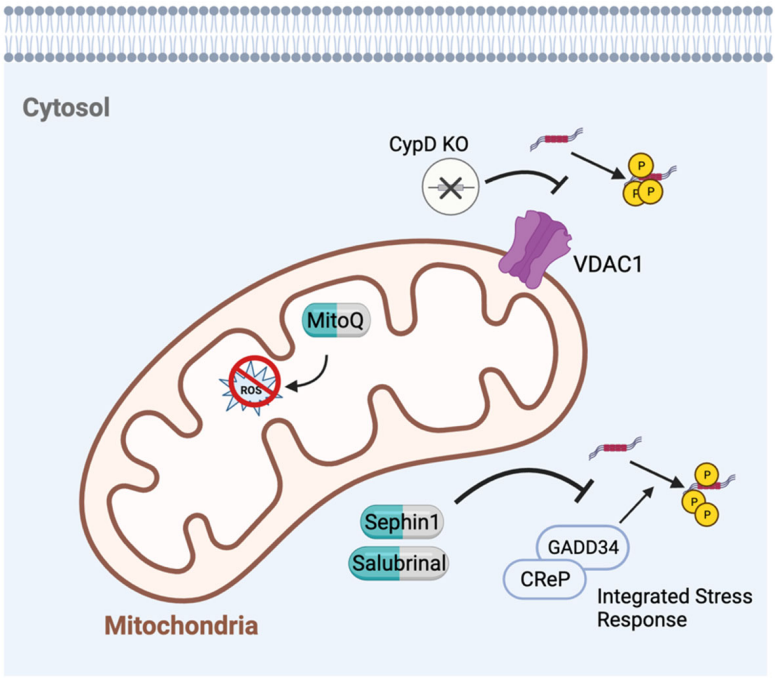


Figure 7. Therapeutic strategies to address structural dysfunction related to tau pathology. Gene regulators (CypD KO), antioxidants (MitoQ), and enzyme inhibitors (sephin1, salubrinal) regulate the VDAC1 hyperphosphorylation pathway, inhibit ROS generation, and inhibit the integrated stress response-driven hyperphosphorylation of tau[53-56]. Created with BioRender.com. CReP: Constitutive repressor of eIF2α phosphorylation; GADD34: growth arrest and DNA damage-inducible protein 34; KO: knockout; ROS: reactive oxidative species; VDAC1: voltage-dependent anion channel protein 1.

The pathway by which this hyperphosphorylation occurs is not fully elucidated, but research does indicate potential for therapeutic targets involved in mitochondrial dysfunction[171-173]. Mitochondrial dysfunction leads to a marked increase in oxidative stress and disruption of protein synthesis[173]. Therefore, therapeutics targeting these processes can inhibit tau aggregation and dimerization[54]. To test the efficacy of the ISR as a therapeutic target, cells were treated with the drugs salubrinal, sephin1, and guanabenz, the first two of which inhibit growth arrest and DNA damage-inducible protein 34 (GADD34) and the last of which inhibits the constitutive repressor of eIF2α phosphorylation (CReP)[54]. GADD34 and CReP dephosphorylate eIF2α, so inhibition of these two factors leads to the deactivation of eIF2α and, thus, ISR promotion[54]. ISR inhibitor-treated cells were shown to have elevated levels of tau dimerization, as quantified by marker fluorescence, reaffirming the anti-AD function of the ISR[54]. More generally, targeting GADD34 or CReP has been shown to help restore cognitive function and long-term memory formation[54]. Enrichment of these two phosphatases that antagonize phosphorylation of eIF2α regulated the ISR and allowed for some reversal of cognitive dysfunction characteristic of AD and other neurodegenerative diseases[54]. The ISR plays a complex role in the onset of neurodegenerative disorders and warrants additional research into therapeutic targets to reduce tau pathology[54,171-173].

ROS

Tau pathology results in mitochondrial damage and dysfunction, stimulating ROS production and further driving pathologic tau propagation[171]. This oxidative stress is a characteristic driver of AD pathology and other neurodegenerative disorders[4,7,171]. ROS generation threatens cell viability and, thus, represents another central target[171]. A gene expression study in humans above age 40 indicated that age-downregulated genes in the brain were subject to advanced oxidative DNA damage compared to age-

nonaffected genes[55,174]. Certain promotor regions rich in guanine and cytosine were particularly prone to oxidation, inhibiting their transcription[55]. The use of mitoquinone (MitoQ) and vitamin E - as well as many other reducible antioxidants - in the mitochondria have been demonstrated to effectively scavenge ROS in cellular models of acute oxidative stress and offers another approach to limiting the tau-induced progression of AD symptoms[55,56].

Tau is bound to MTs via an MT-binding domain generally consisting of 4 MTBRs: R1, R2, R3, and R4[175]. As discussed earlier, the breakdown of these interactions is a hallmark of AD, so it can be useful to investigate this mechanism in more detail as it relates to the production of ROS[7,175,176]. The R2 and R3 MTBRs are consistently dimerized after exposure to copper ions, whereas without copper ions, dimerization occurs to only a limited extent[175]. This dimerization occurs via a disulfide bond, which is likely enhanced by the redox activity of copper ions[175]. *In vitro* treatment with histidine, anthranilic acid, and salicylic acid all modulate these copper-driven redox interactions and reduce overall ROS levels, indicating potential therapeutic value as regulators of inflammation[176]. Without a cysteine residue, which is responsible for the disulfide bond between peptides, the histidine imidazole group generally facilitated R1/ R4 Cu^{2+} redox chemistry[176]. Subsequent metalation of R1 and R4 resulted in conformational and structural changes in tau, ultimately driving observable peptide aggregation after MTBR incubation in copper ion solution[175]. Given their efficacy in scavenging ROS, metal chelators could also act as active agents for protecting against tau-induced AD progression[175].

A range of trials have already verified the efficacy of antioxidants in preclinical and clinical trials for subjects with age-related neurodegeneration. Studies dating to the 1990s have indicated that treatment with the antioxidant vitamin E lowers AD incidence and slows AD progression in patients[177,178]. More recent studies assessing the effect of antioxidant dosage on tau accumulation have indicated the successes of a range of antioxidants - resveratrol, catechins, curcumin, and many more - that bind free radicals to prevent the proliferation of ROS and lipid peroxidation while also challenging Aβ plaque formation[179-184]. In rodent models of AD, resveratrol increased the expression of antioxidant enzymes in neural tissue and enhanced spatial abilities[180]. In another study, mice dosed with green tea catechins exhibited greater spatial cognition and increased antioxidative defenses, protecting the hippocampus and cerebral cortex[182]. Curcumin likewise inhibited protein oxidation and lipid peroxidation in liver mitochondria, though challenges in therapeutic delivery have hindered its usage as an AD deterrent[181,183,184]. Lipoic acid and Coenzyme Q have also exhibited potent redox functions in other models of neurodegenerative disease and have shown some promise of neuroprotection in AD models, making them worthy of further investigation[185-187]. Found in the mitochondria, lipoic acid has been shown to lower oxidative stress marker levels and upregulate the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which drives antioxidant production[186]. Both agents have offered significant potential, given successes in traversing the BBB and reducing oxidative stress[185,186]. It must be noted that when administered alone, these antioxidants slowed the progression of AD in only a limited fashion[187]. Using antioxidants in conjunction with other active agents will likely bolster success rates.

Microtubule modulation

MTs

Tau protein binds to the N- and C-terminals of MTs, with binding to the former associating directly with the cell membrane and regulating the spacing between MTs[85,188,189]. Tau may bind MTs directly via positively charged repeat sequences within its microtubule-binding domain (MBD) that are attracted to the negatively charged side chains on tubulin[189]. When completely dephosphorylated, tau has a high affinity for binding to MTs. As discussed earlier, tau phosphorylation is mediated by protease-activated receptor 1 (PAR1) kinases, calcium/calmodulin-dependent kinase II, and tyrosine kinases[116]. The activation of tau

phosphorylation-associated kinases like Cdk-5 and GSK-3β induces hyperphosphorylation, which results in tau dissociation from MTs[36,85]. Upon dissociation from MTs, tau protein can misfold and become toxic seeds secreted from the cell[85,190,191].

The well-elucidated role of MTs in tau propagation offer MTs as yet another potential target; potentially, the stabilization of MTs can prevent dissociation from the tau protein that triggers pathogenic tau seeding [Figure 8][85,190,191]. Abeotaxane (TPI-287) is a known microtubule stabilizer, as is Davunetide (AL-108)[190,191]. Initial trials demonstrated high tolerance for Davunetide with some observed anaphylactic responses to Abeotaxane; neither yielded significant improvements in cognitive function[190,191]. Davunetide warrants further exploration given its strong tolerance; further investigation must be performed to assess the impact of MT stabilizing on cognitive restoration in AD patients[191].

Abnormal increases in tau phosphorylation lead to decreased MT binding and higher tau levels in the cytosol, promoting tau self-interactions that result in tau aggregation[115]. Hyperphosphorylated tau aggregates can lead to further tau dissociation from MTs[178]. Tau cleavage contributes to the formation of toxic seeds, which can lead to cognitive impairment by impairing synaptic transmission[116]. More precisely, caspase-2 has been found to cleave tau preferentially at its D421-S422 and D314-L315 residues to induce downstream toxicity and degeneration[85,192-194]. After dissociating from MTs, tau invades healthy dendritic spines, impairing synaptic transmission and driving neuronal loss in the hippocampus[194].

Nuclear modulation

Poly (ADP-ribose) polymerase-1

Poly (ADP-ribose) polymerase-1 (PARP-1) and histone 1 (H1) are widely expressed across the genome[195-198]. Changes to these can alter chromatin structure on a large scale[195-198]. These two factors compete for binding to nucleosomes and exhibit a reciprocal relationship, with H1 depleted at actively transcribed gene promoters where PARP-1 binding is enriched[199]. PARP-1 is responsible for PARylation and other important processes like DNA damage repair, RNA processing, and parthanatos[200-202]. An overactivation of PARP-1 has been observed in the brain of AD patients, especially in the frontal and temporal lobe; multiple PARP-1 mediated pathways have been suggested as possible factors that promote neurodegeneration like metabolic disorder-related NAD+ depletion and glycolysis arrest[57]. In stroke cases, the genetic deletion of PARP-1 is a neuroprotective mechanism to prevent PARP-1 from activating the parthanatos pathway[203]. A recent study evaluated the therapeutic potential of PARP inhibitors and assessed their contribution to mechanisms involved with AD progression[57]. Activated PARP-1 also colocalizes with tau and promotes the formation of NFTs, contributing to AD symptoms[57].

The study found that pharmacological inhibition and RNAi-mediated knockdown of the PARP-1 gene can both achieve a decrease in locomotor impairments induced by Aβ42[57]. In the study, olaparib, an approved cancer treatment drug, and MC2050, known for its neuroprotective effects against Aβ peptides-induced neurotoxicity, were assessed [Figure 9][57,203]. The formation of Aβ-positive toxic aggregates were reduced following treatment with both inhibitors[57]. RNAi-mediated gene silencing of PARP-1 restored motor dysfunction in a drosophila model, confirming their drug studies[57]. In the drosophila model, drug administration allowed for the recovery of NAD+ to normal levels and inhibited PARylation, thereby lowering toxicity. The inhibitors also diminished the presence of transposable elements, which are mobile genetic sequences characteristic of neurodegeneration[57,204]. Effective mediation of Aβ aggregates with genetic silencing of PARP-1 or PARP-1 inhibitors is a promising treatment model in the context of tau propagation, given the similar mechanisms of protein aggregation[5,57].

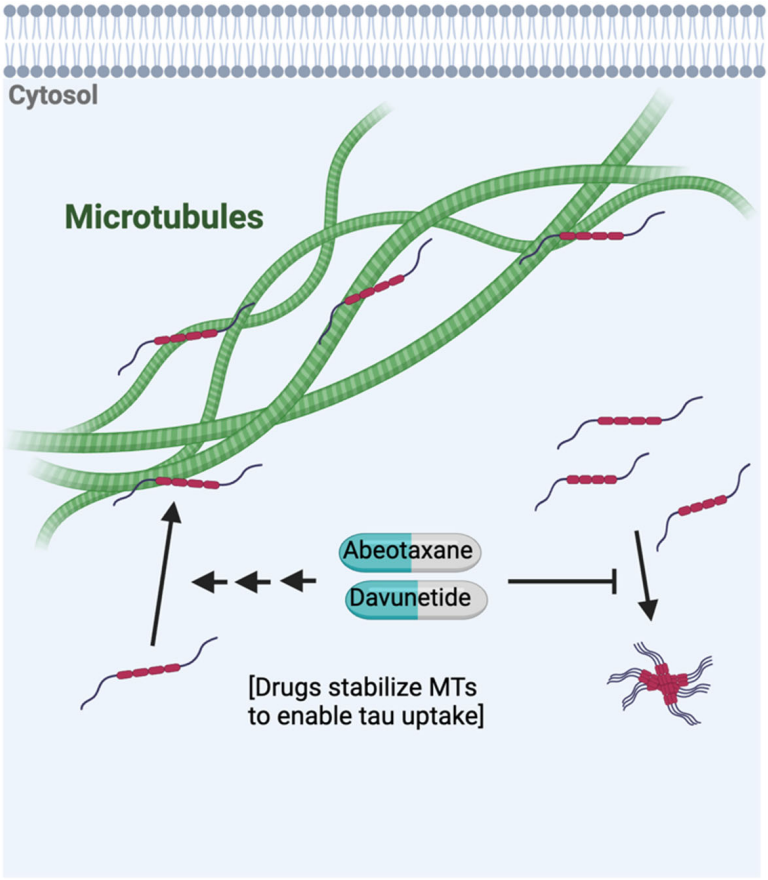


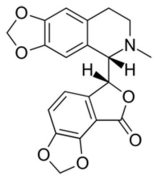
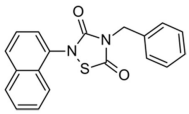
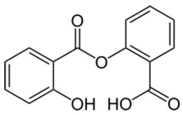
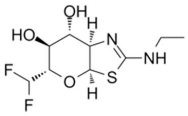
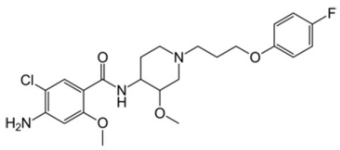
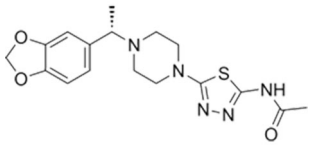
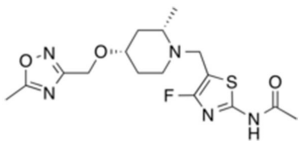
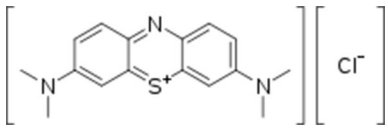
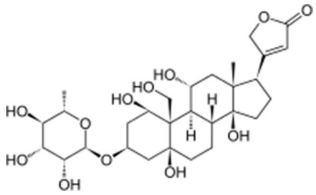
Figure 8. Microtubule stabilizers Abeotaxane and Davunetide promote monomeric uptake out of the cytoplasm onto the MTs to reduce tau aggregation; free-floating tau is more prone to aggregation[85,190,191]. Created with Biorender.com. MTs: Microtubules.

MULTITARGET AGENTS

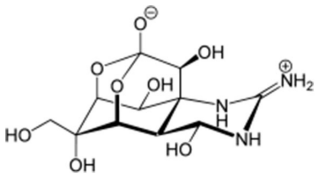
As discussed, a successful treatment to mitigate and treat neurodegeneration of AD will likely consist of a multifaceted plan to target multiple mechanisms and receptors involved in tau pathology [Table 1]. Traditionally, drugs are strictly tailored towards a single receptor for targeted therapeutic effects. However, a novel class of multitarget drugs capable of inducing a more dramatic therapeutic effect may be more suited for the challenge of neurodegeneration[20]. With an intimately related network of cellular pathways leading to the aggregation and propagation of tau as well as neuroinflammation, such agents can induce multiple effects at the cellular level while sidestepping some of the complexities of tuning combination therapies.

Multitarget drugs can be developed through two primary means: (1) repurposing existing drugs by rescreening against other targets or (2) synthesizing a chimeric drug with multiple active moieties tailored to different binding targets[20]. Approach one consists of mass screening of existing drugs to identify secondary target hits. Approach two is a technical challenge but, in theory, poses greater potential. A challenge in executing approach two is retaining the functionality of dual ligands and ensuring intended pharmacodynamic profiles for targeting disparate mechanisms.

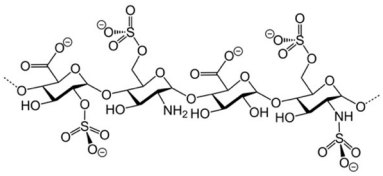
Table 1. Summary of potential therapeutic agents for tau pathology

Target	Intervention	Mechanism	Structure
1. Mediate gene expression			
MAPT gene	ASO's (BIIB080)[32,33]	Bind mRNA, mediate expression/splicing	N/A
2. Modulate post-translational modifications and aggregation			
GABAAR	Bicuculline[22]	Inhibit phosphorylation of tau	Bicuculline  Tideglusib 
GSK-3β	GSK-3β KO or inhibitors (tideglusib)[39,40,75]	Inhibit tau kinase, phosphorylation	
SIRT1, p300 acetyltransferase	SIRT1 upregulation[69], salsalate[70]	Inhibit acetylation, promote deacetylation	Salsalate  MK-8719 
TRIM1, 5-HT4 (Ubiquitin-proteasome system)	Proteasome promoters (cisapride)[73,74]	Promote proteasomal tau degradation	Cisapride 
O-GlcNAcase	O-GlcNAcase inhibitors (MK-8719[75], ASN120290[76], LY3372689[77])	Promote O-GlcNAcylation, inhibit phosphorylation	ASN120290  LY3372689 
Tau aggregates	Methylene Blue (LMTM)[78-80]	Reverse tau aggregation	Methylene blue 
3. Inhibit extracellular uptake and release			
LRP1	Cap LRP1 lysine residues (LRP1 KO)[43,44]	Inhibit receptor-tau binding	Ouabain 
Na+/K+-ATPase complex	Ouabain[47], TTX[46]	Inhibit NKA expression, block tau release	
AMPA receptor	Tetanus toxin, TTX[46]	Block tau release through channel	
LAG3	anti-LAG3 antibodies (410C9, C9B7W)[98-100]	Inhibit receptor-binding and transmission	
HSPGs	Sodium chlorate, heparin, Ext1 KO (HSPG synthetic enzyme)[45]	Inhibit receptor-tau binding	

TTX

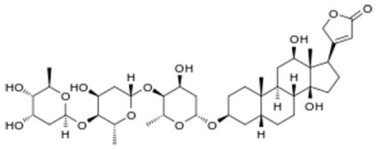


Heparin

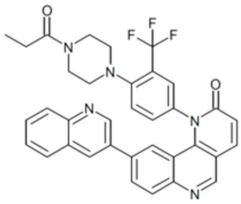


4. Modulate immune response

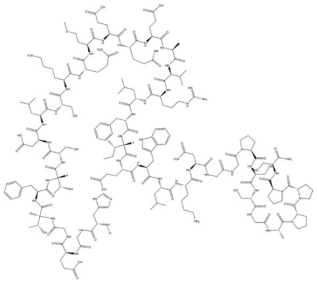
TREM2	Anti-inflammatory molecules[122-125]	Promote TREM2 expression	Digoxin
NF-κB	NLY01[131], IκB kinase KO[126,127]	Inhibit tau seeding	
TFEB/mTORC1	Digoxin, Torin 1[25,129,130]	Inhibition of neurotoxic astrocytes	
GLP1R	NLY01[131], Exendin-4[133]	Activate GLP1R to clear aggregates and reduce neurotoxicity	



Torin 1

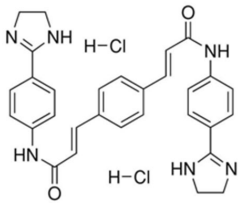


Exendin-4



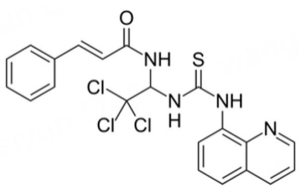
5. Inhibit intracellular organelle exchange

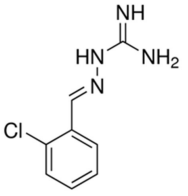
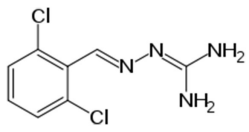
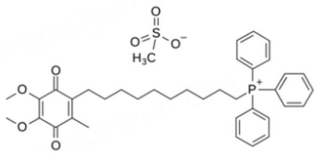
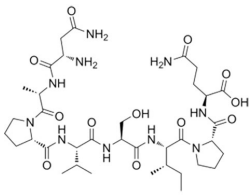
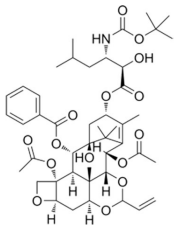
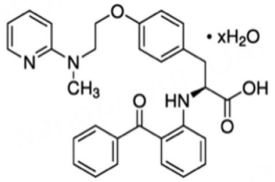
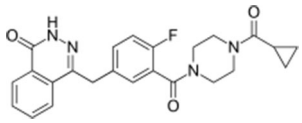
Tunneling nanotubes	Myosin-10 regulation[48-50]	Inhibit TNT formation	GW4869
Exosomes	nSMase2 inhibition-GW4869 or siRNA silencing[52]	Inhibition of exosome synthesis	



6. Modulate structural dysfunction

VDAC1 response	CypD KO[53]	Retain VDAC1 pathway viability	Salubrinal
Integrated stress response	Salubrinal, sephin1, guanabenz[54]	Regulate ISR and tau phosphorylation	
ROS	MitoQ[56], Vitamin E[177,178]	Scavenge ROS, reduce dimerization	



			Sephin1 	Guanabenz 
			MitoQ 	
7. Modulate microtubule stability				
MTs	Davunetide[191], Abeotaxane[190]	Stabilize MTs, reduce tau dysfunction	Davunetide 	Abeotaxane 
8. Modulate nuclear response				
PARP1	Olaparib, MC2050[57]	Rescue motor function, reduce toxicity	MC2050 	
			Olaparib 	

Chemical structures not accessible online were omitted. ASO: Antisense oligonucleotide; CypD: cyclophilin D; GABAAR: γ-aminobutyric acid subtype A receptor; GLP1R: glucagon-like peptide-1 receptor; GSK-3β: glycogen synthase kinase-3β; HSPGs: heparan sulfate proteoglycans; ISR: integrated stress response; KO: knockout; LAG3: lymphocyte-activation gene 3; LMTM: leuco-methylthioninium bis(hydromethanesulphonate); LRP1: lipoprotein receptor-related protein 1; MAPT: microtubule-associated protein tau; MitoQ: mitoquinone; MTs: microtubules; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NKA: Na/K-ATPase; PARP1: poly (ADP-ribose) polymerase-1; ROS: reactive oxidative species; SIRT1: deacetylase protein sirtuin 1; TFEB: transcription factor EB; TNT: tunneling nanotube; TREM2: triggering receptor expressed on myeloid cells 2; TTX: tetrodotoxin; VDAC1: voltage-dependent anion channel protein 1.

Efforts to develop such drugs to challenge tau pathology are ongoing. One encouraging study has developed 2,4-thiazolidinedione agents that act as dual GSK-3β and tau aggregation inhibitors[205]. In advanced *in vitro* studies, these drugs effectively lowered the assembly of tau aggregates and inhibited GSK-3β while improving neuron viability. This model also showed promise in BBB passage in *in vitro* membrane models. Another group is working to develop a joint Aβ-tau drug, utilizing triazinones to inhibit both GSK-3β and

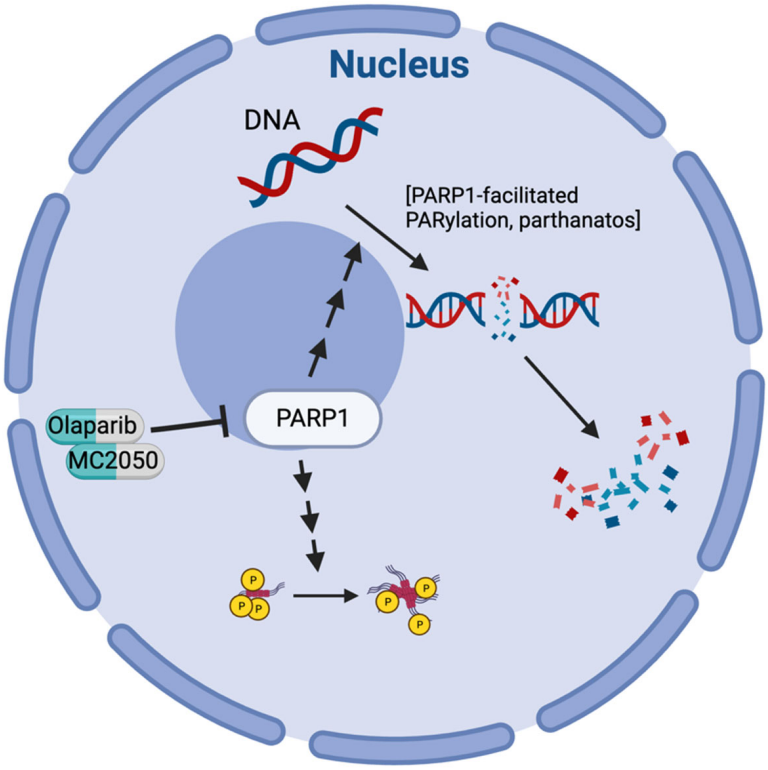


Figure 9. PARP-1 inhibitors (1) inhibit PARP-1-promoted phosphorylation of tau and (2) inhibit the PARP-1 facilitated PARylation of neural DNA to retain neural viability[57,203]. Created with BioRender.com. PARP-1: Poly (ADP-ribose) polymerase-1.

aspartyl protease β-secretase (BACE-1) - an enzyme responsible for Aβ formation[206]. In doing so, they hope to counter AD's two major protein drivers of AD for a more holistic treatment.

Discussion

Targeting tau

Evidence suggests that targeting tau pathology may be a promising approach to mitigating symptoms of AD and other forms of dementia[6,9]. The role of Aβ plaques in AD has been well documented, with tau only being heavily implicated in the past two decades[6]. However, while Aβ plaques are closely tied to the neuronal toxicity associated with AD, treatments focused on targeting these plaques failed to restore cognitive function. The tau protein appears to be more closely tied to the cognitive decline seen in AD patients and, as such, appears to be a better candidate for treatments to inhibit the progression of AD pathology[9]. Anti-tau therapies have thus far failed to induce notable clinical benefits, but there remains justification to continue pursuing tau as a therapeutic target, albeit with a slightly different approach[34]. Several anti-tau drugs that have fallen short target the full-length monomeric form irrespective of phosphorylation or aggregation state[34]. More specific epitope-targeting might yield greater efficacy in reducing pathogenic tau, though given the many isoforms and pathological forms of tau, targeting a certain tau epitope or aggregation form tends to fall short in treating mid-stage and late-stage AD[34].

Given the diverse mechanisms involved in the aggregation and propagation of tau, as well as Aβ buildup, the most effective treatment may involve multiple drugs that target different stages of tau expression as well as Aβ plaque seeding[4,6,8,9]. The two proteins, tau and Aβ, both play a role in neuronal damage and neuroinflammation, so both may prove critical in the campaign against tauopathies[4,6,9]. The mechanism of

tau dysfunction can be classified into broad categories, namely, post-translational modifications and aggregation, receptor-mediated uptake into cells and extracellular release, direct intercellular vesicular transport, immune cell mediation (astrocytes and microglia), mitochondrial stress response, and microtubule breakdown[8,22-26,207]. For this range of mechanisms, drugs to modulate gene expression or induce anti-inflammatory pathways, nanoparticle or viral vector carriers of siRNA to induce gene silencing or block receptors, and antibody blockers, among others, present ways of modulating existing pathways[52,61]. Many of the discussed treatments have shown glimmers of potential in translational models of dementia but still require years of further translational and clinical testing. The optimal treatment will likely comprise multiple of these treatment classes to pursue a multifaceted approach to countering tau propagation in AD[34].

Traversing the BBB

Though not addressed in this review, successful entry of drugs into the CNS and across the BBB is a requisite for the effective treatment of neuronal tauopathies[29]. Optimizing the lipid solubility of a nanoparticle drug encapsulation can allow for effective transport across the BBB while maintaining the drug payload[29,208]. This goal continues to plague campaigns against AD pathology; BBB drug delivery technology is needed to ensure effective transmission of anti-tau and anti-Aβ drugs across the brain parenchyma from the blood, yet as of 2020, less than 1% of all drug delivery studies for AD utilized BBB drug delivery systems[208]. Drug content in the cerebrospinal fluid (CSF) is sometimes errantly used as a proxy for BBB transmission; however, a distinction must be made between blood-CSF passage and blood-to-brain interstitial fluid passage, with far fewer drugs penetrating the latter than the former[208,209]. Effective BBB transmission is as critical to the efficacy of anti-tau drugs as the mechanism of anti-tau aggregation or anti-tau propagation itself. With regard to drug delivery methods, which could consist of nucleic acids or antibodies, mitochondria may prove useful as a drug delivery tool because they can be horizontally transferred between different cells and provide an effective, sustained release mechanism internally[209]. Mitochondrial-targeting drugs are worth exploring because of this intercellular transmission and the dysfunction that often afflicts the mitochondria in neurodegenerative disorders[209]. Introducing healthy mitochondria as treatment in AD mice has demonstrated a decrease in neuronal loss and gliosis[210]. Studies have demonstrated the efficacy of a range of small molecules, salts, metal complexes, and peptides in targeting mitochondria, most of which can help transport loaded nanoparticles to the mitochondria to achieve the desired release[209].

Personalized treatment and detection

A host of discoveries offer greater insight into the nature of tau in AD and treatment options. As noted earlier, brain imaging and detection technology to diagnose AD have advanced in recent years. Plasma phosphorylated-tau181, a marker of pathologic tau, is highly predictive of and specific to AD neuropathology years before post-mortem examination[17]. Meanwhile, tau-positron emission tomography (PET) levels have outperformed amyloid-PET and MRI imaging in forecasting cognitive decline in the preclinical and prodromal stages of AD[211]. With several clinical studies utilizing CSF biopsies and PET imaging markers for tau detection redefining AD as a driver of diverse cognitive defects, the value of a tau-centric approach to AD grows[212,213]. With this success, tau biomarkers are increasingly being explored. MTBR-tau 243 levels have been linked to a longitudinal increase with insoluble tau aggregates in the CSF, representing the latest progress in high-precision detection with potential applications in interventional trials and patient diagnosis[214]. Beyond mere detection, accurate characterization of protein pathology can enable the successful elimination of abnormal proteins related to AD from the patient's brain cells[215]. By utilizing tau-PET or other imaging technologies in conjunction with advanced removal techniques, the removal of early-stage tau accumulations can be achieved[215]. As new therapies and more effective anti-tau

targets are discovered, personalized treatment options for AD in clinical treatment will continue to grow.

DECLARATIONS

Authors' contributions

Literature review and manuscript organization: Kammula SV, Tripathi S
Draft manuscript preparation: Kammula SV, Tripathi S
Figure creation: Kammula SV
Manuscript revisions: Kammula SV, Wang N
Review direction and journal correspondence: Mao X, Dawson TM, Dawson VL
All authors have approved the final version of the manuscript.

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

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