Published in final edited form as:

Alzheimers Dement. 2016 October ; 12(10): 1051–1065. doi:10.1016/j.jalz.2016.06.006.

Therapeutic Strategies for the Treatment of Tauopathies: Hopes and Challenges

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Abstract

A group of neurodegenerative diseases referred to as tauopathies are characterized by the presence of brain cells harboring inclusions of pathological species of the tau protein. These disorders include Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) due to tau pathology, including progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Tau is normally a microtubule-associated protein that appears to play an important role in ensuring proper axonal transport, but in tauopathies tau becomes hyperphosphorylated and disengages from microtubules, with consequent misfolding and deposition into inclusions that mainly affect neurons, but also glia. A body of experimental evidence suggests that the development of tau inclusions leads to the neurodegeneration observed in tauopathies, and there is a growing interest in developing tau-directed therapeutic agents. The following review provides a summary of strategies under investigation for the potential treatment of tauopathies, highlighting both the promises and challenges associated with these various therapeutic approaches.

Introduction

The presence of inclusions comprised of the tau protein [1, 2] within brain cells is a hallmark pathological feature of a group of progressive neurodegenerative diseases referred to as tauopathies, which include Alzheimer's disease (AD) and a major class of frontotemporal degeneration (FTD), such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and Pick's disease, which are associated with underlying tau pathology [3]. Further, while single or repetitive traumatic brain injury (TBI) may lead to AD or other neurodegenerative diseases [4, 5], recent studies suggest that a distinct tauopathy known as chronic traumatic encephalopathy (CTE) may result from repetitive brain trauma, especially in contact sports like football [4, 6, 7]. Moreover, there are a host of other very rare familial and sporadic neurodegenerative tauopathies that are characterized by

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prominent or mainly tau pathology [8]. Finally, a tauopathy now known as pathologic aging related tau pathology (PART), which was previously referred to as tangle predominant senile dementia, may or may not be associated with cognitive impairments or other clinical manifestations [9, 10]. Although most of the non-AD tauopathies are orphan diseases, there may be compelling economic and scientific reasons for conducting clinical trials of disease modifying therapies in these disorders, as illustrated for PSP [11].

Tau is normally a microtubule (MT)-associated protein that is thought to provide stability to axonal MTs [12, 13], where it may also affect axonal transport through modulation of MT motor function [14–16]. In humans, tau exists as 6 isoforms that are generated through alternative mRNA splicing of three exons, one of which encodes a microtubule-binding sequence such that the resulting protein has either 3- or 4-MT-binding repeat domains (i.e., 3-R or 4-R tau), as well as 0, 1 or 2 alternatively-spliced amino-terminal exon sequences. Tau becomes hyperphosphorylated in all tauopathies, which promotes its disengagement from MTs [17–20]. Hyperphosphorylated tau subsequently forms inclusions that are found predominantly within neurons, where they are referred to as neurofibrillary tangles (NFTs) when found within the neuronal soma and neuritic threads when found in dendritic processes [8, 21]. There is compelling evidence that tau hyperphosphorylation and the subsequent formation of higher order multimeric structures leads to neuronal dysfunction and death. For example, there is a strong correlation between the extent of tau pathology and the degree of dementia in AD patients [22–24], and mutations within the tau gene are known to cause forms of frontotemporal lobar degeneration referred to as frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) or familial FTLT-Tau [25, 26]. The mechanism by which hyperphosphorylated and misfolded tau species lead to neuronal dysfunction is still uncertain, but the prevailing hypothesis is that one or more misfolded tau species causes a gain-of-function toxicity [27]. An alternative and not mutually exclusive theory is that reduced binding of hyperphosphorylated tau to axonal MTs results in an alteration of MT structure or function that affects axonal transport, ultimately leading to neurotoxicity. Furthermore, in different variants of FTLT-Tau disorders, as exemplified by CBD, glial tau pathology is very abundant in grey and white matter regions [3] and there is increasing recognition of other types of aging-related astroglial tau pathology [28]. However, the significance of glial tau pathology in mechanisms of neurodegenerative tauopathies and aging-related cognitive decline is still enigmatic. Nonetheless, given the high likelihood that pathologic tau species play a critical role in the onset and progression of neurodegeneration, there is great incentive to identify approaches that will mitigate gain-of-function and/or loss-of-function toxicities.

The objective of this review is to provide brief summaries of a number of tau-directed therapeutic strategies currently being pursued within academic and industry laboratories. As of this writing, most of these endeavors are still at a pre-clinical stage, although there are a growing number of clinical trials examining tau-directed therapeutics (see Table 1). Thus, there is a growing sense of optimism that a disease-modifying treatment will ultimately be identified for neurodegenerative tauopathies, although currently there is no known effective treatment for any neurodegenerative tauopathies or other forms of FTD.

1. Modulating Post-Translational Modifications of Tau

Tau undergoes a number of post-translational modifications that can modulate the function, turnover or multimeric assembly of the protein. In addition to phosphorylation, these include acetylation [29, 30] glycosylation [31, 32], methylation, nitration and sumoylation [33, 34], and several of these modifications are being investigated as potential targets for therapeutic intervention [35], as detailed further below.

a. Inhibiting Tau Phosphorylation

Phosphorylation is by far the most well-studied post-translational modification of tau, as it has been known for some time that tau hyperphosphorylation is a feature of all tauopathies. Tau is phosphorylated even in the absence of disease, but in tauopathies the extent of phosphorylation is increased ~4-fold [36–38]. At least 40 phosphorylation sites have been described for tau, and up to 25 of these may undergo increased phosphorylation within tauopathy brains [39–41]. A number of studies have revealed that hyperphosphorylation of tau greatly reduces its ability to bind to MTs [18–20], thereby leading to the hypothesis that MT structure and/or axonal transport may be affected in tauopathies [42–44]. In addition, increased phosphorylation of some ser/thr residues of tau has also been reported to increase the propensity of the protein to assemble into the fibrils that comprise NFTs and neuropil threads [45, 46]. In addition to directly enhancing tau misfolding, the increased cytosolic tau concentrations that result from hyperphosphorylated tau disengaging from MTs could also promote a concentration-dependent fibrillization of tau. Thus, increased tau phosphorylation could contribute to both loss-of-function and gain-of-function toxicities.

There has been considerable interest in identifying inhibitors of the kinases that catalyze tau phosphorylation, particularly because the pharmaceutical sector has prior experience in the development of kinase inhibitors, albeit largely for the treatment of cancers. However, there is still uncertainty as to which kinase(s) are most relevant to tau phosphorylation in neurons, and several candidate ser/thr kinases have been implicated, including glycogen synthase kinase-3 β (GSK-3 β), cell cycle-dependent kinase 5 (CDK5), MT-affinity regulated kinases (MARKs), protein kinase A (PKA), mitogen-activated kinases (MAPKs) and others [47–49]. Among these, the most well-studied and arguably most validated are GSK-3 β and CDK5. It is beyond the scope of this brief review to summarize all of the data supporting the involvement of these kinases in tauopathies, and the reader is referred to recent reviews focused on tau phosphorylation and inhibitors of putative tau kinases [50, 51]. However, it can be briefly noted that both GSK-3 β and CDK5 co-localize with tau tangles in AD brain [52–55], and there are convincing data showing that altered expression of GSK-3 β [56–58] or p25 [59, 60], an activator of CDK5, affect tau pathology in transgenic (tg) mouse models. Inhibitors of GSK-3 β , including lithium chloride [61–63] and certain synthetic small molecules [61, 64], have been demonstrated to decrease tau phosphorylation and/or tau deposits in tg mouse models of tauopathy, and lithium advanced to clinical testing in AD patients. However, no improvements in cognitive outcomes were observed in a Phase 2 clinical study [65]. Additionally, a non-competitive GSK-3 β inhibitor (tideglusib) was recently evaluated in Phase 2 testing in PSP and AD patients, and it also failed to improve clinical outcomes [66, 67]. Given the fact that several pharmaceutical companies have

previously reported tau kinase inhibitor programs, the relative paucity of such compounds advancing to clinical testing points to 1) the continued uncertainty around which kinase(s) should be preferentially targeted in tauopathies, 2) the known difficulty in developing selective kinase inhibitors, and 3) the safety challenges associated with prolonged inhibition of kinases, such as GSK-3 β [68], that modify multiple proteins and cellular pathways.

b. Inhibiting Tau O-linked Glycosylation

Because of the aforementioned challenges in developing safe and effective tau kinase inhibitors, an alternative but related strategy is the modulation of tau O-glycosylation. There is evidence of tau being modified via addition of one or more N-acetylglucosamine moieties to ser and/or thr residues (O-GlcNAc) [31, 32, 69]. Importantly, O-glycosylation prevents the modified amino acid, and perhaps other nearby ser and thr residues, from being phosphorylated with what appears to be a reciprocal relationship between the extent of tau O-glycosylation and phosphorylation. Moreover, O-glycosylation of tau appears to lower its propensity to form oligomers and fibrils [70, 71]. These observations suggest that increasing O-GlcNAc modifications of tau could reduce the negative effects attributed to hyperphosphorylation; i.e., tau disengagement from MTs and fibrillization. As it is difficult to increase enzyme activity pharmacologically, current research activities have focused not on enhancing O-GlcNAc transferase activity, but rather on inhibiting the O-GlcNAcase (OGA) enzyme that is responsible for the removal of GlcNAc groups from modified ser/thr residues. Notably, an inhibitor of this enzyme, known as thiamet-G, has been demonstrated to reduce tau phosphorylation and decrease insoluble tau in tg mouse models of tauopathy [71–73]. However, one recent report noted that although thiamet-G treatment resulted in improved motor performance and survival in a tau tg mouse model, there was not an increase in tau O-glycosylation nor a decrease in tau phosphorylation, leading the authors to suggest that the salutary effects were likely mediated by changes in O-glycosylation of proteins other than tau [74]. The explanation for the discrepant findings with thiamet-G in tau tg mouse models is presently unknown. More recently, a mass spectrometry study of tau modifications has suggested that only one amino acid is O-glycosylated with very low stoichiometry in wild-type and APP tg mice, raising questions of whether inhibition of O-GlcNAcase would have a significant effect on tau phosphorylation [34]. At this time, it would seem that the inhibition of O-GlcNAcase has conceptual appeal, but additional studies are required to further validate this approach and provide clarity as to whether the reported positive effects of thiamet-G in tau tg mice result from a direct increase of tau O-GlcNAc modifications, or perhaps from enhanced O-glycosylation of other proteins. Moreover, as noted for tau kinase inhibitors, there is a concern that inhibition of O-GlcNAcase could lead to side-effects, as the glycosylation of multiple proteins would be affected by inhibition of this enzyme.

c. Inhibiting Tau Acetylation

In the past several years, it has become clear that another potentially important post-translational modification of tau is lysine acetylation. Tau acetylation was first described in 2010 [29], where a number of residues were identified that could be acetylated, and shortly thereafter a report demonstrated that acetylation of lysine 280 (K280) of tau enhanced fibrillization and altered MT interaction [30]. In addition to direct conformational effects on

tau, acetylation also appears to reduce tau degradation, likely because the acetylation of lysines prevents ubiquitination and consequent proteasomal catabolism [29]. Among the acetylated residues that appear to regulate tau degradation is K174, as a recent publication [75] revealed that mutation of this residue to glutamine (an acetyl-lysine mimic) decreased tau turnover and induced cognitive deficits in mice. Moreover, treatment of tau tg mice with the drug salsalate caused inhibition of p300 acetyltransferase activity, with reduced tau K174 acetylation, decreased total tau levels and a diminution of hippocampal neuron loss [75]. As salsalate is also a cyclooxygenase inhibitor, it is possible that some of the observed improvements in the treated mice also resulted from a reduction in inflammatory eicosanoids. Nonetheless, these data suggest that acetyltransferase inhibition might hold promise for the treatment of tauopathies, although this optimism is somewhat tempered by the knowledge that p300 acetylates a plethora of proteins in addition to tau, and thus inhibition of this enzyme could result in numerous cellular changes. However, the observation that salsalate treatment in the aforementioned study appeared to be well tolerated by tau tg mice suggests that inhibition of this enzyme may be possible without undue side effects, and additional studies will be required to provide further evidence of the relative safety and efficacy of this therapeutic approach.

2. Inhibiting Proteolytic Processing of Tau

A number of studies suggest that tau cleavage by proteases such as caspases, calpain, thrombin, cathepsins and endopeptidases contributes to the generation of pathologic tau species. Perhaps the most widely studied cleavage of tau is at Asp⁴²¹, resulting in a slightly truncated form of tau (Tau or c-tau). This cleavage can be mediated *in vitro* by caspases-3, -7 and -8, and less efficiently by caspases-1, and -6 [76, 77]. The formation of this truncated tau species has been demonstrated to be increased by A β peptides in cultured neurons [77], and its presence increases the rate and extent of tau fibril assembly *in vitro* [76, 77], suggesting Tau acts as a “seed” for fibril formation. The Asp⁴²¹ form of tau has also been detected in the AD brain [76, 78, 79], specifically in NFTs and dystrophic neurites in the CA1 layer of the hippocampus [76, 77], where it also co-localizes with activated caspase-3 [76]. There is an inverse correlation between the number of cells immunoreactive for this tau fragment and cognitive function [76]. Moreover, Tau has been detected in other tauopathies such as Pick’s disease, PSP and CBD [79, 80]. Tau has also been detected in tau tg mouse models, where it appears to facilitate aggregation of endogenous tau [76, 81, 82]. More recently, it was shown in a tau tg mouse model that tau cleavage by caspase-3 was facilitated by mitochondrial carrier protein apoptosin, which may be a risk factor for PSP, and that overexpression of apoptosin led to increased levels of Tau and exacerbated tau pathology in these mice [80].

Tau is also subject to cleavage at Asp⁴⁰² [83] and Asp¹³ by caspase-6 [84]. Activated caspase-6 and caspase-6 cleaved tau (Tau Casp6) have been detected consistently in tangles, neuritic plaques and neuropil threads in mouse models of tauopathy (reviewed in [85]) and in AD brains, where their occurrence correlates inversely with cognitive performance [86, 87]. Tau Casp6 also appears to be specifically elevated in AD CSF [87]. Tau cleaved at Glu³⁹¹ is also found in tangles in AD brains and has been suggested to be a cleavage product of Tau mediated by an unknown protease [88].

Tau cleavage by calpain, as well as by lysosomal asparagine endopeptidase (AEP), results in neurotoxic fragments [89–93]. Notably, the level of some of the calpain-cleaved tau species can be reduced in a tau tg mouse model by overexpression of the specific calpain inhibitor, calpastatin [94]. Moreover, overexpression of calpastatin in these mice led to a decrease in tau hyperphosphorylation and aggregation, with a delay in disease onset and restoration of life span [94]. Similarly, knocking out AEP in a mouse model of tauopathy resulted in a decreased tau phosphorylation and synapse loss, with an improvement in cognition [93].

Finally, tau proteolysis by thrombin [95–97] and cathepsins [98–100] has also been described. Thrombin has been found in the AD brain associated with NFTs, as well as senile plaques [101]. Thrombin was also found to cleave tau in a cell model expressing an inducible variant of the tau repeat domain (Tau_{RD}), with a thrombin inhibitor preventing the fragmentation of Tau_{RD} [102]. Interestingly, in this same cell model it was found that cathepsin-L cleaves the tau fragment produced by thrombin [103]. Finally, cathepsin D is expressed in the brain [104–106] and is found to be increased in the brains of tau tg mice [106], with its inhibition preventing the formation of phosphorylated tau fragments in rat hippocampal cultures [107].

These various studies all build a case for tau proteases and certain specific proteolytic products as possible therapeutic targets. However, the large number of putative enzymes implicated in the cleavage of tau makes the selection of the most appropriate protease targets difficult. For example, AEP, caspases and calpain can cleave tau independently of one another [93], with all leading to fragments that are pro-aggregating or toxic. Moreover, all of the proteases implicated in tau cleavage also act on a number of additional protein substrates, so there may be negative consequences of prolonged inhibition of many or most of these enzymes. Thus, although there is a growing body of evidence that the proteolytic processing of tau likely contributes to pathological processes, additional studies will be required to gain a better understanding of the most important and druggable of these many targets. Finally, as discussed below, while tau fibrillization can occur with tau fragments, neither tau cleavage nor other posttranslational modifications are required for tau monomers to assemble into tau filaments like those seen in AD and other tauopathies.

3. Inhibiting Tau Fibrillization

Given the evidence of tau inclusions contributing to neurodegeneration in the various tauopathies, there has been a longstanding interest in targeting the tau fibrillization process to inhibit formation of intracellular tau aggregates. Fibrillization of tau and other amyloidogenic proteins under well-defined but varying in vitro conditions appears to occur through a common mechanism consisting of a slow-to-develop nucleation (lag) phase followed by a rapid fibril growth (elongation) phase [108, 109]. Once formed, fibrils can be stained by thioflavine (Th) dyes, such as ThS or ThT, which recognize cross- β -fibril structures [110, 111]. Whereas the events that precipitate tau fibrillization in disease remain unclear, normal tau proteins without any posttranslational modifications, including partial proteolysis, can be made to readily fibrillize in vitro into ThS+/ThT+ structures in the presence of heparin or other anionic molecules [112, 113]. This feature has allowed

laboratories to assay a number of compounds for their ability to halt or interfere with this assembly process through disruption of protein-protein interactions.

Several small molecules have been identified that can inhibit tau fibrillization in vitro, including methylene blue (MB) [114], the cyanine dye, N744 [115], as well as a number of polyphenols, porphorins, rhodanines, anthraquinones, quinoxalines, pyrimidotriazines, and aminothienopyridazines (ATPZs) [116–121]. MB is a phenothiazine that has been used clinically for decades to treat malaria and methemoglobinemia, and recent studies have revealed that it prevents tau multimerization through a non-specific oxidation of cysteine residues within 4-R tau, resulting in a compacted tau structure that is refractory to fibrillization [118, 122]. Interestingly, MB may not inhibit 3-R tau fibrillization since it promotes the formation of an intermolecular disulfide-linked dimer that is fibrillization-competent [118]. MB has been demonstrated to reduce aggregated tau in organotypic slice cultures from a tg tauopathy mouse model [123], and has also been shown to inhibit tau aggregate formation in a preventative study in tau tg mice, although the drug did not work in an interventional study design [124]. Interestingly, MB was suggested to enhance protein degradation systems in this study [124], so it is possible that the in vivo benefits of MB do not rely entirely on inhibition of tau fibrillization. MB has progressed to clinical trials and a Phase II study suggested that MB (138 mg/day) halted disease progression in patients with moderate AD compared to placebo-treated controls [125]. However, higher doses were not efficacious and complications regarding absorption and drug stability were observed [125]. A related but distinct molecule, LMTX, has been described to possess improved absorption, tolerability, and bioavailability compared to MB [126] and has advanced to Phase III clinical trials in AD and FTD patients.

A variety of additional small molecules have been described as tau fibrillization inhibitors. For example, the cyanine dye N744 has been shown both to inhibit aggregation of 4-R tau in vitro and to disaggregate mature fibrils [115, 127]. However, this compound has also been found to form self-aggregates at high concentrations which enhance tau fibrillization [128], complicating its potential use as a therapeutic agent. A number of phenothiazines (including MB), as well as certain polyphenols and porphyrins, have also been shown to be capable of inhibiting heparin-induced tau filament formation [116]. All compounds which possessed inhibitory activity in tau aggregation assays in this study also inhibited the formation of A β fibrils [116]. This highlights the potential for a multi-targeted approach against both tau and A β fibrils, the key pathological features of AD, but also points to the lack of specificity of these compounds in disruption of protein-protein interactions. A screening assay identified a number of anthraquinones, including some clinically utilized anti-cancer agents, that were capable of both inhibiting aggregation of tau and dissolving pre-formed tau aggregates in cell-free systems [120]. Furthermore, select compounds decreased tau aggregation in a neuroblastoma cell-line (N2a) [120]. Similarly, rhodanine-based compounds were shown to inhibit the formation of tau fibrils and promote the disassembly of tau filaments in cell-free systems and in tau aggregate-bearing N2a cells [119]. However, rhodanines were determined to have a minor effect on tau-induced MT assembly [119], which could prove detrimental in cellular systems.

A high-throughput screen performed in our laboratory identified additional classes of compounds, including quinoxalines and pyrimidotriazines, which possessed inhibitory activity in a tau fibrillization assay [129]. A number of false-positive hits were identified, including many of the previously described tau aggregation inhibitors, as compound activity was found to be dependent on the generation of peroxides in the presence of the reducing agent DTT utilized in the assay [129]. A subsequent screening campaign led to identification of ATPZs as another compound class capable of inhibiting tau aggregation [117, 118]. ATPZs demonstrated tau specificity in that they were much more potent at inhibiting the formation of tau fibrils than A β fibrils [117]. However, the ATPZs were later found to prevent tau fibrillization by the same non-specific oxidative mechanism as MB [118].

In summary, significant challenges exist to the development of tau fibrillization inhibitors for clinical use. Most of the described inhibitors act via unknown mechanism(s) of action, and several are known promiscuous molecules with potential for off-target effects. Moreover, certain of these compounds appear to work through non-specific redox mechanisms [122, 129] that could potentially affect multiple cellular proteins. Indeed, pharmacological inhibition of protein-protein interaction has proved challenging over the years, particularly with regard to achieving required selectivity and potency [130]. However, the demonstration that MB has been used clinically for years without evidence of significant side effects suggests that compounds that affect tau fibrillization via non-specific oxidative mechanisms may be safely tolerated, and thus warrant further investigation as potential therapeutic agents for the treatment of AD and related tauopathies.

4. Improving Cellular Proteostasis

The ubiquitin-proteasome system (UPS) and autophagy-lysosome system (ALS) are two of the major pathways involved in maintaining cellular protein homeostasis and their lowered efficiency has been implicated in the pathophysiology of tauopathies. As summarized below, multiple literature reports suggest that improving the activities of the UPS and/or ALS to clear tau oligomers and aggregates may serve as a therapeutic strategy for the treatment of tauopathies.

Tau has been shown to be a client of the chaperone proteins that constitute the UPS [131–134] and is subject to degradation by the proteasome. Hsp90 inhibition was shown to increase the levels of Hsp70 and reduce levels of total and phosphorylated tau in cultured neurons and CHO cells expressing tau carrying the P301L mutation found in FTDP-17 [135]. Mice that overexpress inducible Hsp70 also show a significant decrease in both soluble and insoluble tau [136]. The effects of Hsp70 family proteins seem to be isoform specific, as Hsp72 can recruit carboxyl terminus of Hsp70 interacting protein (CHIP) in the presence of tau, thus enabling tau ubiquitination and subsequent degradation [137, 138]. Inhibition of Hsp90 via a small molecule inhibitor resulted in a decrease in phosphorylated tau in HeLa cells overexpressing tau and in a mouse model of tauopathy, likely due to an increase in the expression of CHIP [139]. CHIP-mediated ubiquitination of phosphorylated tau results in decreased cell death [140, 141] and deletion of CHIP results in the accumulation of hyperphosphorylated tau [142, 143]. Interestingly, the Hsp70 family member, Hsc70, interacts with tau with greater affinity than Hsp72 and stabilizes tau [137,

144], and expression of a dominant-negative variant of Hsc70 led to clearance of tau via the proteasome in HEK293T cells, as well as in brain tissue [145].

Proteasome function is also subject to the process of aging, as activity of brain proteasomes declines in aged and in AD brains [146], where activity of the proteasome is markedly decreased in regions where NFTs are abundant [147]. The latter finding is consistent with the observation that aggregated tau interacts with the proteasome and inhibits its function *in vitro* [148]. A more recent study found that delivering purified human proteasomes via silica nanoparticles to cells expressing inducible aggregated tau enhanced tau degradation, without affecting endogenous proteasome substrates [149].

Although the UPS may be the major pathway by which hyperphosphorylated or initially misfolded tau species are degraded, removal of tau aggregates such as NFTs by the UPS is unlikely given that the proteasome cannot physically accommodate large complexes. Thus, modulation of autophagy may be a preferred therapeutic strategy for more advanced tauopathy, particularly since it has been suggested that aggregated proteins may impair the function of the UPS [150]. In *Drosophila* expressing wild-type or mutant forms of tau, toxicity is alleviated by induction of autophagy by rapamycin [151]. Similarly, induction of autophagy in mouse models of AD has also resulted in decreased tau pathology [152]. In mice expressing P301S tau, treatment with the autophagy activator, trehalose, resulted in a reduction in insoluble tau and improved neuron survival in the cortex, whereas no improvement was observed in motor impairment due to lack of activation of autophagy in the spinal cord [153]. Ameliorative effects of activating autophagy with trehalose were also observed in a mouse model of tauopathy with parkinsonism [154] and in a neuronal model of tauopathy [155]. Treatment of P301S mice with rapamycin resulted in fewer cortical tau tangles, a reduction in tau hyperphosphorylation and lowering of insoluble tau in the forebrain, effects observed with preventive as well as late interventional administration of the drug [156]. Lithium can also enhance autophagy in addition to affecting GSK-3 β -mediated phosphorylation of tau, and its application to P301L mice resulted in reduced tau phosphorylation and an improvement in motor deficits [157], although the pleiotropic activity of lithium chloride make it difficult to discern the relative contributions of reduced GSK-3 activity versus improved autophagy in the improved outcomes.

Modulation of the UPS and autophagy as a therapeutic strategy for tauopathies is conceptually appealing. In fact, numerous Hsp90 inhibitors are in clinical trials as anti-cancer agents [158]. However, many of these candidate drugs are unable to cross the blood-brain barrier (BBB) and since they engage a large number of client proteins, there is a concern about their ability to specifically affect misfolded tau [135, 159]. Importantly, there is some evidence that such specificity might be achievable, as it appears that at least certain Hsp90 inhibitors show a higher binding affinity to Hsp90 that is in complex with misfolded tau [139]. Similarly, care will have to be taken in modulation of Hsp70 activity since different isoforms have differing effects on pathological tau [137]. Targeting autophagy also presents challenges, as it is a key cellular proteolytic system that is involved in the clearance of multiple proteins, as well as damaged organelles, and hence increasing autophagy may result in unwanted side effects. Also, components of the autophagy pathway are involved in other cellular functions. For example, rapamycin affects mammalian Target of Rapamycin

(mTOR), which plays a role in growth and metabolism [160], and the use of autophagy enhancers in a manner that is mTOR-dependent might result in untoward effects. Finally, although enhancement of autophagy has shown promise in models of AD and tauopathy, it has been suggested that autophagic protein degradation within lysosomes is impaired in AD [161, 162], and thus further initiation of autophagy may prove to be ineffective. In conclusion, although the evidence for beneficial effects after increasing cellular proteostasis in mouse models of tauopathy are compelling, there are still questions about the viability of these approaches for the treatment of human tauopathies.

5. Modulating Tau Expression

As noted in the introductory comments, tau-mediated neurodegeneration has been postulated to occur via both gain-of-function and/or loss-of-function toxicities. The belief by many that the former is the primary mechanism underlying neuronal dysfunction and death in tauopathies has led to the suggestion that a reduction of cellular tau levels could prove efficacious in the treatment of these diseases. This concept was borne largely from studies utilizing tau knockout mice, where beneficial effects have been observed when these mice are crossed with APP tg mice, as summarized briefly below. Moreover, the tau knockout mice are thought to be relatively normal and free of significant morbidities, although this view may be somewhat simplistic, as there is evidence of age-related deficiencies in these mice [163–166]. Furthermore, as with all constitutive knockout mice, there is the possibility that developmental compensatory changes mask the consequences of tau knockout, and that knockout in adulthood would result in a more severe phenotype.

Among the first studies to suggest that a reduction of tau levels may prove beneficial were those in which crossing of tau knockout mice with APP tg mice led to a reduction of behavioral deficits normally observed in the APP mice [167]. Notably, tau reduction did not alter A β plaque burden in the APP mice, suggesting that the effects of tau were not at the level of A β processing or deposition [167]. Moreover, tau reduction protected both wild-type and APP transgenic mice from excitotoxic insult [167]. Subsequent studies suggested tau involvement in mediating A β -induced changes in synaptic function [168], with tau reduction leading to lower epileptiform activity in APP transgenic mice [169] that may be linked to the Kv4.2 potassium channel [170]. More recently, it has been demonstrated that a reduction of endogenous tau using anti-sense oligonucleotides (ASO) delivered via intracerebroventricular infusion attenuated chemically-induced seizures in wild-type mice without other obvious detrimental cognitive effects after 1.5 months of treatment [171]. This study provides support for the concept that tau expression can be reduced in adult mice, at least for a period of time, without severe side effects and thus provides hope that a similar outcome might be observed in humans treated with tau ASOs or other agents that lower tau expression. With regard to ASO treatment for CNS disease, it is known that these agents do not readily cross the BBB, so administration would likely require direct intrathecal infusion to directly access the CSF from where the ASO could enter the brain. This methodology has worked relatively well in distributing ASOs to the brains of rhesus monkeys, although drug exposure appears to be greater in the spinal cord and cortical regions, with lesser activity in the midbrain and brainstem [172, 173]. Thus, the utilization of ASO technology to lower tau expression in tauopathies appears to be a tractable therapeutic approach, although there are

still uncertainties about the long-term safety of lowering tau and whether ASOs will distribute sufficiently throughout the regions of the brain susceptible to tau pathology.

6. Decreasing MT Dynamics

Neuronal tau normally binds to and stabilizes MTs and is believed to play an important role in the transport of critical cellular proteins and organelles along axons [174, 175]. In neurodegenerative tauopathies, hyperphosphorylated tau disengages from MTs and is sequestered into pathological aggregates, which may contribute to MT instability and increased dynamicity (i.e., greater degree of MT growth and disassembly), with associated axonal transport deficits [8, 21, 176]. In this regard, MT deficits have been observed in animal models of tauopathy [177–179] and there is evidence of MT alterations in the brains of AD patients [180, 181]. Moreover, tau that is isolated from AD brain shows a reduced ability to bind to MTs [17]. These observations suggest that MT-stabilizing agents, such as those used in the treatment of cancer, might compensate for axonal abnormalities that arise from tau dysfunction in tauopathies [42].

The first study to directly test whether a MT-stabilizing agent might prove beneficial in a tauopathy model was conducted in 2005, where paclitaxel was administered to tau tg mice that developed tau inclusions and neuronal loss primarily in the spinal cord and brainstem [177]. Treatment with weekly paclitaxel for 3 months resulted in improved fast axonal transport and neuron survival, in addition to mitigating tau pathology [177]. However, paclitaxel is not BBB permeable [43], and the observed improvements in this study were likely dependent upon uptake of drug at neuromuscular junctions with retrograde transport to affected motor neurons in this mouse model [177]. Relatively few MT-stabilizing drugs have been described to penetrate the BBB, although several examples of the epothilone class show excellent brain exposure [43], and doses of epothilone D (EpoD) that were ~100-fold lower than previously utilized in cancer trials were shown to be effective in reducing tau pathology and restoring MT content and function in multiple tau tg models with brain tauopathy [178, 179, 182]. In these studies, EpoD was found to be efficacious in both preventative and interventional settings, and EpoD subsequently progressed to clinical testing in mild AD patients. This Phase 1b trial demonstrated that low doses of EpoD could be safely tolerated, but no clinical benefit or improvement in CSF biomarkers were observed over the 9 weeks of the study. Recently, the taxane TPI-287 has progressed to Phase 1 clinical testing in moderate AD patients and, in a separate trial, in CBD and PSP patients (see clinicaltrials.gov). Although most taxanes do not cross the BBB, TPI-287 was shown to have good brain exposure [183]. There are no published reports of TPI-287 being tested in a tau tg mouse model, but the compound reduced brain colonization of metastatic breast cancer cells in mice [183].

In addition to the aforementioned small molecule MT-stabilizing drugs, the peptide NAP (davunetide) was reported to be a MT-stabilizing agent that reduced tau pathology and cognitive decline in 3xTg-AD mice [184, 185], which display both amyloid and tau pathology. Moreover, NAP improved axonal transport and synaptic function in a *Drosophila* model of tauopathy [186]. However, NAP had no effect on phosphorylated tau levels in the latter study [186]. NAP is a small peptide (NAPVSIPQ) derived from the activity-dependent

neuroprotective protein (ADNP) and it is believed to be the primary region responsible for the neuroprotective function of ADNP [187]. Interestingly, NAP has been reported to bind to tubulin and promote MT assembly [188, 189], but neuroprotection has been attributed to additional mechanisms, including interaction with inflammatory mediators and regulation of P53 [190]. Phase 1 clinical trials demonstrated no apparent side effects of intranasally administered davunetide and a subsequent Phase 2 clinical trial in patients diagnosed with amnesic mild cognitive impairment (aMCI) indicated significant improvement in cognitive testing in patients receiving davunetide for 8 or 16 weeks, but not at 12 weeks. However, a one-year Phase 2/3 trial in PSP patients failed to show improvement in the two primary efficacy measures [11], and at this time there are no reports of further clinical development of davunetide.

Challenges to the advancement of MT-stabilizers for the treatment of tauopathies include the toxicity associated with the anti-mitotic properties of these agents, as well as the lack of a good biomarker for MT target engagement in human patients. Whereas MT-stabilizing compounds have demonstrated impressive results in improving axonal function and reducing tau pathology in multiple tau tg models without notable side effects, the failure of davunetide in clinical testing raises concerns about the potential of this therapeutic strategy. However, davunetide appears to act by a number of mechanisms, and whether its primary activity in vivo relates to MT-stabilization is unknown. Furthermore, the failure of EpoD to improve clinical or biomarker outcomes in a Phase 1b study likely reflects the short 9 week trial period, as typical AD trials intended to demonstrate clinical efficacy are typically 18–24 months. In this regard, the ongoing trials with TPI-287 are also reported to be of 9 week duration. Important considerations in the development of additional MT-stabilizing drug candidates will be maximizing brain concentrations while minimizing peripheral exposure, so as to avoid toxicities related to the anti-mitotic activity of such molecules (e.g., neutropenia) and effects on peripheral nerves (i.e., peripheral neuropathy).

7. Tau Immunotherapy

As of this writing, arguably the most actively pursued strategy for reducing tau pathology in neurodegenerative disease tauopathies is immunotherapy, as evidenced by the growing literature on this topic as well as the number of early stage clinical programs [191]. The rapid ascendance of this approach is notable given the fact that, until quite recently, many researchers did not believe tau immunotherapy was likely to be a viable therapeutic tactic because the intracellular location of tau inclusions was thought to preclude accessibility to antibodies. As antibody concentration in the brain is generally only ~0.1% of that in blood, there were questions of whether effective intraneuronal antibody concentrations could be achieved in the brain, although there is some evidence that antibodies can be internalized by neurons [192, 193]. However, the concern of antibody access to the cytosol of affected neurons or glia has been mitigated by recent evidence demonstrating that the stereotypical pattern of pathology progression in AD and other neurodegenerative disorders likely results from a cell-to-cell transmission of pathologic protein species [194]. This prion-like spreading mechanism has been demonstrated in multiple tau transgenic mouse studies [195–200], and suggests that a pathologic form of extracellular tau may be released that is

accessible to antibodies within the brain interstitial fluid, thereby providing an increased impetus to investigate the potential of immunotherapy treatments for tauopathies.

The initial studies examining the potential of tau immunotherapy in vivo using active vaccination revealed that wild-type mice inoculated with recombinant tau had significant morbidities, including the formation of phosphorylated tau tangle-like accumulations in neurons, gliosis and evidence of axonal damage [201]. However, shortly thereafter a report [202] indicated that vaccination of JNPL3 tau tg mice with a phospho-peptide containing the epitope recognized by the PHF1 tau antibody resulted in a reduction of PHF1-positive tau in the brains of the vaccinated mice, as well as a reduction in misfolded tau as identified with the MC1 antibody. However, there did not appear to be an overall reduction of insoluble tau. A subsequent vaccination study [203] with a mixture of phospho-tau peptides reported a significant reduction in tau pathology without adverse events. Although these latter studies suggest that vaccination with tau peptides may be safely tolerated, a more recent study [204] indicated that repeated immunization with phosphorylated tau peptides induces neuroinflammation that manifested as a paralytic phenotype, suggesting that active tau vaccination programs should be closely monitored for adverse events.

More recently, nearly all studies on tau immunotherapy have focused on passive immunization with a variety of tau monoclonal antibodies (mAbs). Since 2010, there have been a large number of publications reporting on the effects of various tau mAbs in various mouse models of tauopathy. The reader is referred to recent reviews specifically directed to this literature for greater detail [191, 205]. In summary, most of the mAbs that were utilized in these studies targeted either phospho-tau epitopes believed to be increased within tau inclusions in tauopathies, or conformational epitopes found within misfolded tau. The strategy here is to target neoepitopes generated in the disease state while avoiding mAbs that bind to normal tau. Nearly all studies reported some degree of reduction in phospho-tau species, and typically some reduction of total insoluble tau was achieved, although in many instances the extent of this decrease was relatively modest. Taken together, the passive immunization studies provide a reasonably compelling case that tau immunization could be an attractive therapeutic strategy for tauopathies, and as a result, a number of pharmaceutical and biotechnology companies have active programs in this area [191], with both active and passive immunization strategies now under clinical investigation (Table 1). However, there are still uncertainties about what species/forms of pathological tau might actually be transmitted from cell-to-cell, so the selection of the preferred tau epitope(s) to target for the generation of potentially therapeutic mAbs is at this point empirical. However, focusing on the production of mAbs that target or preferentially recognize neoepitopes generated in the disease state has plausible appeal. Moreover, there have been reports that tau might be released in exosomes [206, 207], which would largely negate the premise that released tau is more accessible to antibodies than that within the neuronal cytosol. Nonetheless, there is a great deal of anticipation and hope in the AD and tauopathy communities that one or more these drug candidates will prove to be efficacious.

8. Conclusions

As summarized in the preceding sections, there are a number of therapeutic strategies under investigation directed toward reducing the contribution of tau pathology to neurodegenerative processes in AD and other tauopathies. These approaches are summarized in tabular form in Table 2, with a listing of perceived positive aspects of each tactic, as well as challenges. Arguably, the identification and validation of druggable targets that affect tau pathology has proven to be much more difficult than for the A β senile plaque pathology of AD. In the latter, the identification of the β - and γ -secretase enzymes involved in A β cleavage from the amyloid precursor protein led to the rapid initiation of drug discovery programs targeting these proteolytic systems, although there has yet to be clinical success with drugs directed to these enzymes. Thus, while there is evidence of tau proteolysis, it is still debatable as to how much such cleavage contributes to pathology in tauopathies. Furthermore, the large number of candidate proteases has resulted in uncertainty as to which might be the most important enzyme target. A similar uncertainty exists for the tau kinases, as a large number of enzymes have been implicated in the hyperphosphorylation of tau. In contrast, the identity of tau enzyme targets that regulate post-translational O-glycosylation and acetylation are generally well understood, but these modify a large number of proteins, raising questions of whether prolonged modulation of these enzymes will lead to side-effects. A similar concern can be raised regarding the modulation of targets linked to cellular proteostatic systems, such as those involved in proteasomal protein degradation or autophagy, where specificity of action may be difficult. It is likely that many of these concerns have led to pharmaceutical interest in non-enzymatic strategies, including reduction of tau expression and tau immunotherapy. As noted, tau anti-sense oligonucleotide approaches are being considered, and a large number of ongoing tau immunotherapy trials should soon provide initial indications of the merits of this strategy. In addition, there is still interest in the potential of brain-penetrant MT-stabilizing agents in compensating for axonal transport deficiencies that may occur in tauopathies, with TPI-287 presently in Phase 1 clinical testing. Finally, MB has been shown to affect tau pathology in several models and Phase 3 clinical trials are presently ongoing with the related molecule, LMTX.

Historically, one of the key limitations for all neurodegenerative diseases drug discovery programs has been an absence of pharmacodynamic markers of target engagement [208]. However, the emergence of reliable biomarkers to measure A β in CSF and its deposition in brain is rapidly changing how clinical trials of new A β -focused therapeutic trials are being conducted and they have ushered in an entirely new approach to clinical trials of A β therapies, i.e. prevention trials based on A β biomarker positivity in the absence of clinically manifest AD [208]. The good news is that promising biomarkers to monitor the burden of tau pathology also are available now [209, 210] to similarly facilitate clinical trials of therapies that target pathological tau. Hence, the design of how these trials are done should begin to change and improve very rapidly while also making possible the design and implementation of prevention trials for neurodegenerative tauopathies. Finally, another very new approach to AD therapy that is beginning to be discussed in the research and regulatory communities is the use combination therapy, which could also be considered for tauopathies [211]. For example, by combining a tau immunotherapy intervention with a MT stabilization

therapy, one could potentially address both toxic tau gain- and loss-of-function. Thus, although there are considerable challenges to developing tau-based therapeutics, the large number of strategies under active investigation provide hope that one or more efficacious drugs will be identified in the not too distant future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the NIA and NINDS for previous and continued funding, and the Marion S. Ware Alzheimer Disease Foundation and Woods Foundation for their support. We are also grateful for the philanthropic gifts from the Karen Cohen Segal and Christopher S. Segal Alzheimer Drug Discovery Initiative Fund, the Paula C. Schmerler Fund for Alzheimer's Research, the Barrist Neurodegenerative Disease Research Fund, the Eleanor Margaret Kurtz Endowed Fund, the Mary Rasmus Endowed Fund for Alzheimer's Research, and Mrs. Gloria J. Miller and Arthur Peck, M.D.

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Table 1

Current clinical programs utilizing tau-directed therapeutic agents.

Therapeutic Type	Name	Company	Clinical Stage
Vaccine	AADvac-1	Axon Neuroscience	Phase 1
Vaccine	ACI-35	AC Immune/Janssen	Phase 1
Antibody	BMS-986168	Bristol-Myers Squibb	Phase 1
Antibody	ABBV-8E12	AbbVie/C2N	Phase 1
Microtubule Stabilizer	TPI-287	Cortice Biosciences	Phase 1
Tau aggregate inhibitor	TRx0237	TauRx	Phase 3

Table 2

Summary of tau-directed therapeutic strategies with associated opportunities and challenges.

Target/Strategy	Opportunities	Challenges
Tau Kinases	Enzyme targets; Industry expertise; Clear disease relevance	Which kinase(s)?; Drug selectivity; On/off-target toxicities
O-GlcNacase (OGA)	Enzyme target; Prototype drug tested in mouse models	Further target validation; Multiple proteins modified by OGA
Tau Acetyltransferase(s)	Enzyme target; Increasing literature reports of relevance	Further target validation; Multiple proteins modified by acetyltransferase(s)
Tau Cleavage	Enzyme targets; Compelling literature	Which protease(s)?; Multiple proteins cleaved by candidate proteases
Tau Fibrillization	Clear disease relevance; Multiple reported inhibitors; LMTX in P3 testing	Difficult to inhibit protein-protein interactions; Many non-drug-like example inhibitors
Proteostasis	Clear disease relevance; Conceptually appealing	What is best target?; Can proteostatic systems be safely modulated?
Tau Expression	Amenable to ASO approaches; Intriguing in vivo data	Long-term safety of tau reduction; ASO brain distribution
Microtubule stabilizer	Reasonably compelling target validation; TPI-287 in P1 testing	Long-term safety
Tau Immunotherapy	Target validation; Industry expertise; Passive immunization generally safe	Best epitope(s)?; Sufficient antibody exposure in brain; Exosomal release of tau?