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Advances in tau-based drug discovery

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Abstract

Introduction—Tauopathies, including Alzheimer's disease (AD) and some frontotemporal dementias, are neurodegenerative diseases characterised by pathological lesions comprised of tau protein. There is currently a significant and urgent unmet need for disease-modifying therapies for these conditions and recently attention has turned to tau as a potential target for intervention.

Areas covered—Increasing evidence has highlighted pathways associated with tau-mediated neurodegeneration as important targets for drug development. Here, the authors review recently published papers in this area and summarise the genetic and pharmacological approaches that have shown efficacy in reducing tau-associated neurodegeneration. These include the use of agents to prevent abnormal tau processing and increase tau clearance, therapies targeting the immune system, and the manipulation of tau pre-mRNA to modify tau isoform expression.

Expert opinion—Several small molecule tau-based treatments are currently being assessed in clinical trials, the outcomes of which are eagerly awaited. Current evidence suggests that therapies targeting tau are likely, at least in part, to form the basis of an effective and safe treatment for Alzheimer's disease and related neurodegenerative disorders in which tau deposition is evident.

Keywords

aggregation; Alzheimer's disease; autophagy; chaperone; immunotherapy; phosphorylation; tau; tauopathy; therapeutic

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1. Introduction

Tauopathies are a family of neurodegenerative disorders including Alzheimer's disease (AD), frontotemporal dementia associated with Parkinsonism linked to tau mutations on chromosome 17 (FTDP-17T), Pick's disease, progressive supranuclear palsy, and corticobasal degeneration. While tauopathies display distinct pathological signatures and clinical symptoms, they are all characterised by the progressive development of intracellular inclusions of the microtubule-associated protein tau [1;2].

AD is the most common tauopathy, affecting approximately 20% of the population at the age of 80 [3]. Familial early onset AD accounts for less than 1% of all cases and is caused by mutations in presenilins (PS) 1 and 2 or the amyloid precursor protein (APP), leading to increased β -amyloid ($A\beta$) production [4]. There is no known genetic basis for the majority of AD cases, although several risk factors have been identified [5-7]. However, despite the fact that one of the characteristic features of AD is the presence of tau pathology, to date no mutations have been detected in the *MAPT* gene, which encodes tau, in AD. As a result, the role of tau abnormalities in neurodegenerative disease was largely overlooked for several years while attempts were made to characterise the mechanism by which $A\beta$ causes neurodegeneration.

Direct evidence that tau directly causes neurodegenerative disease came from the discovery of tau mutations in families with FTDP-17T [8]. These findings heralded a new generation of research directed at uncovering the mechanisms underlying tau-associated neuronal loss. To date, some 38 different mutations in the *MAPT* gene have been identified (Fig 1A); these are either exonic (missense, deletions or silent) or intronic mutations. Almost all of the mutations are present in the region of the *MAPT* gene that encodes the C-terminal half of tau, with clusters in and around the microtubule-binding domains, and in the splice-donor site following exon 10. Tau is alternatively-spliced and two major classes of isoforms result, each with either three (3R) or four (4R) microtubule-binding repeat domains (Fig. 1). Several of the tau mutations cause increased exon 10 retention, which results in increased production of 4R relative to 3R tau isoforms. The remaining mutations affect the protein sequence such that the mutant tau displays a decreased ability to bind to microtubules and/or an increased propensity to form filamentous aggregates (reviewed in [9]).

Increasing evidence indicates that tau plays a critical role in mediating neurodegeneration and cognitive decline in AD [10;11], perhaps by acting synergistically with $A\beta$ [12]. These findings, combined with evidence that amyloid deposition occurs years before clinical diagnosis and shows little correlation with subsequent cognitive decline [13] has ensured that identification of tau-based therapies has become a priority in the search for disease-modifying treatments for neurodegenerative tauopathies.

1.1. Tau biology and function

Tau is a predominantly neuronal protein, which under normal conditions is highly soluble. A total of six different isoforms of tau are expressed in the adult human CNS by alternative splicing of the *MAPT* gene (Fig 1B). Splicing of exons 2 and 3 results in the inclusion or exclusion of 0 (0N), 1 (1N) or 2 (2N) N-terminal inserts in the expressed tau protein. Splicing of exon 10 gives rise to 3R and 4R tau isoforms, which differ in their relative abilities to bind to microtubules. In normal human brain the ratio of 4R to 3R tau is approximately one, whereas in many tauopathies, this ratio is altered. Thus progressive supranuclear palsy and corticobasal degeneration exhibit over-expression of 4R tau isoforms, in contrast Pick's disease is characterised by an increase in 3R tau isoforms [1;14;15].

Tau interacts with microtubules through its C-terminal microtubule-binding domain, which helps to stabilise the cytoskeleton and regulate axonal transport [16]. This domain has also been reported to interact with a number of other proteins including PS-1 [17] and protein phosphatases [18], as well as RNA [19] and DNA [20]. The N-terminus of tau has been shown to interact with neuronal membranes [21;22] and the Src homology (SH)3 domain of plasma membrane-associated signalling proteins [23]. Furthermore, tau has recently been suggested to have distinct dendritic functions, acting to target the non-receptor tyrosine kinase Fyn post-synaptically, regulating N-methyl-D-aspartate (NMDA) receptor subunit 2 (NR2) phosphorylation and interactions between NMDA receptors and the post-synaptic density (PSD) protein PSD-95 [24].

Tau also contains a proline-rich domain harbouring many potential phosphorylatable residues. Both the phosphorylation status and isoform expression of tau are developmentally regulated, events believed to be related to the increased plasticity required during embryogenesis and early development. During embryonic development a single tau isoform, ON3R, is expressed and at this time relatively elevated tau phosphorylation is observed [14]. In contrast, all six tau isoforms are present in normal mature human brain, at which time the amount of phosphorylation detected is decreased [14;25].

In tauopathy brain, despite heterogeneity between and within various diseases, deposited tau is invariably hyperphosphorylated. The insoluble, paired helical filament (PHF)-derived tau extracted from AD brain, is highly phosphorylated, with at least half of all of the phosphorylatable residues being phosphorylated. This contrasts with the few phosphorylation sites identified in normal soluble tau (Figure 1B) [25]. An imbalance in the action of various protein kinases and phosphatases is believed to induce tau hyperphosphorylation in disease, and several candidate enzymes have been identified that likely contribute to these events, including proline-directed kinases glycogen synthase kinase-3 (GSK-3), and cyclin-dependent kinase 5 (cdk5), non-proline directed kinases, such as casein kinase 1 (CK1), microtubule affinity-regulating kinase (MARK) and cyclic AMP-dependent protein kinase (PKA), tyrosine kinases including Fyn, Abl, and Syk, and the protein phosphatases (PP) 1, 2A and 5 [1;14;26]. It remains to be established if the overall phosphorylation state of tau or phosphorylation at specific residues is important in disease pathogenesis; however, there is evidence that phosphorylation of individual residues can significantly impact tau function. For example, phosphorylation of serines 262 and 214 has the effect of removing tau from microtubules and rendering tau less susceptible to degradation [27].

In addition to abnormal phosphorylation, tau proteins in tauopathy brain can be modified by a number of other ways, including N- and C-terminal proteolytic cleavage, altered conformation, nitration, glycosylation, acetylation, glycation, ubiquitylation, aggregation and filament formation [14;28]. The relationship between these abnormal, disease-associated tau species and the importance of specific modifications for tau-associated neurodegeneration is the subject of continued research. Uncovering the mechanisms underlying aberrant tau processing in disease could therefore reveal novel therapeutic targets. For the purposes of this review, we will focus primarily on modifiers of tau phosphorylation and aggregation since these have received the most attention to date.

1.2 Tau biology and function in disease

Both loss and gain of toxic tau function are hypothesised to contribute to neurodegeneration. The arguments for each of these are summarised below.

1.2.1. Loss of function of tau protein in neurodegenerative diseases—The most well characterised roles of tau are those of stabilising microtubules and regulating axonal

transport. Increased tau phosphorylation such as occurs in neurodegenerative disease causes tau to detach from microtubules, which leads to the disassembly of microtubules and disruption in the structure of the neuronal cytoskeleton [29]. In addition, hyperphosphorylated tau encourages breakdown of the microtubule network by sequestering normal tau and other microtubule-associated proteins [30]. Deficits in axonal transport have also been linked directly to alterations in tau phosphorylation [31;32], another loss of function that might contribute to axonal degeneration and eventual neuronal death.

1.2.2 Toxic gain of function of tau protein—The aberrant processing of tau in disease is believed by many to cause the generation of a toxic tau species, although there are different opinions concerning which form of tau may be involved. Recent work indicates that pre-fibrillar tau, rather than the neurofibrillary tangles (NFTs) that characterise many tauopathies, might possess some inherent toxicity [33-36]. In addition, over-stabilisation of microtubules, due to increased production of 4R tau isoforms, could reduce the required plasticity of the microtubule cytoskeleton in some tauopathies, which may represent a detrimental gain of tau function [31]. Furthermore, reduced degradation or clearance of aggregated tau might contribute to a ‘clogging’ of microtubules and consequent disruption in axonal transport. Finally, recent work has demonstrated that the accumulation of hyperphosphorylated tau in intact dendritic spines results in a disruption of synaptic function through impaired trafficking and/or synaptic anchoring of glutamate receptors [37]. Thus, tau-mediated synaptic dysfunction may be one of the earliest events in the pathogenesis of tauopathies.

2. Tau-based therapeutic strategies

Here, we summarise the main strategies that have been proposed as approaches to ameliorate tau-associated neuronal death. These include inhibitors of tau phosphorylation, aggregation blockers, promoters of tau clearance, tau immunotherapies, and approaches targeting tau pre-mRNA to regulate tau splicing. We also discuss strategies designed to tackle loss of function of tau protein, such as microtubule-stabilising agents. Where information on the stage of clinical development of particular drugs/strategies is available, this has been stated.

2.1 Tau phosphorylation inhibitors

2.1.1 Tau kinase inhibitors—Of the various candidate tau kinases identified to date, GSK-3 has been the focus of the majority of inhibitor studies, and several classes of GSK-3 inhibitors have now been investigated including lithium chloride (LiCl) [38], indirubins [39], paullones [40], thiadiazolidinones (TDZDs) [41], maleimides [42], and thiazoles [43], all of which are described here.

LiCl inhibits GSK-3, amongst many other cellular targets. *LiCl* has a potency of approximately 3.3mM K_i against GSK-3 and it competes with Mg^{2+} ions, rather than with ATP, resulting in disrupted catalytic function [44]. Many proof of concept studies in rodent models of neurodegeneration have reported that GSK-3 inhibition by *LiCl* serves both to reduce tau phosphorylation and tau-associated neurodegeneration [45-47]. Interestingly, *LiCl* treatment also reduces $A\beta$ production, both in cells and in a mouse model of AD [48], suggesting that this compound might have the capacity to target both tau and amyloid pathologies in AD. Despite these promising findings, it is unlikely that *LiCl* will be considered for treating tauopathy patients since, not only does it have a diverse range of actions, but also it is not well tolerated at therapeutic doses in the elderly. Indeed, the results of a small clinical trial of Alzheimer patients treated with lithium for ten weeks resulted in no cognitive improvement after treatment [49], although the extent of target engagement, GSK-3 inhibition, in this study was not determined.

Indirubin compounds can cross the blood brain barrier (BBB), are ATP competitive and interact with the ATP binding site to inhibit both GSK-3 and some cdk5 [39]. Indirubin-3-monoxime effectively reduces phosphorylation of human tau at several epitopes in an insect cell line (Sf9 cells) [39]. A related compound, 6-bromo-indirubin-3'-oxime (6-BIO) also reduces tau phosphorylation in cultured rodent cortical neurons, however this was associated with a reduction in the amount of total tau in these cells [51].

Paullones, a family of benzazepinones, includes the ATP competitive inhibitor alsterpaullone. The efficacy of alsterpaullone is 10-fold higher for GSK-3 than most cdk5 [52] and its administration reduces endogenous tau phosphorylation at putative GSK3 sites in the hippocampus of postnatal rats [50]. This compound also effectively and dose-dependently reduces tau toxicity in human tau-expressing drosophila [53]. The structure of alsterpaullone suggests it is likely to be BBB permeable and have good tissue retention [52].

Thiadiazolidinones (TDZDs) are not ATP competitive, and derivatives such as TDZD-8 (Noscira) show high levels of selectivity for GSK-3 with an IC₅₀ in the μ M range [54]. In a cell model of Parkinson's disease, TDZD-8 reduced GSK-3 activity, tau phosphorylation and the associated neurotoxicity [55], indicating the therapeutic potential of this compound. Furthermore, in a mouse model of tauopathy a second generation TDZD with an improved absorption, distribution, metabolism and excretion profile, NP031112 (Noscira), reduced somato-dendritic accumulation of phosphorylated tau, decreased A β deposition and glial infiltration, and significantly ameliorated neuronal death in a time- and dose-dependent manner [56]. NP031112 (Noscira) is currently in clinical trials for AD [54].

Maleimides of two major classes, bis-arylmaleimides and anilino-arylmaleimides, have been reported to be potent GSK-3 inhibitors. Of the bis-arylmaleimides, SB-216763, and SB-415286 (GlaxoSmithKline) show high selectivity for GSK3 against a panel of kinases [52]. SB-216763 reduces tau phosphorylation in postnatal rats [50], and reverses A β -induced tau phosphorylation, caspase-3 activation and nuclear DNA fragmentation [57]. However, it is worth noting that in the latter study, SB-216763 did not reverse either A β -induced gliosis or cognitive dysfunction.

Thiazoles, of which AR-A014418 (AstraZeneca), a nitrothiazole urea, is perhaps the most well-known example, compete with ATP for binding to GSK-3. Unlike most GSK-3 inhibitors, these compounds are highly selective for GSK-3 when compared to a range of other kinases [43]. AR-A014418 reduces tau phosphorylation and aggregation in a mouse model of tauopathy [47], and increases tau exon 10 inclusion in cultured neurons [58]. Thus, AR-A014418 may reduce the abnormal GSK-3-directed phosphorylation of tau, as well as having an additional impact in tauopathies where alternative splicing is dysregulated. AZD1080, a compound structurally related to AR-A014418, entered Phase I clinical trials for AD in 2007, however this programme has since been discontinued [59].

Several other classes of compounds that inhibit GSK-3 have been reported including 3-aminopyrazinyl-2-carboxamides and oxindolequinazoline, both of which are ATP-competitive, and show some selectivity for GSK-3 [52]. The major problem with developing inhibitors of GSK-3 β for the chronic treatment of Alzheimer's Disease is that this kinase plays a key role in multiple biological pathways and thus avoiding the on-target toxicity associated with GSK3 inhibition, even with highly selective compounds, remains a significant challenge.

In addition to selective GSK-3 inhibitors there is some momentum for investigating non-selective kinase inhibitors since it is possible that reducing the overall level of tau phosphorylation may be beneficial in tauopathies. This approach has the advantage that relatively moderate suppression of kinase activity might also lead to a reduction in the on

and off-target side-effects associated with kinase inhibitors that produce more complete suppression of a specific kinase. In support of this view, treatment of tau transgenic mice with SRN-003-556 (Sirenade Pharmaceuticals), a synthetic agent that inhibits GSK-3, extracellular-regulated kinase 2, cdk 1, PKA and protein kinase C with approximately equal efficacies, reduces the amount of hyperphosphorylated tau in these mice. Importantly, the severe motor deficits typical of these animals also improved [60]. However, since cross-talk between several tau kinases has been reported [61], 'broad-range' kinase inhibitors will still require careful screening to determine not only the precise kinases targeted, but also the net impact on overall tau phosphorylation.

2.1.2. Tau phosphatases—In addition to tau kinases, imbalances in protein phosphatase activity could be responsible for the aberrantly high levels of tau phosphorylation observed in many neurodegenerative diseases. A prominent role for protein phosphatases in the regulation of tau phosphorylation *in vivo* was demonstrated by Gong et al. [62] who demonstrated reduced PP1 and PP2A activities in AD brain, and more recently by Planel et al. [63] who showed that inhibition of PP2A overrides inhibition of cdk5 and GSK-3 in starved mice.

Current strategies to target dysregulation of protein phosphatase activity focus on the use of agents that restore PP2A activity, which would decrease tau phosphorylation. For example, memantine, an NMDA receptor antagonist used clinically in the treatment of AD, was reported to elevate hippocampal PP2A activity and decrease tau phosphorylation both in cells and in rat brain slices [64]. Thus, cell and BBB permeable memantine-based derivatives may have some utility for the treatment of tau-associated neurodegeneration. An alternative strategy to increase PP2A activity is to inhibit its demethylation. Signum Biosciences in collaboration with Princeton University have identified at least one compound, SIG-1012, that blocks PP2A methyltransferase (PPME) activity and thus efficiently increases PP2A methylation and activity in hippocampal neurons. Administration of SIG-1012 has been shown to lower tau phosphorylation in rats [65].

2.2 Tau disaggregating agents

The accumulation of neurofibrillary pathology in neurons is associated with cognitive decline and neurodegeneration [66]. Several classes of compounds, including those identified in recent screens [67-70], have now been identified that inhibit tau aggregation and/or disassemble existing filaments *in vitro*. These include several chemical classes including phenothiazines, cyanine dyes, aminothienopyridazines (ATPZs), anthraquinones, phenylthiazolyl-hydrazides (PTH), and rhodanines.

Phenothiazines, include methylene blue (Rember™), an inhibitory scaffold that supports tau aggregation antagonist activity. Rember™ (TauRx) has been reported to attenuate the rate of cognitive decline in AD patients over approximately one year in one Phase 2 study [71]. Moreover, this compound was recently reported to rescue cognitive deficits and block neurodegeneration in the conditional rTg4510 mouse model of tauopathy [72]. TauRx are presently seeking investment to fund a Phase 3 study.

Cyanine dyes include N744, which when used at low concentration reduces tau aggregation in organotypic slice cultures prepared from tau transgenic mice [73;74]. Sub-stoichiometric concentrations of N744 have also been shown to disaggregate pre-formed tau filaments in cultured neurons [74;75].

Aminothienopyridazines (ATPZs) were first identified in a screen of 292,000 small molecules designed to identify drug-like agents that inhibit tau assembly [67]. Subsequent development of ATPZ analogues revealed several compounds which, at a 1:1 molar ratio

with tau, effectively prevented tau fibrillization when used in a heparin-induced tau fibrillation assay in which truncated tau with the P301L mutation was the substrate [76]. Importantly, the majority of ATPZ analogues tested showed a high blood to plasma ratio when administered to mice intraperitoneally, indicating good blood-brain-barrier permeability [76]. Furthermore, efficacious brain concentrations of several ATPZ compounds were observed following their oral administration to mice, indicating the good oral bioavailability of these agents [76]. Testing of these compounds in a mouse model of tauopathy will reveal important information concerning their potential as therapeutic agents for the treatment of AD and related tauopathies.

Anthraquinones, including the cancer treatments daunorubicin and adriamycin, were identified by Pickhardt et al. [77] as inhibitors of tau aggregation. Subsequent cell-based studies revealed some differences in the affinity of different class members for 3R and 4R tau, tau fragments and the holoprotein [77]. Several compounds in this class inhibit PHF formation and reduce cell death in neuroblastoma cells inducibly expressing tau, with the majority having IC50s in the low μM range. Furthermore, the anthraquinone emodin significantly reduces tau aggregate load in neuroblastoma cells expressing FTDP-17T mutant tau [78;79].

Phenylthiazolyl-hydrazides are another group of compounds first identified by the Mandelkow group following a screen of 200,000 compounds [69]. The most active compound, Bsc2463 and several derivatives, disaggregate tau and reduce cell death in a cellular model, with IC50 values in the μM range. Further development of these compounds is now required to determine if their solubility and BBB permeability is sufficient to warrant further clinical investigation.

Rhodanines include epalrestat, an aldose reductase inhibitor shown to be well tolerated by patients when used long-term [80]. Up to 70% inhibition of tau aggregation, low toxicity and an ability to disassemble pre-formed tau fibrils have been reported with selected rhodanine-based compounds in cells [81]. Further investigation of this class of compounds is also required to determine if they have the characteristics necessary for clinical use.

2.3 Promotion of tau degradation and clearance

In addition to reducing the abnormal processing of tau, several alternative approaches to reduce tau toxicity are currently in development, where the focus is on increasing the proteolysis or cellular clearance of abnormal tau species. The two main strategies in this area are elevating the phosphorylation-dependent proteosomal degradation of tau by molecular chaperones including heat-shock protein (Hsp) 90, and enhancing tau aggregate degradation by macroautophagy.

2.3.1 Enhancing the degradation of phosphorylated tau by Hsp90—Molecular chaperones such as Hsp70 and Hsp90 modulate the cellular trafficking and function of client proteins such as tau, as well as assisting client protein folding by reversibly binding to unfolded and misfolded proteins to prevent their aggregation. Hsps commonly form large complexes with co-chaperones such as CHIP, an E3 ubiquitin ligase, and these are often organised by Hsp90 [82]. Inhibition of Hsp90 ATPase function was thus hypothesised to prevent the misfolding of tau being rectified by chaperones, leading to it being ubiquitinated and targeted for subsequent degradation by the proteasome.

Several studies have now demonstrated the validity of such an approach. Dickey et al. [83] identified a number of low molecular weight compounds that inhibit Hsp90 ATPase activity (Biogen Idec) and these enhance the proteosomal degradation of tau. In particular, many of these compounds selectively reduce the amount of tau phosphorylated at specific serine and

threonine residues or tau present in specific pathological conformations when used in CHO cells expressing human tau. Furthermore, inhibition of Hsp90 activity with the brain-permeable inhibitor EC102 reduces the burden of phosphorylated tau in affected brain regions of transgenic mice expressing the entire wild-type human tau gene [84]. Similar findings have been reported with the geldanamycin derivative 17-(allylamino)-17-demethoxygeldanamycin (17AAG) in mice expressing mutant human tau [85]. Interestingly, 17AAG shows preferential affinity for complexes associated with misfolded proteins suggesting that 17AAG may not interfere with other physiologically important Hsp90-client protein interactions [29].

2.3.2 Promoting tau clearance by macroautophagy—Tau can be degraded both by the proteasome and by the macroautophagy-lysosomal system. The proteasomal system prevents the entry of large oligomers or aggregated proteins, cleaving only single proteins for steric reasons, whilst protein aggregates can be cleared by the autophagosome. The induction of macroautophagy with rapamycin is reported to reduce the accumulation of insoluble tau and exert neuroprotective effects in flies expressing wild-type and mutant human tau [86], although these findings have yet to be substantiated. These findings indicate that promoting macroautophagy may have utility for the treatment of tauopathies.

2.4 Tau immunotherapy

Vaccination approaches for the treatment of tauopathies have gained much attention recently since these allow the opportunity to remove specific forms of pathological proteins. Indeed, active immunization with a synthetic phosphorylated tau peptide, corresponding to the PHF1 epitope, which is present on NFTs, reduces tau aggregation and slows the development of NFT development in mice expressing P301L tau [87]. The reduced development of tau pathology observed following immunisation was associated with improved behavioural phenotypes. More recently, when mice co-expressing two FTDP-17T tau mutants were immunised with three distinct phosphorylated tau peptides, significant reductions in NFT burden were reported in the absence of marked neuroinflammatory responses [88]. Interestingly, in the latter work, there was an increase in the amounts of lysosomal proteases suggesting the possible involvement of the lysosomal-autophagy pathway in the clearance of tau following immunisation. Thus, tau immunotherapy may represent a novel therapeutic approach for the treatment of tauopathies. It is worth noting, however, that immunisation of wild-type mice with unphosphorylated full-length recombinant human tau protein was reported to result in neuroinflammation, the development of NFTs and axonal degeneration [88].

2.5 Microtubule-stabilising agents

To address the loss of tau in relation to its microtubule-stabilising function in disease, several approaches to reduce microtubule depolymerisation have now been investigated.

Paclitaxel and related taxol derivatives were the first microtubule-stabilising agents to be tested in mouse models of tauopathy. Zhang et al., [89] reported that a three month treatment with paclitaxel reverses fast axonal transport deficits, increases microtubule density and improves locomotor function in transgenic mice over-expressing wild-type (0N3R) human tau. However, paclitaxel has limited brain bioavailability, and furthermore, peripheral neuropathy has been reported following its administration to cancer patients [90].

A synthetic neuronal tubulin-binding peptide, NAPVSIPQ (NAP), which reaches therapeutic concentrations in the CNS when administered intranasally [91], has been investigated in several different transgenic mouse lines which recapitulate various aspects of human tauopathies. NAP administration to mice over-expressing three mutant transgenes, human

APP, PS and tau (3 x Tg-AD), and to mice over-expressing mutant human tau alone, reduces the development of tau pathology in association with improved microtubule stability [91-93], and cognitive function [93]. Following good safety and tolerability profiling in Phase I testing, NAPVSIPQ has now entered clinical trials for elderly patients with mild cognitive impairment (Allon Therapeutics Inc) [94].

Finally, Brunden et al. [95] have recently reported that administration of Epopthilone D to tau transgenic mice results in improvements in CNS axon integrity, increased microtubule content, and an amelioration of memory impairments. However, no significant impact on tau phosphorylation was observed, suggesting that any neurotoxic effects of tau other than those related to its interactions with microtubules may not be targeted by this compound.

2.6 Modulators of tau pre-mRNA splicing

Elevated 4R tau expression in several of the tauopathies is linked to disease pathogenesis. This could be due to over-stabilisation of microtubules, perturbation of axonal transport, or promotion of tau aggregation. Therefore, correcting aberrant production of 4R tau isoforms may represent another disease-modifying approach for the 4R-dominant tauopathies.

In rat PC12 cells that express predominantly 4R tau, blocking access of the splicing machinery to tau pre-mRNA using antisense oligoribonucleotides that target the 5' or 3' splice junction of E10, results in a relative reduction of 4R tau expression [96], although the problem of how such oligonucleotides would be delivered to the brain still needs to be resolved. Another promising strategy is to reprogramme tau mRNA using spliceosome-mediated RNA *trans*-splicing technology (SMaRT™). In a proof of concept study, tau pre-mRNA was modified in cells using SMaRT, in this case to increase the conversion of 3R tau to 4R tau encoding RNA with approximately 30% efficiency [97]. If appropriate delivery methods can be developed it is conceivable that *trans*-splicing strategies could be used to efficiently correct aberrant tau E10 splicing, and hence the 4R/3R tau protein ratio, *in vivo*. Furthermore, this technology could be extended further to repair FTDP-17T mutations at the RNA level by downregulating expression of the mutant tau while simultaneously increasing expression of the corrected tau transcript.

3. Conclusion

A number of tau-based strategies have shown promise as potential disease-modifying therapies for neurodegenerative tauopathies. Indeed, the variety of different approaches allows a comprehensive coverage of potential tau abnormalities in disease, targeting the over-expression of specific tau isoforms and mutations, abnormal tau phosphorylation and aggregation, improving tau clearance and correcting tau-induced microtubule instability (Fig. 2).

Many of the studies summarised here are pre-clinical, using cell and/or animal models of tauopathy, however several small molecules have now reached the early stages of clinical development. These include the GSK-3 inhibitor NP031112 (Noscira), the tau aggregation inhibitor Rember (TauRx) and the microtubule stabilizing peptide NAPVSIPQ (Allon Therapeutics Inc.). Should good safety and tolerability data be forthcoming, the efficacy of at least some of these compounds in modifying the course of AD will be known in due course.

Importantly, many of these reports have indicated a clear and strong association between the amelioration of tau pathology, improved cognitive function and reduced neuronal loss. This data cements the hypothesis that tau plays a central role in mediating neurodegeneration in

tauopathies such as AD, and strengthens the case for investigating tau-based therapeutic strategies in neurodegenerative disease drug discovery programmes.

4. Expert opinion

Until recently, the search for disease-modifying therapies for AD has concentrated upon means to reduce the accumulation of A β and amyloid in the CNS by inhibiting amyloidogenic processing of APP to reduce A β production, or by increasing A β clearance from the CNS using immunotherapy. Early developmental clinical trials of active immunotherapy to remove A β showed some promise in that A β deposits were reduced following A β vaccination, however, in the clinical trials reported to date severe inflammatory problems ensued in a proportion of patients, thus halting the trials prematurely [98]. Passive A β immunotherapy appears to reduce many of the adverse side effects reported in active vaccination programmes, and a number of these have now entered Phase III clinical trials [99].

However, it is not clear if lowering CNS amounts of A β will be sufficient to modify neurodegeneration and/or cognitive decline in AD. Instead, increasing evidence suggests that tau is a key mediator of A β toxicity [12], and thus therapies targeting tau are perhaps more likely to modify the course of AD rather than those targeting A β alone. In support of this idea, reductions of both soluble A β and tau, but not A β alone, are required to ameliorate cognitive decline in the 3 x Tg-AD mouse model of AD [100]. In addition, reducing endogenous tau amounts prevents behavioural deficits and improves survival in transgenic models of AD over-expressing mutant human APP [10;24].

Thus, reducing tau-associated neurodegenerative events is a key area for therapeutic intervention. As described above, a variety of strategies have now been employed in laboratory studies to reduce or prevent tau-mediated neurodegeneration, with some of these having now entered clinical trials. However, the clinical development of kinase inhibitors has been plagued by problems with on and off-target toxicity, whereas many tau aggregation blockers possess poor brain penetrance [101]. Another consideration with molecules that disaggregate tau is the potential for increasing cellular amounts of potentially neurotoxic pre-aggregate tau oligomers [101]. Disaggregating agents may also suffer from a lack of specificity, since many of these target particular protein structures (e.g. β sheets) rather than being specifically directed at disease-related proteins of interest. In addition, microtubule-stabilising agents often show a lack of impact on the development of tau pathology in animal models [95], thus any additional non-microtubule-associated and pathogenic influence of disease-associated tau species may not be addressed with these compounds.

4.1 Clinical translation of tau-based therapies

Biomarkers are increasingly used to follow the progression of AD, and in some cases to support early diagnosis of the disease [102;103]. However, to accelerate the clinical translation of tau-based therapies, it is essential that sensitive and specific biomarkers are available to allow the measurement of drug interactions with targets, and the impact of treatment on downstream pathophysiology. The development of such biomarkers, and their use in validated animal models, will allow a faster selection of candidate treatments, and appropriate dose ranges, for clinical development.

A large number of studies have reported elevated total and/or phosphorylated tau in CSF from tauopathy patients (reviewed in [104]). Indeed, there are highly significant correlations between CSF phospho-tau amounts and hippocampal atrophy in patients with mild cognitive impairment [105], suggesting that increased amounts of phosphorylated tau in tauopathy patients may be a reliable biomarker of neurodegeneration. However the relationship

between CSF tau amounts and the accumulation of potentially pathogenic tau species in the CNS is rather unclear, and therefore the utility of CSF tau measurements to assess the efficacy of tau-based therapies has not yet been established.

CNS tau deposits can be detected directly using PET imaging biomarkers, such as FDDNP [106], to allow direct assessment of the disease-modifying capacity of potential tau-based treatments. However, FDDNP and related agents are not specific to tau, also detecting A β deposits. The detection of neuroinflammatory responses by PET are also considered a reliable, though not specific, biomarker of disease progression in many neurodegenerative diseases. Interestingly, PET was recently used to show glial infiltration in pre-symptomatic FTDP-17T patients [107], suggesting that neuroinflammatory biomarkers may have utility for tracking the therapeutic efficacy of drugs that target tau.

There is also a lack of reliable target engagement tauopathy biomarkers that allow assessment of the interaction between drugs and their target protein. For example, biomarkers are required that allow measurement of interactions between tau aggregation blockers and polymerised tau. Rojo et al. [108] have recently reported that the benzimidazole derivatives, lanzoprazole and astemizole, selectively detect pathological oligomers of tau. The development of these compounds as PET biomarkers will have great utility as a target engagement and/or mechanism biomarker for strategies targeting oligomeric tau.

4.2 Further considerations for clinical trials using tau-based therapies

When considering the impact of recent clinical trials in AD, it is important to consider that the development of abnormal tau species and aberrant A β production in AD likely precedes the onset of dementia by up to a decade [109]. Thus, by the time a clinical diagnosis has been made, patients are in the mid-late stages of disease progression, at a time when the synapto- and neuro- toxic events downstream of A β and/or tau are already well established. Therefore, while they may be effective when used during the early stages of disease, the effectiveness of strategies targeting tau or A β may be only moderately, if at all, effective once AD has progressed to a stage where dementia is apparent. Indeed, biomarker trajectories suggest that anti-A β therapies will be most useful when used in pre-dementia patients [110], and it is possible that some of the tau-based therapies discussed here may also be most effective in prodromal patients. Thus, ideally, clinical trials for tau modifying agents would recruit both patients with AD, and those with mild cognitive impairment, in order that the disease-modifying impact and/or neuroprotection afforded by novel tau-based therapies can be observed at mid-late disease stage, as well as during stages of disease where neurodegenerative events are likely to be considerably more tractable.

In addition, there may be a need to stratify patients into sub-groups, since the abundance of tau, A β , TDP-43 and synuclein pathology varies considerably across cases [111], and the development of such AD pathology is modified by many factors including ApoE genotype and the presence of cerebrovascular disease [112]. Indeed, one recent study suggests that, on the basis of CSF biomarkers, five subgroups of sporadic AD can be identified, including one in which no increase in the level of CSF tau is found [113]. Such stratification of cases may aid in the interpretation of results from clinical trials using novel tau-based disease-modifying strategies.

Finally, the main focus of many tau-based drug discovery programmes is AD, however tau-based therapy proof of concept studies in rarer tauopathies such as PSP may be extremely valuable. There is no co-existing pathology in PSP to confound the interpretation of results, and the disease progresses rapidly allowing the potential for a greater range of treatment effect than would be observed in many other tauopathies. Several new disease-modifying

approaches are in Phase II/III clinical trials for PSP, including kinase inhibitors, coenzyme Q10, and inhibitor of mitochondrial complex 1 [114].

Article highlights box

- There is a significant and urgent unmet need to develop disease-modifying treatments for Alzheimer's disease and related tauopathies
- Increasing evidence indicates that the microtubule-associated protein tau plays a central role in the pathogenesis of neurodegenerative tauopathies.
- Tau abnormalities in disease may mediate neuronal loss by a loss of essential function (microtubule stabilisation, axonal transport, post-synaptic receptor localisation), or by a toxic gain of function (production of neurotoxic tau species, over-stabilisation of microtubules, impaired glutamate receptor trafficking).
- Several approaches to reduce tau-associated neurodegeneration are under investigation including targeting tau pre-mRNA to maintain an appropriate balance of 3R to 4R tau isoforms, reducing abnormal tau phosphorylation and aggregation, promoting tau clearance, and improving microtubule stability.
- On the basis of successful animal studies, a synthetic peptide that stabilises microtubules, and treatments that block PP2A methylation and inhibit GSK-3 activity to reduce pathological tau phosphorylation have entered clinical trials.
- Novel functions of tau are still being elucidated, for example the role of tau at dendritic synapses, and future drug discovery efforts may focus on disease-associated abnormalities that are related to these alternative functions of tau.

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8. Abbreviations

Aβ	beta-amyloid
AD	Alzheimer's disease
ADME	absorption, distribution, metabolism and excretion
APP	amyloid precursor protein
ATP	adenosine triphosphate
ATPZ	aminothienopyridazines
BBB	blood brain barrier
Cdk	cyclin-dependent kinase
CHIP	C terminus of Hsc70-interacting protein
CHO	Chinese hamster ovary
CK	casein kinase
CNS	central nervous system
CSF	cerebrospinal fluid

Dyrk	dual specificity tyrosine phosphorylation regulated kinase
fMRI	functional magnetic resonance imaging
FTDP-17T	Frontotemporal dementia with Parkinsonism associated with tau mutations on chromosome 17
GSK-3	glycogen synthase kinase-3
Hsp	heat shock protein
LiCl	lithium chloride
MAPT	microtubule-associated protein tau
MARK	microtubule-associated protein (MAP)-microtubule-affinity regulating kinase
MRI	magnetic resonance imaging
NFT	neurofibrillary tangle
NMDA	N-methyl-D-aspartate
NR	NMDA receptor
PET	positron electron tomography
PHF	paired helical filament
PKA	cyclic AMP-dependent protein kinase
PP	protein phosphatase
PS	presenilin
PSD	post-synaptic density
PTH	phenylthiazolyl-hydrazides
SH	Src homology
TDZD	thiadiazolidinone

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Figure 1.

(A) The human tau gene showing exons –1 to 14. Exons 1, 4, 5, 7, 9, 11, 12 and 13 are translated in all tau isoforms. Exons 2, 3 and 10 are alternatively spliced to generate six tau isoforms in the human CNS. The 38 known exonic and intronic FTDP-17T tau mutations are shown. (B) The six isoforms of human CNS tau, showing the regulated alternative splicing of exons 2, 3, and 10. Absence of N1 and N2 gives rise to 0N tau, inclusion of N1 produces 1N, and inclusion of both N1 and N2 generates 2N tau isoforms. R1-R4 represent the four imperfect repeat microtubule-binding domains, R2 being encoded by exon 10. Isoforms lacking R2 produce 3R tau and inclusion of R2 results in 4R tau isoforms. The phosphorylation sites on tau identified in Alzheimer brain are indicated below the 2N4R tau isoform. S, serine; T, threonine; Y, tyrosine.

Figure 2.

Illustration of the various tau-based therapeutic strategies currently under investigation. Modifying tau alternative splicing can be achieved by targeting tau pre-mRNA, phosphorylation and aggregation can be reduced by the use of immunotherapy, kinase inhibitors, PP2A inducers and disaggregating agents, tau clearance and degradation can be enhanced by targeting the proteasome and lysosomal-autophagy machinery, and loss of the microtubule binding function of tau can be corrected by microtubule-stabilising agents.