

ALZHEIMER'S DISEASE AND TREATMENT




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Therapeutic Strategies Targeting Tau Protein: Implications for Alzheimer's Disease

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Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative condition with clinical features that include memory loss and cognitive deterioration. It belongs to the class of proteinopathies, thus being characterized by the aggregation of misfolded proteins. Intracellular Neurofibrillary Tangles (NFT), consisting of a highly phosphorylated form of the microtubule-bound tau protein, and extracellular amyloid plaques mainly composed of β -amyloid peptide appear to endorse the histopathology of AD. The process of association/dissociation of tau protein with Microtubules (MT) causes its involvement in MT dynamics and in neuronal axonal transport.

Considering the development of novel therapeutic agents in AD, tau aggregation inhibitors have been largely studied, including Glycogen Synthase Kinase-3 β (GSK-3 β) inhibitors and phosphatase activators, responsible for tau phosphorylation or dephosphorylation, respectively. Leuco-methylthioninium and its derivatives that directly inhibit the assembly of tau protein into filaments both *in vitro* and *in vivo* are among the most promising compounds for treatment of AD, being currently in phase III clinical trials. Small molecules have also been explored as MT stabilizing agents in the treatment of tauopathies, with some notable results based in animal models, such as Epothilone D. Furthermore, a correlation has been reported between the decrease in *O*-linked β -*N*-acetylglucosamine (*O*-GlcNAc) in the brain of AD patients with the promotion of tau aggregation and MT destabilisation, turning glycosidase *O*-GlcNAcase (OGA) into a promising therapeutic target.

With several clinical trials of amyloid-targeting therapies failing recently, tau has become one of the most actively pursued therapeutic targets for AD. Presently, new tau-based therapies are emerging such as immune therapy, MT stabilizers, OGA inhibitors and tau aggregation inhibitors. Regarding immunotherapy, two vaccines against phosphorylated pathological tau protein are currently under clinical trials. This review summarizes the tau-related etiology of AD and its therapeutic implications, focusing on recent findings of promising drugs and strategies targeting tau protein.

Abbreviations

AAV: Adeno-associated virus; A β : Amyloid- β ; Abl: Abelson; AD: Alzheimer's disease; AMP: Adenosine monophosphate; AMPK: AMP-activated protein kinase; ATP: Adenosine triphosphate; BBB: Blood brain barrier; cAMP: cyclic AMP; CBD: Corticobasal degeneration; CBP: CREB-binding protein; CDK5: Cyclin-dependent kinase 5; CHIP: Carboxy terminus of HSP70-interacting protein; CREB: cAMP response element binding protein; CSF: Cerebrospinal fluid; DNA: Deoxyribonucleic acid; EMA: European Medicines Agency; Fc: Fragment crystallizable region; FDA: Food and Drug Administration; FTD; Frontotemporal dementia; FTDP-17: Frontotemporal dementia and parkinsonism linked to chromosome 17; GlcNAc: β -*N*-acetylglucosamine; GSK-3 β : glycogen synthase kinase-3 β ; HAT: Histone acetyl transferase; HDAC: Histone deacetylase; HSF1: Heat shock factor 1; HSP: Heat shock protein; I2PP2A: Inhibitor-2 of PP2A; IgG, Immunoglobulin G; iPSC: Induced pluripotent stem cells; LCMT1: leucine carboxyl methyl transferase 1; LMT: leucomethylthioninium; LMTB: leucomethylthioninium bromide; LMTM: leucomethylthioninium mesylate; MAPK: Mitogen-activated



protein kinase; MARK: Microtubule affinity regulating kinase; MCI: Mild cognitive impairment; MT: Microtubule; MTC: methylthionium chloride; mTOR: Mammalian target of rapamycin; NAD: Nicotinamide adenine dinucleotide; NFT: Neurofibrillary tangles; NMDA: *N*-methyl-D-aspartate; NSAID: Non-steroidal anti-inflammatory drugs; *O*-GlcNAc, *O*-linked β -*N*-acetylglucosamine; OGA: *O*-GlcNAcase; OGT: *O*-GlcNAc transferase; p-tau: phosphorylated tau; PBMC: peripheral blood mononuclear cells; PHF: Paired helical filaments; PME-1: Phosphatase methylesterase-1; PP2A: protein phosphatase 2A; PSP: Progressive supranuclear palsy; SIRT1, sirtuin 1; TAI: tau aggregation inhibitor; UPS: Ubiquitin-proteasome system.

Introduction

Alzheimer's Disease (AD), a progressive neurodegenerative disorder characterized by memory loss and cognitive impairment, is the most common cause of dementia in the elderly. It is estimated that 50 million people worldwide are living with dementia and the number is expected to rise up to 152 million in 2050 due to demographic changes and longer life expectancy, with a profound impact on health, economic and societal costs [1]. Currently, drugs for AD therapy are limited to cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and *N*-methyl-D-aspartate (NMDA) receptor antagonists (memantine), which only provide short-term symptomatic relief and do not alter disease progression. Delaying onset of AD for 5 years could potentially result in 40% lower prevalence in 2050 [2]. However, there is a modest pipeline of drugs in development for AD [3-6].

Extracellular senile plaques composed of Amyloid β (A β) peptide and intracellular Neurofibrillary Tangles (NFT) made of Paired Helical Filaments (PHF) of hyperphosphorylated tau protein are the pathological hallmarks of AD [7-9]. The progression and anatomic distribution of NFT, but not amyloid load, has been found to correlate with the duration and severity of dementia in AD [7,8]. However, pathophysiological brain alterations and neuronal degeneration can occur decades before manifestation of the symptoms and clinical diagnosis [5,7].

Tau is a soluble microtubule-associated protein that is sorted into neuronal axons in physiological conditions [10,11]. In AD, hyperphosphorylation and aggregation of tau leads to impaired Microtubule (MT) interaction and tau missorting into the somatodendritic compartment with loss of axonal integrity [12]. Furthermore, the truncated repeat domain tau fragment containing the MT-binding region found in the core of PHF is able to catalyze and propagate the aggregation of normal soluble tau into proteolytically stable tau oligomers, which can propagate to healthy neighboring neurons in a prion-like manner [13,14]. It has also been recently suggested that hyperphosphorylation may determine both the spread and the morphology of tau pathology [15]. Self-propagation of tau pathology further accelerates the progression of the disease [16]. Besides AD, other neurodegenerative disorders are characterized by tauopathy, such as Progressive Supranuclear Palsy (PSP), Frontotemporal Dementia (FTD), Pick's disease, argyrophilic grain disease, and Corticobasal Degeneration (CBD) [8,11]. Dominant mutations in tau have been identified in FTD and parkinsonism linked to chromosome 17 (FTDP-17) [8,11].

The pivotal role of tau in the pathogenesis of AD, combined with recent failures of several drugs targeting the A β pathway in clinical trials, led to an increasing interest in therapeutic strategies directed against tau [8,9,17-20]. Current approaches mainly focus on inhibition of tau hyperphosphorylation, prevention of tau aggregation, promotion of MT stabilization and enhancement of tau clearance. Several drugs developed or repurposed toward tau pathology are represented in Figure 1. Tau-directed immunotherapy is a promising approach for treating AD and

other tauopathies due to the self-propagating nature of misfolded tau, and both active and passive immunization strategies are being actively pursued [21-23].

Drugs targeting tau pathology

Microtubule stabilizers

In physiological conditions, tau is normally bound to MTs in axons, modulating tubulin assembly and MT stability [10,11]. However, hyperphosphorylation of tau in tauopathies reduces the tubulin binding affinity of the protein and detaches normal tau from MTs, leading to MT destabilization and impaired axonal transport [10,11]. Thus, MT-stabilizing agents that can compensate for the loss of tau function and restore axonal transport have therapeutic potential in AD and other tauopathies.

Several classes of MT-stabilizing natural products are known, which include the taxane family comprising the antimetabolic drug paclitaxel employed in cancer chemotherapy [24]. Paclitaxel binds to the lumen of MTs at the taxane binding site, similarly to tau protein, and can displace tau from MTs [24]. The therapeutic potential of paclitaxel in neurodegenerative tauopathies was revealed in the T44 transgenic mice affected by spinal cord tau pathology [25]. Administration of paclitaxel by intraperitoneal injections (10 or 25 mg/m² weekly) for 12 weeks was found to increase MT density and restore fast axonal transport in spinal axons, ameliorating motor impairments [25]. However, paclitaxel has poor Blood-brain barrier (BBB) permeability and has been associated to severe side-effects, namely neutropenia and peripheral neuropathy, which precludes its use in brain tauopathies [24,25]. Synthetic derivatives of the taxane diterpenoids with fewer side-effects have been obtained, and the abeo-taxane TPI-287 is currently under phase 1 studies to determine its efficacy, safety, tolerability and pharmacokinetic properties after intravenous infusion in mild-to-moderate AD patients (NCT02966666) and other tauopathies (NCT02133846) (Table 1).

Similarly to paclitaxel, natural polyketide macrolactones bind to the taxane binding site promoting tubulin polymerization and MT-stabilization [24]. Epothilone D (BMS-241027) is a brain-penetrant natural macrolide found to improve MT density, axonal integrity, and cognition in PS19 tau transgenic mice after intraperitoneal administration at low weekly doses (1 or 3 mg/kg) for 3 months without showing systemic toxicity [26]. The therapeutic effects were observed both before and after the onset of tau pathology in aged animals [27]. A phase 1 study (NCT01492374) was conducted in mild AD patients to evaluate the tolerability and safety of epothilone D, as well as the effects of the drug on Cerebrospinal Fluid (CSF) biomarkers, with lower and less frequent doses (Table 1) than those used in phase 2 oncology trials to avoid neurological side effects, but further clinical trials were not pursued [9,17].

Dictyostatin, another antimetabolic macrolide, was found to cross the BBB *in vivo* exhibiting prolonged brain retention and activity [28]. Dictyostatin-treated aged PS19 tau transgenic mice

displayed improved MT density and reduced axonal dystrophy with a reduction of tau pathology and increased hippocampal neuronal survival compared to vehicle-treated PS19 mice [29]. However, dose-limiting peripheral side effects were observed, with higher doses being associated with gastrointestinal complications and body weight loss [29].

Davunetide (NAP, AL-108) is a neuroprotective octapeptide (NAPVSIQ) derived from activity-dependent neuroprotective protein with neurotrophic activity *in vitro* in rat hippocampal and cortical cultures [30]. In the triple transgenic mouse model of AD, intranasal administration of davunetide protected cognitive functions and reduced both A β accumulation and tau hyperphosphorylation, which is associated with MT dysfunction and defective axonal transport [30]. Intranasal davunetide also protected memory, increased soluble tau and reduced tau hyperphosphorylation *in vivo* in a tauopathy mouse model [30]. In a *Drosophila* model of tauopathy, characterized by prominent axonal transport defects, davunetide prevented and reversed behavioral phenotypes even after they became established [30]. Davunetide neuroprotection has been associated to increased MT end-binding protein-tau interaction, enhancing MT-stability and protecting axonal transport [31].

In phase 1 clinical trials, davunetide was found to cross the BBB after nasal and systemic administration [32]. A 12-week phase 2 study (NCT00422981) to evaluate the safety, tolerability and efficacy of intranasal administration of davunetide to patients with Mild Cognitive Impairment (MCI), frequently a prodrome to AD, showed that the drug was generally safe and well tolerated with signs of potential benefit on attention and working memory [33]. A subsequent phase 2/3 trial (NCT01110720) to evaluate the efficacy and safety of intranasal davunetide in PSP patients showed that the drug was well tolerated but did not provide beneficial treatment for PSP [34], while another phase 2 pilot study (NCT01056965) to evaluate the safety and tolerability of davunetide in tauopathy patients and to determine changes in CSF tau levels has recently been completed (Table 1).

Phosphorylation inhibitors

Glycogen synthase kinase-3 β inhibitors

Tau phosphorylation regulates binding of the protein to MTs but hyperphosphorylation causes tau to lose its biological activity [10,11]. Most of the putative phosphorylation sites for tau are in the proline-rich region near the MT-binding domain, except for Ser262, Ser293, Ser324 and Ser356 (KXGS motif) in the repeat domains [10,11]. The phosphorylation state of tau correlates with its MT-binding affinity and also with its propensity to aggregate. Phosphorylation at Ser199/Ser202/Thr205, Thr212/Ser214, Thr231/Ser235, Ser262/Ser356 and Ser396/Ser404 is associated with sequestration of tau from MTs while missorted dendritic tau in AD is phosphorylated at Ser202/Thr205 [10,11]. Phosphorylation at Thr231, Ser396/Ser404 and Ser422 increases the fibrillogenic tendency of tau and enhances formation of PHF [10,11]. Levels of phosphorylated tau at Thr181 are specifically elevated in the CSF of AD patients, being a widely used biomarker of AD [35].

Several kinases of different classes are involved in tau phosphorylation, including proline-directed serine/threonine protein kinases, such as Glycogen synthase Kinase-3 β (GSK-3 β), Cyclin-dependent Kinase 5 (CDK5) and Mitogen Activated Protein kinases (MAPK), non-proline directed serine/threonine protein

kinases, such as Microtubule Affinity-regulating kinase (MARK) responsible for phosphorylation at Ser262/Ser356, and tyrosine protein kinases [10,11]. Among those, GSK-3 β plays a major role in tau phosphorylation in the brain, both in physiological and pathological conditions. This enzyme phosphorylates tau on Thr181, Ser199, Ser202/Thr205, Thr231 and Ser396/Ser404, among other sites commonly found phosphorylated in PHF [10,11]. Moreover, phosphorylation at Thr231 induces a conformational change associated with *trans*-to-*cis* isomerization that exposes tau residues at the C-terminus for subsequent phosphorylation [10,11]. Both GSK-3 β levels and activity are elevated in the AD brain [10,11], suggesting the therapeutic potential of GSK-3 β inhibitors.

Lithium and valproic acid (usually formulated as divalproex sodium) are GSK-3 β inhibitors with a long record as mood stabilizers for the chronic treatment of bipolar disorder. Elevated GSK-3 β levels in Peripheral Blood Mononuclear Cells (PBMC) of bipolar disorder subjects are documented [36]. Preclinical evidence of the neuroprotective effects of sodium and valproate have been obtained in neuronal cell cultures and animal models. In FTDP-17 and GSK-3 β overexpressing transgenic mice, chronic lithium treatment prevented tau hyperphosphorylation and NFT formation, reduced amyloid burden, and improved memory deficits [36]. Valproate markedly decreased GSK-3 β and CDK5 activities, and reduced the levels of tau phosphorylation in human neuroblastoma SH-SY5Y cells treated with okadaic acid [37]. Based on their neuroprotective activity and pleiotropic effects, lithium and valproic acid have been proposed as promising disease-modifying therapeutic interventions for AD and related tauopathies.

A recent systematic review and meta-analysis of randomized, placebo-controlled trials suggest good tolerability and beneficial effects of lithium treatment on cognitive performance in subjects with MCI and AD dementia [38]. However, an open-label study with low-dose lithium for up to 1 year in 22 patients with mild-to-moderate AD did not show cognitive function improvement [39]. Similarly, in a 10-week trial (ISRCTN72046462) with 71 mild AD patients, lithium treatment had no effect on cognitive performance, GSK-3 β activity, or CSF levels of Phosphorylated Tau (p-tau) [40]. The negative data could be due to the small sample in the first study [39] or the short duration of the treatment in the second trial [40], since in a subsequent 12-month phase 2 study (NCT01055392) with 45 amnesic MCI patients, lithium treatment was associated with significant decrease in CSF levels of p-tau and better cognitive performance with good overall tolerability and an adherence rate of 91% [41]. The effects of long-term, low-dose lithium administration on renal, thyroid, immune, and glycemic functions were also evaluated [42]. No significant changes were detected in renal function while increases in the number of neutrophils, serum thyroid-stimulating hormone and body weight were observed [42], as well as a reduction of glucose metabolism in the cerebellum and hippocampus, in a small sample of older adults [43]. A phase 3 study (NCT02601859) evaluating lithium as a GSK-3 β inhibitor in MCI is currently recruiting while a phase 4 study (NCT03185208) has been designed to assess lithium efficacy in preventing cognitive impairment in amnesic MCI (Table 1).

Valproic acid has also been used for the management of behavioral and psychological symptoms associated with dementia, and as an anticonvulsant drug in the treatment of epilepsy [37]. In a phase 3 study (NCT00071721) enrolling 313 patients with moderate AD, valproate treatment for 24 months did not

delay the emergence of agitation or psychosis and was not able to slow cognitive decline [44], being associated with increased brain volume loss [45]. Another clinical trial (NCT00385710) conducted in 28 patients with PSP showed no significant differences between the valproate-treated and the placebo groups [46].

Several natural products that selectively inhibit GSK-3 β have been discovered, mainly of marine origin, and synthetic GSK-3 β inhibitors belonging to different chemical classes have been prepared, such as aminopyrimidines, paullones, maleimides and other indole derivatives, oxadiazoles, thiazoles, thiadiazolidinones, and halomethyl ketones [47]. A potent and selective GSK-3 β inhibitor, AZD1080 (Figure 1), has been synthesized which showed good oral bioavailability and the ability to cross the BBB [48]. The oxindole ring of AZD1080 binds to the ATP pocket of the catalytic domain of the enzyme, inhibiting tau phosphorylation and rescuing synaptic plasticity deficits in the rodent brain *in vivo* [48]. A subsequent phase 1 multiple ascending dose study in 224 healthy volunteers showed prolonged suppression of GSK-3 β activity in PBMC [48] but the trial was discontinued due to nephrotoxic side effects of the drug [49].

Tideglusib (Figure 1), an ATP-non-competitive and irreversible GSK-3 β inhibitor of the thiadiazolidinone family [49], significantly reduced the levels of tau phosphorylation and amyloid deposition, reduced neuronal loss and ameliorated memory deficits upon sustained oral administration to double transgenic AD mice for 3 months [50]. Oral administration of tideglusib at 50 to 1200 mg daily doses to 152 healthy volunteers showed no relevant side effects except for some moderate and fully reversible increases in serum transaminases [51]. A pilot study (NCT00948259) with escalating doses of tideglusib in mild to moderate AD patients (Table 1) showed that the treatment was well tolerated [51]. However, a subsequent phase 2 trial (NCT01350362) evaluating the efficacy of tideglusib in mild AD failed to show clinical benefit after 26-week treatment [52]. Another phase 2 trial (NCT01049399) to assess the efficacy and safety of tideglusib as an orphan drug for PSP also displayed no significant differences when compared to placebo after 52 weeks of treatment [53].

Phosphatase activators

Abnormal phosphorylation of tau in AD can result from an imbalance between the activity of protein kinases and protein phosphatases involved in tau phosphorylation and dephosphorylation, respectively [10,11]. While multiple distinct protein kinases are involved in tau phosphorylation, the major phosphatase implicated in tau dephosphorylation, Protein Phosphatase 2A (PP2A), is responsible for over 70% of tau dephosphorylation [54]. Additionally, PP2A is also involved in dephosphorylation and inactivation of protein kinases involved in tau phosphorylation, which are over-activated in the AD brain whereas PP2A levels and activity are deficient [54]. Thus, enhancing PP2A activity is a feasible alternative to kinase inhibition for reducing tau phosphorylation.

Several PP2A activating natural compounds have been identified, including antioxidant polyphenolic compounds, diterpenes and α -tocopherol analogs [54]. Activation of PP2A can be achieved directly or indirectly, through regulation of endogenous inhibitory proteins of PP2A activity or modulation of post-translational modifications such as carboxyl methylation of the catalytic subunit, which is controlled by Leucine Carboxylmethyltransferase (LCMT1) and protein phosphatase methylesterase

(PME-1) [54]. Reduced PP2A methylation has been implicated in cancer and AD, and high-throughput screening uncovered aza- β -lactams and sulfonyl acrylonitriles as potent and selective inhibitors of PME-1, the enzyme responsible for demethylation of the carboxyl terminus of PPA2 [55]. Memantine, the NDMA-receptor antagonist currently used in AD therapy, has been found to inhibit abnormal hyperphosphorylation of tau and prevent cell death in rat hippocampal slices pretreated with the PP2A inhibitor okadaic acid [56] by blocking inhibitor-2 of PP2A (I2PP2A)-induced inhibition of PP2A activity [57]. On the other hand, biguanides like antidiabetic drug metformin (Figure 1) and its derivative phenformin induce PP2A activity and reduce tau phosphorylation at PP2A-dependent epitopes in murine primary neurons from wild-type and human tau transgenic mice, both *in vitro* and *in vivo*, via inhibition of PP2A proteasomal degradation [58].

Sodium selenate is a specific agonist for PP2A able to stabilize PP2A-tau complexes, reducing tau hyperphosphorylation and completely abrogating NFT formation both *in vitro* and *in vivo* [59]. Oral administration of sodium selenate to tau transgenic mice mitigated tau pathology, prevented neurodegeneration and improved memory and functional deficits in AD mouse models [59,60]. Moreover, activation of PP2A by sodium selenate *in vivo* is presumed to be selective towards phosphorylated tau with no apparent side effects associated with indiscriminate PP2A activation [60]. A phase 2a study recently conducted in Australia with 40 mild-to-moderate AD patients to assess the safety and tolerability of 24-week treatment with sodium selenate (VEL015) at doses up to 30 mg per day (Table 1) showed that the therapy was safe and well-tolerated [61].

Acetylation modulators

Tau acetylation has recently emerged as a potential regulatory modification implicated in AD and related tauopathies [62, 63]. Acetylation of Lys259/290/321/353 (KXGS motifs) occurs in the human brain in physiological conditions, preventing tau hyperphosphorylation and aggregation, and is reduced in the AD brain [11,64]. On the other hand, acetylation at Lys174 in the proline-rich region reduces tau turnover and induces cognitive deficits *in vivo* [65] while acetylation at Lys274 and Lys280 within the MT-binding domain neutralizes the positively charged lysine residues and impairs tau-MT interactions, being a common feature across a wide range of human tauopathies, including AD [62,66]. Moreover, acetylation at Lys280 has been found to precede and enhance tau fibrillization *in vitro*, being a pathogenic modification associated with intracellular NFT formation [62, 63,66]. Therefore, acetylated tau levels represent potential biomarkers for the diagnosis of AD and related tauopathies while strategies aiming at tau acetylation inhibition that can restore MT stability and simultaneously ameliorate tau pathology are promising therapeutic approaches towards AD [63].

Tau can be acetylated by the histone acetyltransferases (HAT) p300 or CREB-binding protein (CBP) and deacetylated by histone deacetylases (HDAC), namely HDAC6 or NAD⁺-dependent sirtuin 1 (SIRT1) [11,67]. The roles of these enzymes in AD are controversial since increased HAT-mediated acetylation and decreased SIRT1 levels are implicated in AD pathology but HDAC6 inhibitors have neuroprotective effects [64,68]. Cytosolic HDAC6 is overexpressed in the AD brain and selective HDAC6 inhibitors have been found to simultaneously promote acetylation and prevent phosphorylation of tau on KXGS motifs, reducing the propensity of tau to aggregate and enhancing tau clearance [64]. Overall, these findings highlight the complexity

of post-translational modifications regulating tau function and the neuronal toxicity of different tau species.

Several HDAC inhibitors initially developed for cancer chemotherapy can restore the learning and memory deficits in AD mouse models and have been repurposed for AD therapy [64]. Tubastatin A and ricolinostat (ACY-1215) (Figure 1), two selective HDAC6 inhibitors, reduced tau hyperphosphorylation, amyloid load, and rescued cognitive deficits in AD transgenic mice without observable adverse effects [69]. Moreover, decreasing HDAC6 activity also promoted MT stability and rescued impaired mitochondrial trafficking through enhanced tubulin acetylation [64,69].

Decreased SIRT1 levels in the AD brain are associated with enhanced accumulation of PHF [70]. Resveratrol (Figure 1) has been identified as a potent SIRT1 activator [68] able to reduce hippocampal neurodegeneration, preventing learning impairment and decreasing the acetylation of SIRT1 substrates in the inducible p25 transgenic mouse model of AD and tauopathies [71]. In a recent phase 2 study (NCT01504854), oral administration of resveratrol to mild-to-moderate AD patients for 52 weeks (Table 1) attenuated cognitive decline and decreased CSF A β_{42} levels but did not alter tau levels [72,73].

Inhibition of p300 activity has been achieved with salsalate (Figure 1), a prodrug of salicylic acid belonging to the class of non-steroidal anti-inflammatory drugs (NSAID). In the PS19 transgenic mouse model of FTD, oral administration of salsalate (225 mg/kg) after disease onset lowered acetyl-Lys174 tau levels, reduced NFT formation, rescued memory deficits and prevented hippocampal atrophy at a human equivalent dose lower than the one usually prescribed for osteoarthritis or rheumatoid arthritis (3 g/day) [65]. A 12-month phase 1b study (NCT03277573) enrolling 40 mild-to-moderate AD patients to evaluate the safety and tolerability of salsalate at a daily dose of 3 g (Table 1) is currently recruiting. Another phase 1, open-label pilot study (NCT02422485) to evaluate the safety and tolerability of oral salsalate at a dose of 2250 mg/d for 6 months in 10 PSP patients is also recruiting.

O-GlcNAcase inhibitors

Tau can also undergo a post-translational modification involving β -*N*-acetylglucosamine (GlcNAc) attachment to serine or threonine residues through an *O*- β -glycosidic bond (*O*-GlcNAcylation) in the nucleocytoplasmic compartment. Addition and removal of *O*-linked β -*N*-acetylglucosamine (*O*-GlcNAc) is catalyzed by *O*-GlcNAc Transferase (OGT) and *O*-GlcNAcase (OGA), respectively [74,75]. *O*-GlcNAcylation reduces tau propensity to aggregate and negatively regulates tau phosphorylation in a site-specific manner both *in vitro* and *in vivo* [75]. Abnormal hyperphosphorylation of tau can result from the decreased levels of *O*-GlcNAcylation in the human AD brain induced by deficient brain glucose metabolism common in AD and other tauopathies [75]. Hence, selective OGA inhibitors that increase *O*-GlcNAc-tau levels in the brain and prevent pathological tau phosphorylation are a potential disease-modifying approach to AD [76]. Moreover, recent studies suggest that pharmacological inhibition of OGA is implicated in enhanced autophagy in the brain, thus also promoting turnover of tau aggregates [77].

Several OGA inhibitors have been developed, most of them being analogs of *O*-GlcNAc designed as transition state mimics, such as the 2-aminothiazoline thiamet-G (Figure 1), a potent and selective inhibitor of human OGA with a K_i value of 21

nmol/L. Oral administration of thiamet-G to healthy rats (200 mg/kg) increased *O*-GlcNAc-tau levels and reduced pathological phosphorylation of tau at Thr231 and Ser396 in the rat cortex and hippocampus [76]. Chronic treatment with thiamet-G increased *O*-GlcNAc-tau, hindered tau oligomerization and decreased neuronal cell loss in several tau transgenic mouse models [78-80].

Another brain penetrating OGA inhibitor, ASN120290 (ASN-561), was found to markedly increase *O*-GlcNAc-tau levels, reduce abnormally phosphorylated tau and prevent NFT formation after daily administration to P301S tau transgenic mice for 14 weeks in a preventative study without observable adverse events [81]. Increased *O*-GlcNAcylation in PBMC correlated well with brain *O*-GlcNAcylation and was developed as target engagement biomarker for phase 1 studies [81]. Preclinical data obtained with MK-8719, a competitive reversible inhibitor of human OGA, showed significant increase in *O*-GlcNAcyl-tau levels in brain tissue and reduced tauopathy in Tg4510 mouse brain [82]. MK-8719 was designated as orphan drug for PSP and early clinical results from a phase 1 study in healthy adults revealed that single oral doses of MK-8719 up to 1200 mg were generally well-tolerated while positron emission tomography studies demonstrated target engagement of the OGA enzyme in the brain [82].

Tau aggregation inhibitors

Several small-molecule compounds able to prevent tau aggregation and/or dissolve already formed aggregates have been identified in cell-based and *in vitro* screening assays, which include rhodanines, phenylthiazolyl hydrazides, *N*-phenylamines, anthraquinones, (thio)carbocyanines, phenothiazines, flavonoids and polyphenols [83]. However, for many tau aggregation inhibitors (TAIs) there is a lack of correlation between *in vitro* and *in vivo* efficacy, and further *in vivo* studies are required to demonstrate the efficacy and safety of TAIs. Currently, the most advanced TAIs for AD therapy in clinical development are Methylthioninium Chloride (MTC), also known as methylene blue, and its derivatives [9]. MTC (Figure 1) is a phenothiazine dye that exists as a blue cation under physiological conditions but can be reduced to an uncharged and colorless form, Leucomethylthioninium (LMT), depending on pH and redox environment [84]. Previously used as an antimalarial agent, MTC is an FDA-approved drug for the treatment of methemoglobinemia that has been repurposed for AD and related tauopathies following reports on its ability to reverse the proteolytic stability of PHF isolated from AD brain tissue by blocking tau-tau binding *in vitro* without disrupting tau-tubulin interactions [9,84,85].

The first-generation TAI developed, TRx0014 (Rember), was a purified formulation of MTC which was evaluated as monotherapy in a 24-week phase 2 clinical trial (NCT00515333) enrolling 321 patients with mild-to-moderate AD, randomly assigned to placebo or 30, 60, or 100 mg MTC capsules three times per day (corresponding to 69, 138 and 228 mg daily of methylthioninium, respectively), followed by an open-label study (NCT00684944) for up to 12 months. Only the 138 mg daily dose showed potential benefits on cognitive performance of moderate AD subjects [86]. Gastrointestinal and urinary disorders were the most common side-effects reported [86]. The lack of efficacy of the highest dose was attributed to impaired delivery due to delayed release of the hard gelatin capsule formulation and dose-dependent absorption limitations in the presence of food [86,87]. Another randomized phase 2 study (NCT02380573) designed to investigate the effects of methylene blue in cognitive perfor-

mance of healthy aging, MCI and AD subjects after 2-week and 12-week treatment is currently underway with simultaneous administration of the sodium channel inhibitor phenazopyridine hydrochloride to alleviate methylene blue side-effects on the urinary tract.

In order to overcome the pharmacokinetic limitations of MTC, which requires the presence of food or enzymatic reduction to uncharged LMT (Figure 1) in the gut prior to absorption [87], TRx0237 (LMTX) was developed as second-generation TAI, corresponding to reduced methylthioninium stabilized in the form of crystalline leucomethylthioninium salts, either as dihydromesylate or dihydrobromide derivatives [84,85]. Both the Leucomethylthioninium Mesylate (LMTM) and Leucomethylthioninium Bromide (LMTB) salts retained TAI activity *in vitro* [85] and *in vivo* [88]. At the pH 6.8 of the small intestine, these salts are mainly deprotonated to yield neutral LMT, which improves intestinal absorption and bioavailability [87].

Several phase 3 studies were conducted to evaluate the efficacy and safety of TRx0237 mesylate (LMTM) in the treatment of AD and behavioral FTD patients (Table 1), some of them on acetylcholinesterase inhibitors and/or memantine. All studies employed oral tablets instead of capsules and active placebo containing 4 mg of TRx0237 as a urinary and fecal colorant to help maintain blinding. Gastrointestinal and urinary disorders were again the most common adverse events registered for the higher doses tested [89, 90]. The 12-month trial in behavioral FTD (NCT01626378) did not achieve the co-primary endpoints [90] and the 15-month trial in mild-to-moderate AD (NCT01689246) also showed disappointing results [89]. However, in the latter case post-hoc analysis revealed an unexpected benefit in the monotherapy subgroup, including the low control dose (active placebo), when compared with the subgroup in add-on therapy [89]. On the other hand, encouraging results were obtained in the 18-month trial (NCT01689233) in mild AD patients randomly assigned to 100 mg or 4 mg (control) of LMTM twice daily. Significant cognitive and functional outcomes were observed in favor of LMTM monotherapy compared to add-on therapy and also suggested that the control low-dose might be as effective in monotherapy as the higher LMTM dose [90]. To further test this hypothesis, a phase 2/3 study (NCT03446001) to evaluate the efficacy of TRx0237 at a daily dose of 8 mg in patients with mild AD is currently recruiting.

Chaperone modulators

Enhancing misfolded or aggregated tau clearance offers another therapeutic strategy to target aberrant tau. The Ubiquitin-Proteasome System (UPS) and the autophagy-lysosome system are the major pathways for degradation of misfolded and aggregated proteins in eukaryotic cells [91]. Proteasome activity is decreased in the AD brain, contributing to the accumulation of misfolded proteins and protein aggregates, including PHF and NFT. Molecular chaperones, which regulate protein folding and client maturation, can also target misfolded proteins for refolding or proteasome-mediated degradation, representing a potential target for therapeutic intervention [92,93]. The Heat Shock Proteins (HSP), particularly HSP70 and HSP90, are a major class of molecular chaperones that assist in protein folding, transport and degradation, playing an important role in the regulation of aberrant intracellular proteins, like tau [93]. Elevated expression of HSP has been found in cancer and several neurodegenerative diseases like AD and related tauopathies, suggesting that chaperone dysregulation may contribute to the accumulation of misfolded protein aggregates in proteinopa-

thies [92,93]. Moreover, in cells overexpressing wild-type tau, molecular chaperones have also been found to promote tau association with MTs [94].

HSP90 is involved in protein stabilization and refolding while HSP70 promotes proteasomal degradation via protein ubiquitination mediated by the carboxy terminus of HSP70-interacting protein (CHIP), a co-chaperone for both HSP70 and HSP90 [93, 95]. CHIP modulates HSP90 function and determines the refolding or degradation of HSP70/90 client proteins [95]. CHIP recognizes and selectively ubiquitinates tau phosphorylated at pathological proline-directed Ser/Thr sites, such as pSer202/pThr205 and pSer396/pSer404, and conformationally altered tau species while leaving normal phosphorylated tau unaffected [96]. On the other hand, tau phosphorylated by MARK within KXGS motifs, such as pSer262/pSer356, is not ubiquitinated by CHIP and is more resistant to proteasomal degradation [96]. CHIP overexpression decreased hyperphosphorylated tau levels and rescued mitochondrial transport deficits in human H4 neuroglioma cells [97]. Most chaperones and cochaperones, including HSP70, HSP90 and CHIP, are up-regulated in the AD brain whereas proteasome activity is decreased, contributing to the accumulation and aggregation of hyperphosphorylated and ubiquitinated tau in PHF and NFT [93].

Inhibition of the ATPase activity of HSP90 has been shown to hinder the refolding pathway and increase CHIP-mediated proteasomal degradation of p-tau (through HSP70/CHIP complex) in cell culture and to decrease hyperphosphorylated tau levels in a transgenic mouse model of tauopathy, suggesting that HSP90 may protect hyperphosphorylated tau from degradation [93,96,98,99]. Furthermore, it has been suggested that the higher aggregation propensity of P301L mutant tau may reflect a perturbation of its chaperone-assisted stabilization and proteasome-dependent degradation due to the proline-to-leucine mutation at position 301 (P301L) [100]. Thus, proteostasis modulators that inhibit HSP90 binding and stabilization of misfolded tau or promote HSP70/CHIP-dependent ubiquitination and degradation of pathological p-tau species via the proteasome can potentially attenuate accumulation and aggregation of hyperphosphorylated tau [95,99].

Several HSP90 ATPase inhibitors have been developed that bind at either the N-terminus or the C-terminus of the chaperone [99]. The former also activate heat shock factor 1 (HSF1) inducing the expression of other chaperones, such as HSP70 [99]. First generation HSP90 inhibitors were N-terminal ATPase inhibitors based on natural antitumor antibiotics geldanamycin and radicicol bearing benzoquinone and resorcinol scaffolds, respectively, as nucleotide mimics [99]. In cell cultures transfected with tau constructs, geldanamycin led to 80% reduction in levels of insoluble tau aggregates and a concomitant increase in soluble tau levels associated with an increase in MT-bound tau [101]. Treatment of primary cortical neurons with geldanamycin reduced p-tau levels in a dose-dependent manner that inversely correlated with HSP70 induction [102]. Less hepatotoxic ansamycin derivatives developed as second generation HSP90 inhibitors for cancer chemotherapy, like 17-allylamino 17-demethoxy-geldanamycin (tanespimycin) and 17-dimethylamino-ethylamino-17-demethoxy-geldanamycin (alvespimycin), also reduced tau pathology *in vitro* [103] and *in vivo* in a transgenic mouse model of FTD [104]. Synthetic N-terminal ATPase inhibitors of HSP90 have been developed based on the purine scaffold, including highly BBB permeable EC102 [98] and water-soluble PU24FCl and PU-DZ8 derivatives (Figure 1) shown

to facilitate brain clearance of aberrant p-tau in transgenic mouse models of tauopathy [103]. Alternatively, C-terminal HSP90 ATPase inhibitors are being developed based on the coumarine core of novobiocin, a small molecule antibiotic, to avoid toxic adverse events and other off-target effects of N-terminal inhibitors, including induction of the heat shock response [99].

Regarding modulation of HSP70 function, both activators and inhibitors of HSP70 ATPase activity have been identified [95, 105]. Surprisingly, activation of HSP70 ATPase activity preserved cellular tau levels while inhibition of this activity significantly reduced levels of p-tau by blocking the refolding pathway, driving bound tau towards USP-mediated degradation [95,105]. Methylene blue, a phenothiazine dye currently in clinical trials as an inhibitor of tau aggregation, was also shown to inhibit HSP70 although not selectively [105]. Another dye, the rhodocyanine MKT-077 (Figure 1), selectively binds HSP70 in cellular models but lacks BBB permeability [106]. Its derivative YM-08 obtained by replacing the cationic pyridinium moiety of MKT-077 with a neutral pyridine showed enhanced brain penetrance and was not retained in the kidney, inhibiting HSP70 ATPase activity and reducing p-tau levels in cultured brain slices [106].

Autophagy enhancers

Monomeric, misfolded tau can be degraded by the proteasome but not aggregated forms of tau which are preferentially cleared by macroautophagy via the lysosomal pathway [91]. In the AD brain, accumulation of autophagic vacuoles containing protein aggregates in dystrophic neurites due to impaired autophagosome clearance contributes to the neurodegenerative process [107]. On the other hand, expression of beclin-1, a key autophagy regulator also involved in neuroinflammation, is reduced in AD brain tissue leading to decreased autophagosome formation and increased brain levels of pro-inflammatory mediators due to enhanced microglia activity [107]. Thus, autophagy impairment in AD has been associated with disruption in both autophagosome biogenesis and degradation, suggesting that stimulation of autophagy can be neuroprotective against AD and other proteinopathies [91,107].

The immunosuppressant drug rapamycin (sirolimus) can induce autophagy and delay the progression of tauopathy in mutant P301S tau transgenic mice by inhibiting the mammalian target of rapamycin (mTOR), a protein kinase with a major role in the maintenance of protein homeostasis [108]. The mTOR pathway negatively regulates autophagy and mTOR is hyperactive in the brains of AD patients, being a potential therapeutic target for AD. Rapamycin was also neuroprotective in the triple transgenic mouse model of AD, reducing A β and tau pathology in the mouse brain and rescuing cognitive deficits by inducing autophagy [109], however severe side-effects limit its long-term use in AD therapy. Temsirolimus (CCI-779), a prodrug of rapamycin with improved pharmacokinetic profile, lowered tau accumulation and rescued motor dysfunctions in mutant tau transgenic mice by stimulation of mTOR-dependent autophagy [110].

The natural polyphenol resveratrol, which induces autophagy by SIRT1 activation mimicking caloric restriction, has recently been identified as an ATP-competitive inhibitor of mTOR [111]. On the other hand, the antidiabetic drug metformin activates adenosine monophosphate-activated protein kinase (AMPK) which induces autophagy by suppression of mTOR [112]. Although metformin has been shown to reduce abnormally phosphorylated tau levels by increasing PP2A activity [58], other

studies suggest that the drug may exacerbate AD pathology by additionally promoting A β production in the autophagic vacuoles in a transgenic mouse model of AD [112]. A recent phase 2 pilot study (NCT01965756) evaluating the effect of metformin in AD biomarkers (Table 1) showed a slight increase in both total tau and p-tau levels.

Tyrosine kinase inhibitors used in cancer chemotherapy induce autophagy through AMPK activation [113]. These drugs also inhibit Abelson (Abl) kinase, a reversible non-receptor tyrosine kinase activated in neurodegeneration, decreasing the levels of misfolded aggregated proteins by stimulating beclin-mediated autophagy and promoting the maturation of autophagic vacuoles to autophagosomes while simultaneously decreasing blood and brain levels of proinflammatory cytokines [113]. Preclinical studies showed that nilotinib (Figure 1), a second-generation tyrosine kinase inhibitor used in adult leukemia treatment, decreased brain Abl activity and enhanced autophagic clearance of A β and p-tau, reversing cognitive decline in AD mouse models [114]. An open label pilot trial in advanced Parkinson's disease with dementia and dementia with Lewy bodies (NCT02281474), involving 12 patients randomized to low oral doses of 150 or 300 mg nilotinib once daily for 24 weeks, showed that the drug penetrates the BBB, being safe and well-tolerated [115]. Furthermore, nilotinib significantly reduced CSF total tau and p-tau levels, consistent with observed cognitive improvement [115]. Based on these promising results, nilotinib entered a phase 2 clinical trial (NCT02947893) in mild-to-moderate AD patients (Table 1).

Similarly, the natural disaccharide trehalose used as nutritional and food preservative has been shown to enhance beclin expression, reduce insoluble tau levels and improve neuronal survival in the brain of P301S tau transgenic mice by stimulating autophagy through an mTOR-independent pathway [116]. On the other hand, it has recently been demonstrated that trehalose can also promote autophagy by inhibiting glucose transport thus inducing AMPK-dependent autophagy [117].

Several studies and chemical screenings have identified small molecules regulating mTOR-independent autophagy, including some FDA-approved drugs. Pimozide (Figure 1), a conventional antipsychotic drug of the diphenylbutylpiperidine class, reduced toxic forms of tau in the brain of TauC3 transgenic mice after daily intraperitoneal administration and rescued memory deficits by increasing autophagic flux independent of dopamine D₂ receptor inhibition [118]. Pimozide and other calcium channel antagonists have been identified as mTOR-independent autophagy activators increasing autophagosome synthesis by intracellular calcium levels drop and calpain inactivation [119].

Other FDA-approved drugs known to induce autophagy also have pleiotropic actions, such as the mood stabilizers lithium, valproic acid and carbamazepine used in the treatment of bipolar disorders. Lithium induces autophagy through inhibition of inositol monophosphatase which prevents inositol recycling while the anticonvulsant drugs valproic acid and carbamazepine inhibit inositol synthesis thus reducing intracellular inositol levels. Lithium also inhibits GSK-3 β , which activates mTOR and attenuates the overall ability of lithium to enhance p-tau clearance and mitophagy of damaged mitochondria [120]. The mTOR-independent autophagy inducing activity of lithium may contribute to the attenuation of cognitive decline in AD [36,38].

Tau-directed immunotherapy

Active immunization

Immunization with tau peptide vaccines has become an appealing strategy for the treatment of AD and other tauopathies since it allows selective clearance of specific pathological forms of the protein, which may prevent transmission and spreading of tau pathology throughout the brain [21-23]. Most tau immunotherapeutic approaches selectively target individual p-tau epitopes involved in tau pathology, such as pSer396/pSer404, pThr231/pSer235 or pSer422, in order to avoid autoimmune reactions against physiological forms of the human tau protein [21-23]. The first tau-based immunization approach employing dephosphorylated, full length recombinant human tau emulsified in complete Freund adjuvant and with pertussis toxin, caused encephalomyelitis, development of NFT-like pathology and neurologic deficits upon administration to wild-type C57BL/6 mice [121]. However, active immunization of P301L tau transgenic mice with a 30-amino acid peptide comprising the p-tau epitope pSer396/pSer404, prior to the onset of the pathology, reduced aggregated tau in the brain and improved functional impairment without significant adverse events [122]. Active immunization against the pathological p-tau epitope pSer422 in THY-Tau22 transgenic mice also reduced insoluble tau species, which correlated with cognitive improvement [123]. Following immunization, an increase in plasma tau levels was observed suggesting that immunotherapy facilitated tau clearance from the brain toward the periphery [123].

The first active tau vaccine to enter clinical trials, AADvac1, is a synthetic peptide designed to target misfolded tau, comprising residues 294-305 (KDNKHPGGGS) of the tau sequence coupled to keyhole limpet hemocyanin through an *N*-terminal cysteine, using aluminum hydroxide as adjuvant. In a transgenic rat model of AD, vaccination reduced the levels of tau oligomers and the extension of NFT pathology in the animal brains [124]. The vaccine displayed favorable safety and tolerability profile in preclinical studies [124] and proceeded to clinical trials.

The first-in-man study of AADvac1 was a 12-week phase 1 trial (NCT01850238) conducted in Austria to assess safety, tolerability and immunogenicity of the active vaccine in mild-to-moderate AD, followed by another 18-months open label, safety follow-up study (NCT02031198) [125]. From a total of 30 patients receiving 3 subcutaneous injections of AADvac1 at monthly intervals, 29 developed an IgG immune response [125]. The vaccine was considered safe and well-tolerated [125], and entered a 24-month phase 2 study (NCT02579252) enrolling 208 patients with mild AD randomly assigned to subcutaneous injections of AADvac1 (40 µg dosage) or placebo, which is currently ongoing.

The liposomal vaccine, ACI-35, contains a 16-amino acid tetrapalmitoylated peptide with the tau sequence 393-408 incorporating the p-tau epitope pSer396/pSer404 [126]. Vaccination elicited rapid and robust immune response in both wild-type mice and P301L tau transgenic mice, decreased levels of tau aggregates and improved tau pathology without signs of neuroinflammation or other significant adverse events [126]. A phase 1b trial (ISRCTN13033912) is underway to assess the safety profile in mild to moderate AD patients.

The first anti-tau DNA vaccine based on the multiTEP platform, AV-1980D, targeting a tau B cell epitope spanning amino acids 2-18, was shown to induce strong immune responses and to significantly reduce tau pathology in THY-Tau22 mice after intramuscular administration followed by electroporation [127].

The maximum titers of anti-tau antibody were reached after 2 immunizations and remained steady during 5 subsequent monthly immunizations [127]. The data suggest that electroporation-mediated DNA immunization is an attractive alternative to protein-based adjuvanted vaccines for inducing high concentrations of anti-tau antibodies [127].

Passive immunization

Passive immunotherapy, involving direct administration of anti-tau antibodies, can be a more effective and safer alternative to active immunization with tau epitopes due to the weakened immune system of the elderly population. Several monoclonal and polyclonal antibodies have been developed that target specific pathological p-tau epitopes (e.g. PHF-1, recognizes pSer396/pSer404), misfolded conformations (e.g. MC1, recognizes interaction of N-terminus amino acids 5-15 with residues 312-322) or tau oligomers (e.g. T22, recognizes tau dimers, trimers, and tetramers but not monomers or PHF fibrils) [21-23,128]. Antibody specificity for pathological tau can be more important than affinity in therapeutic passive immunization, requiring careful selection of the target tau epitope [129, 130].

On the other hand, the pan-tau antibody B1B076 was found to bind with subnanomolar affinity to human and cynomolgus monkey recombinant tau, recognizing both monomeric and fibrillar tau forms [131]. The safety profile of B1B076 in cynomolgus monkeys after intravenous or subcutaneous administration [131] allowed for its inclusion in a phase 1 study (NCT03056729) to evaluate the safety of single-ascending intravenous B1B076 infusions in healthy volunteers and AD patients. Several other anti-tau antibodies are currently in early clinical trials for AD therapy and other tauopathies (Table 2).

A pioneer study involving weekly intraperitoneal injection of JNPL3 tau transgenic mice with the PHF-1 monoclonal antibody for 13 weeks reduced functional decline and cleared insoluble tau aggregates from the brain when the treatment was started prior to the onset of the disease [132]. Similarly, inoculation with conformational monoclonal antibody MC1 reduced hyperphosphorylated tau levels and delayed the progression of tau pathology in tau transgenic mouse models after disease onset [133]. Recently, an adeno-associated viral (AAV) vector was used to deliver the genes encoding the PHF-1 antibody directly to the hippocampus of P301S tau transgenic mice, which resulted in higher hippocampal antibody levels than those obtained after repeated systemic administration [134]. A significant reduction (> 80%) in hippocampal insoluble tau levels and NFT was observed following direct brain administration of a single dose of AAV-vectored PHF-1, in contrast to systemic passive immunization [134]. Vector-mediated antibody gene delivery, allowing for a sustained and continuous antibody expression after a single administration, might be a promising alternative to passive immunotherapy for the prevention and/or treatment of tauopathies.

Immunotherapy with antibodies targeting misfolded tau has succeeded in reducing tau pathology in several tau transgenic mouse models [130,132,133,135,136] despite tau being an intracellular protein. Several mechanisms have been proposed to explain antibody-mediated clearance of misfolded tau, such as antibody targeting of extracellular tau, leading to inhibition of transcellular spreading of tau aggregate pathology reported in the literature [13,14,135,136]. Alternatively, the anti-tau/pSer422 antibody was shown to bind to membrane-associated

ed tau/pSer422 on the neuronal cell surface after peripheral administration in the triple transgenic AD mouse model, and intracellular clearance of the antigen-antibody complexes contributed to decreased accumulation of tau pathology [137]. A humanized monoclonal antibody targeting the p-tau epitope pSer422, RO6926496 (RG7345) entered phase 1 single-ascending dose trial (NCT02281786) in the UK in healthy male volunteers to compare the safety of multiple intravenous doses of antibody to placebo.

On the other hand, by using full effector and effectorless antibodies against p-tau epitope pSer409, antibody binding of tau was found to be sufficient to prevent spreading of tau pathology, both *in vitro* and *in vivo*, without requiring effector function for microglial uptake and clearance, thus avoiding the release of proinflammatory cytokines [138]. Effector function is mediated by antibody interaction with microglia Fc_γ receptors, which can be modulated by mutations in the IgG fragment crystallizable (Fc) region [138]. The IgG4 backbone weakens activation of microglia Fc_γ receptors, avoiding neuronal toxicity due to inflammatory response [138]. An IgG4 humanized anti-tau monoclonal antibody, RO7105705 (RG6100), was developed to target pathological tau in the extracellular brain environment. The antibody recently completed a phase 1 study (NCT02820896) designed to evaluate the safety of single dose, dose-escalation, and multiple doses of either intravenous or subcutaneous RO7105705 in healthy volunteers and mild-to-moderate AD patients and entered phase 2 (NCT03289143) for evaluation of its efficacy and safety in patients with prodromal to mild AD.

Antibodies that target tau oligomers and other extracellular pathological tau species are ideal candidates for tau-directed immunotherapy [129]. A single intravenous injection containing 60 µg of an anti-tau oligomer-specific mouse monoclonal antibody was able to prevent cognitive impairment and oligomeric tau toxicity in Htau mice following intracerebroventricular injection with brain-derived tau oligomeric seeds [135]. Further studies showed that long-term administration of this antibody was effective as a preventative therapy, preserving memory function and inhibiting accumulation of oligomeric tau but not hyperphosphorylated tau [135].

Extracellular tau aggregates have been implicated in transcellular aggregate propagation of tau pathology *in vivo* [136] and increased Aβ production by inducing neuronal hyperactivity *in vitro* [139]. Extracellular tau is mainly composed of N-terminal tau fragments which have been identified in secreted tau from Induced Pluripotent Stem Cells (iPSC) AD patient-derived cortical neurons in conditioned media [139]. Furthermore, comparative analysis of healthy and AD human CSF showed a significant increase of N-terminal tau fragments in the latter [35,139].

Gosuranemab, an humanized IgG4 monoclonal anti-tau antibody (also known as BIIB092, BMS-986168 or IPN007) that targets residues 9-18 of extracellular N-terminally truncated tau originally isolated from familial AD patient-derived iPSC, was shown to decrease extracellular tau-induced neuronal hyperactivity and soluble Aβ levels in human primary cortical neurons and in the P301L tau transgenic mouse model [139]. Gosuranemab was tested in phase 1 single ascending-dose study in healthy volunteers (NCT02294851) and phase 1 multiple-ascending dose study in PSP patients (NCT02460094) followed by a 18-month open label extension study (NCT02658916) still

ongoing. This biologic was assigned orphan drug status for PSP by both EMA and FDA agencies in 2015. Preliminary data indicated that gosuranemab was safe and well-tolerated, producing marked reduction in CSF free extracellular tau in support for further studies. A phase 2 trial in PSP patients (NCT03068468) is currently recruiting while another listed phase 2 study (NCT03352557) aims to evaluate safety, tolerability and efficacy of intravenous gosuranemab in subjects with MCI due to AD or mild AD (Table 2).

Other antibodies have been developed to target extracellular aggregated tau species. Intraperitoneal injection of HJ8.5, an anti-tau monoclonal antibody recognizing the N-terminal epitope spanning amino acids 25-30 (DQGGYT), to P301S tau transgenic mice at a weekly dose of 50 mg/kg for 3 months significantly reduced insoluble tau, decreased brain atrophy and improved motor impairment [140]. Based on the promising results of HJ8.5 in tau transgenic mouse models, the humanized anti-tau antibody ABBV-8E12 (C2N-8E12) targeting aggregated extracellular tau was developed and data from a single-ascending dose phase 1 study in 30 PSP patients (NCT02494024) revealed that the biologic was safe and well-tolerated, with a half-life of 30 days in plasma and CSF/plasma ratio in the range 0.2–0.4% [141]. An extension study (NCT03413319) to evaluate long-term safety of ABBV-8E12 in PSP was designed. The antibody entered phase 2 studies that are currently enrolling participants to evaluate the safety and efficacy of ABBV-8E12 solution (20 mg/mL) for infusion at 3 different doses against placebo, one in early AD (NCT02880956) and another in PSP (NCT02985879), with an extension study to assess long-term safety and efficacy of ABBV-8E12 in PSP patients (NCT03391765). FDA granted this antibody orphan drug designation for the development of PSP.

Conclusion

Abnormal tau phosphorylation leading to sequestration of tau from MTs and aggregation are critical events in the NFT pathology of AD and related tauopathies. Current tau-target therapies are mainly based in hyperphosphorylation inhibition, MT stabilization, prevention of aggregation and enhanced degradation of misfolded/aggregated tau. Tau-direct immunotherapy promoting clearance of pathological tau species is an attractive approach to prevent and/or stop the progression of AD and other related tauopathies, and the first clinical trials with two active vaccines targeting tau yielded promising results. On the other hand, MT-stabilizing agents already in clinical trials offer the opportunity to restore axonal transport due to loss of tau function.

Methylthioninium, the most advanced TAI in clinical trials, showed a good safety and tolerability profile, but results from a recent phase 2 trial were rather disappointing although a phase 3 trial with a leuco-methylthioninium derivative yielded more promising outcomes. Several protein kinase inhibitors targeting abnormal tau hyperphosphorylation in AD have shown efficacy in preclinical trials. Other post-translational tau modifications, such as acetylation and O-GlcNAcylation, have been recently implicated in the pathophysiology of AD and other tauopathies, opening new avenues for therapeutic intervention. An OGA inhibitor is currently ongoing phase 1 trials while promising results obtained in animal studies with a NSAID prodrug is driving the attention towards tau acetylation inhibitors for AD therapy.

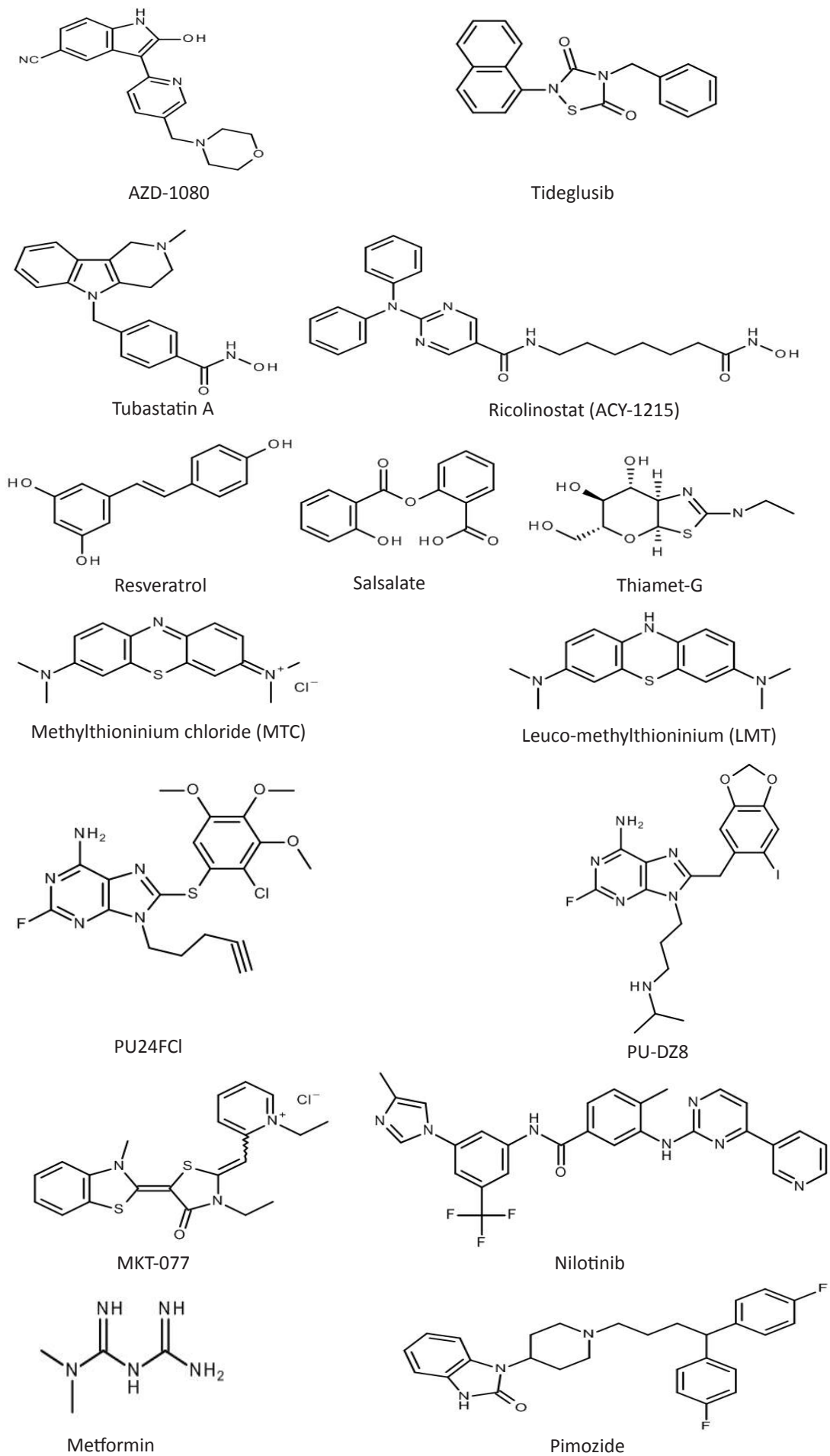


Figure 1: Several drugs targeting tau pathology, some of them in clinical trials.

Table 1: Tau-targeting drugs in clinical trials for AD and related tauopathies.

Drug	Trial identifier ^{a)} (acronym)	Disease ^{b)} (number of participants)	Treatment ^{c)} (total duration)	Stage ^{d)} , completion date
MT-stabilizers				
TPI-287	NCT01966666	Mild-to-moderate AD (n=33)	2, 6.3 or 20 mg/m ² IV q3wk (9 wks)	Phase 1, ongoing
	NCT02133846	Tauopathies (n=44)	2, 6.3 or 20 mg/m ² IV q3wk (9 wks)	Phase 1, ongoing
Epothilone D (BMS-241027)	NCT01492374	Mild AD (n=40)	0.003, 0.01 or 0.03 mg/kg IV qwk (9 wks)	Phase 1, 10/2013
Davunetide (AL-108, NAP)	NCT00422981	MCI (n=144)	5 mg qd or 15 mg bid, IN (12 wks)	Phase 2, 01/2008
	NCT01110720	PSP (n=313)	30 mg bid IN (52 wks)	Phase 2/3, 12/2012
	NCT01056965	Tauopathies (n=12)	15 mg bid IN (12 wks)	Phase 2, 07/2017
GSK-3β inhibitors				
Lithium	ISRCTN72046462	Mild AD (n=70)	42 mg Li ₂ SO ₄ /tab bid, up to 0.5–0.8 mmol/L Li ⁺ in serum (10 wks)	N/A, 07/2005
	NCT01055392	Amnesic MCI (n=80)	150 to 450 mg Li ₂ CO ₃ /tab bid, up to 0.25–0.5 mmol/L Li ⁺ in serum (2 yrs)	Phase 2, 10/2011
	NCT02601859	MCI (n=20)	100 to 200 to 400 mg/d (9 wks)	Phase 3, not yet recruiting
	NCT03185208 (Lattice)	MCI (n=80)	150 mg/d Li ₂ CO ₃ tabs, up to 0.6–0.8 mmol/L Li ⁺ in serum (2 yrs)	Phase 4, recruiting
Valproic acid	NCT00071721 (Valid)	Mild-to-moderate AD (n=313)	250 mg/tab qd, up to 10–12 mg/kg/d (2 yrs)	Phase 3, 12/2009
	NCT00385710	PSP (n=28)	Depakine (2 yrs)	Phase 2, 07/2010
Tideglusib (NP-12, NP031112)	NCT00948259	Mild-to-moderate AD (n=30)	400, 600, 800 or 1000 mg qd, pwd oral susp (20 wks)	Phase 2a, 11/2009
	NCT01350362 (Argo)	Mild-to-moderate AD (n=306)	500 or 1000 mg qd or 1000 mg qod, pwd oral susp (26 wks)	Phase 2, 10/2012
	NCT01049399 (Tauros)	Mild-to-moderate PSP (n=146)	600 or 800 mg qd, pwd oral susp (52 wks)	N/A, 11/2011
PP2A activators				
Sodium selenate (VEL015)	ACTRN 12611001200976	Mild-to-moderate AD (n=40)	320 mcg or 10 mg oral caps tid (24 wks)	Phase 2a, recruiting
SIRT1 activators				
Resveratrol	NCT01504854	Mild-to-moderate AD (n=119)	500 mg/cap qd up to 1 g bid (52 wks)	Phase 2, 03/2014
Acetylation inhibitors				
Salsalate	NCT03277573	Mild-to-moderate AD (n=40)	750 mg/tab, 2 tabs bid (12 mos)	Phase 1b, recruiting
	NCT02422485	PSP (n=10)	2250 mg/d (6 mos)	Phase 1, recruiting
Tau aggregation inhibitors				
Methylene blue (TRx0014)	NCT00515333	Mild-to-moderate AD (n=323)	30, 60 or 100 mg tid, hard caps (24 wks)	Phase 2, 12/2007
	NCT00684944	AD (n=111)	30 or 60 mg gelatin caps tid (12 mos)	Phase 2, 12/2011
	NCT02380573	Healthy ageing, MCI, AD (n=240)	282 mg/d oral plus phenazopyridine HCl 97.5 mg/d (2 or 12 wks)	Phase 2, ongoing
TRx0237	NCT01626378	Behavioral FTD (n=220)	100 mg/tab bid (12 mos)	Phase 3, 02/2016
	NCT01689246	Mild-to-moderate AD (n=891)	75 or 125 mg/tab bid (15 mos)	Phase 3, 11/2015
	NCT01689233	Mild AD (n=800)	100 mg/tab bid (18 mos)	Phase 3, 05/2016
	NCT03446001	Mild AD (n=180)	4 mg/tab bid (6 mos)	Phase 2/3, recruiting

Autophagy enhancers				
Metformin	NCT01965756	MCI and AD	500 mg qd up to 1000 mg bid (8 wks)	Phase 2, 04/2017
Nilotinib	NCT02947893	Mild-to-moderate AD (n=42)	150 mg/caps, 1 or 2 caps qd (12 mos)	Phase 2, recruiting

Abbreviations: NCT: ClinicalTrials.gov number; ISRCTN: International Standard Randomised Controlled Trial Number; ACTRN: Australian Clinical Trials Registration Number; AD: Alzheimer's disease; FTD: Frontotemporal dementia; MCI: Mild cognitive impairment; PSP: Progressive supranuclear palsy; bid: Twice daily; Cap: Capsule; D: Daily; IN: Intranasal; IV: Intravenous; Mo: month; pwd: Powder; qd: Once daily; qod: Every other day; qwk: Every week; q3wk: every 3 weeks; Susp: suspension; Tab: Tablet; Tid: Thrice daily; Wk: Week; Yr: Year. N/A: Not applicable.

Table 2: Clinical trials involving tau-directed immunotherapeutic approaches.

Bioactive	Trial identifier ^{a)} (acronym)	Disease ^{b)} (number of participants)	Treatment ^{c)} (duration)	Stage ^{d)} , completion date
Passive immunization				
BIIB076	NCT03056729	Healthy volunteers, AD (n=56)	SAD, IV inf (20 wks)	Phase 1, ongoing
RO6926496 (RG7345)	NCT02281786	Healthy male volunteers (n=48)	6 SAD cohorts, IV inf (16 wks)	Phase 1, 10/2015
RO7105705 (RG6100)	NCT02820896	Healthy, mild-to-moderate AD (n=74)	6 SAD cohorts IV or SC, 1 or more MAD cohorts IV qw up to 4 doses	Phase 1, 06/2017
	NCT03289143	Prodromal to mild AD (n=360)	3 SAD cohorts, IV	Phase 2, recruiting
Gosuranemab (BIIB092, BMS-986168, IPN007)	NCT02294851	Healthy volunteers (n=65)	SAD, IV inf (8 mos)	Phase 1, 04/2016
	NCT02460094	PSP (n=48)	3 MAD cohorts, IV inj q4wk up to 3 doses	Phase 1, 01/2017
	NCT02658916	PSP (n=48)	4 MAD cohorts, IV inj q4wk (1.5 yrs)	Phase 1, ongoing
	NCT03068468 (Passport)	PSP (n=396)	50 mg/mL IV inf q4wk (48 wks)	Phase 2, recruiting
	NCT03352557 (Tango)	MCI and mild AD (n=528)	3 MAD cohorts, IV inf q4wk (90 wks)	Phase 2, not yet recruiting
ABBV-8E12 (C2N-8E12)	NCT02494024	PSP (n=32)	4 SAD cohorts, IV inf (4 mos) ^f	Phase 1, 08/2016
	NCT03413319	PSP (n=10)	IV inf (up to 2 yrs)	Phase 1, not yet recruiting
	NCT02985879	PSP (n=330)	2 MAD cohorts, 20 mg/mL IV inf (52 wks)	Phase 2, recruiting
	NCT03391765	PSP (n=340)	2 MAD cohorts, 20 mg/mL IV inf (up to 5 yrs)	Phase 2, recruiting
	NCT02880956	Early AD (n=400)	3 MAD cohorts, 20 mg/mL IV inf (96 wks)	Phase 2, recruiting
Active immunization				
AADvac1	NCT01850238	Mild-to-moderate AD (n=30)	1 dose/mo, SC inj (3 mos)	Phase 1, 03/2015
	NCT02031198 (Fundamant)	Mild-to-moderate (n=25)	3 doses, SC inj q4wk plus 1–2 booster doses (18 mos)	Phase 1, 12/2016
	NCT02579252 (Adamant)	Mild AD (n=208)	6 doses, SC inj q4wk plus 5 booster doses quarterly (24 mos)	Phase 2, ongoing
ACI-35	ISRCTN13033912	Mild-to-moderate AD (n=24)	3 dose cohorts, 2, 3 or 5 IV inj (6 mos)	Phase 1b, 06/2017

Abbreviations: NCT: ClinicalTrials.gov number; ISRCTN: International Standard Randomised Controlled Trial Number; AD: Alzheimer's disease; MCI: Mild cognitive impairment; PSP: Progressive supranuclear palsy; Inf: Infusion; inj: Injection; IV: intravenous; MAD: Multiple ascending dose; Mo: Month; qw: every week; q4wk: Every 4 weeks; SAD: Single ascending dose; SC: Subcutaneous; Wk: Week; Yr: Year.

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