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New perspectives on the role of tau in Alzheimer's disease. Implications for therapy[☆]

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ABSTRACT

Alzheimer's disease (AD) and related dementias constitute a major public health issue due to an increasingly aged population as a consequence of generally improved medical care and demographic changes. Current drug treatment of AD, the most prevalent dementia, with cholinesterase inhibitors or NMDA antagonists have demonstrated very modest, symptomatic efficacy, leaving an unmet medical need for new, more effective therapies. While drug development efforts in the last two decades have primarily focused on the amyloid cascade hypothesis, so far with disappointing results, tau-based strategies have received little attention until recently despite that the presence of extensive tau pathology is central to the disease. The discovery of mutations within the tau gene that cause fronto-temporal dementia demonstrated that tau dysfunction, in the absence of amyloid pathology, was sufficient to cause neuronal loss and clinical dementia. Abnormal levels and hyperphosphorylation of tau protein have been reported to be the underlying cause of a group of neurodegenerative disorders collectively known as 'tauopathies'. The detrimental consequence is the loss of affinity between this protein and the microtubules, increased production of fibrillary aggregates and the accumulation of insoluble intracellular neurofibrillary tangles. However, it has become clear in recent years that tau is not only a microtubule interacting protein, but rather has additional roles in cellular processes. This review focuses on emerging therapeutic strategies aimed at treating the underlying causes of the tau pathology in tauopathies and AD, including some novel approaches on the verge of providing new treatment paradigms within the coming years.

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1. Introduction

Tau protein is a microtubule-associated protein (MAP) that under physiological conditions regulates microtubules (MT) assembly, dynamic behavior, and spatial organization [1,2]. More recently, tau has been shown to regulate the axonal transport of organelles, including mitochondria [3]. Tau is encoded by a single

gene from which six major isoforms are expressed in adult brain through alternative splicing [4,5]. The interaction between tau and tubulin is mediated by four imperfect repeat domains (encompassing 31–32 residues) encoded by exons 9–12 [6]. Alternative splicing of exon 10 results in the production of isoforms containing 3 or 4 binding domains (3R and 4R tau) [5]. Adult human brain contains equal amounts of 3R and 4R isoforms whereas foetal brain, however, only expresses 3R tau, demonstrating developmental regulation of exon 10 splicing [7]. Different brain regions also differ in the relative levels of 3R and 4R isoforms with granule cells in the hippocampal formation reported to have only 3R tau [5].

Within neurons, tau is predominantly found in axons [8] as a highly soluble phosphoprotein [9]. Phosphorylation is also developmentally regulated, with a high tau phosphorylation level during embryogenesis and early development, when only the shortest of the isoforms is being expressed. By contrast, adult brain expresses all six isoforms with relatively reduced phosphorylation levels compared with the foetal one (see [10] for a review).

A number of neurodegenerative disorders, including Alzheimer's disease (AD), present prominent tau pathology in the CNS, collectively referred to as tauopathies [11–14]. In tauopathies, the natively unfolded tau protein forms intracellular fibrillar structures of aggregated, hyperphosphorylated, and ubiquitinated tau, which are associated with synaptic and neuronal loss. Upon abnormal phosphorylation, the microtubule-associated protein tau reduces its affinity for and dissociates from microtubules [15–17]. In Alzheimer's disease (AD) brains tau accumulates in the neuronal perikarya and processes as paired helical filaments (PHF) [18,19]. It has been suggested that at the single-cell level the defects start with a modification of tau by phosphorylation, resulting in a destabilization of microtubules giving rise to a “pre-tangle” stage [20]. After this stage, the destabilization of microtubules leads to loss of dendritic microtubules and synapses, plasma membrane degeneration, and eventually cell death [21].

The occurrence of fibrillar tau inclusions in tauopathies strongly supports a key role in the observed clinical symptoms and pathology. As a matter of fact, tau pathology in AD correlates better than amyloid- β (A β) pathology with the cognitive impairment observed in patients [22]. Furthermore, the discovery of mutations within the tau gene that cause a disease known as fronto-temporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [23], demonstrated that tau dysfunction, in the absence of amyloid pathology (as occurs in AD), was sufficient to cause neuronal loss and clinical dementia. Interestingly, tau is needed for A β to cause its toxicity both *in vitro* and *in vivo*, as suggested by the resistance shown by primary cultured neurons from tau knockout mice when exposed to A β [24] as well as by the rescuing premature mortality and preventing memory deficits observed in APP transgenic mice when crossed with tau knockout mice [25]. This supports a central role of tau in mediating A β toxicity in the early pathogenesis of AD.

Taken together, there is a large body of evidence clearly pointing out to a central role for tau in the pathogenesis of several neurodegenerative conditions including AD, laying the groundwork for its use as a target for the development of novel disease modifying therapeutics [26]. Drug discovery and development efforts for AD in the last two decades have primarily focused on targets defined by the amyloid cascade hypothesis, so far with disappointing results. In contrast, despite the fact that the presence of extensive tau pathology is central to the disease, tau-based strategies have received little attention until recently [27].

2. Tau-induced neurodegeneration

The knowledge accumulated in recent years strongly suggest that tau-induced neurodegeneration is most likely a consequence of a combination of loss of (tau) function as well as gain of (toxic)

functions. On one hand, tau detachment from microtubules after hyperphosphorylation (or mutations) causes impaired microtubule function and axonal transport and eventually synaptic dysfunction and neuronal loss [28]. On the other hand, hyperphosphorylated tau molecules tend to self-assemble into filaments such as paired helical filaments (PHF) or straight filaments (SF) forming neurofibrillary tangles (NFT), but hyperphosphorylated tau seem to also have the capacity of sequestering normal tau molecules (and perhaps other microtubule-associated proteins) into the aggregates, which will also have a negative impact on the normal microtubule function. At some point after detaching from microtubules and getting into the aggregation process, tau molecules also suffer other post-translational modifications such as truncation, glycosylation, O-GlcNAcylation, and ubiquitination, which could also contribute to the pathology. For a recent review on the molecular mechanisms by which tau induces neurodegeneration, please refer to [21] or [29].

When establishing new tau-based therapeutic strategies it is critical to clearly identify the specific pathological, neurotoxic form of tau. For a very long time, tangles or fibrils have been considered to be the pathological species, however in recent years it has become clear that, much like amyloid plaques [30], NFTs are the final stage of a pathological process, but the real damage might actually be done by some sort of intermediate hyperphosphorylated, most likely soluble oligomeric tau species [28,31,32]. As a matter of fact, there is some evidence suggesting that NFT might be indeed protective, as tangle-bearing neurons seem to survive for long periods of time [33–35]. Considering our current understanding of the tau pathological pathway, a number of points of therapeutic intervention with disease-modifying potential can be clearly identified. The therapeutic approaches currently being most actively pursued are addressed below.

3. Modulating tau phosphorylation

Although it is still debated whether phosphorylation of tau drives aggregation, or is a consequence of it, the strong correlation that exists between phosphorylation and tau pathology has laid the foundation to look for tau kinase inhibitors as potential therapeutics. However, there is not a single but multiple kinases involved in generating hyperphosphorylated tau *in vivo* [10], raising the issue of whether targeting specific kinases or distinct groups of kinases will be more effective in reducing tau hyperphosphorylation [36]. Several tau kinases, including GSK-3 β , CDK5, and MARK, among others, have all been implicated as potential kinase targets for tau therapeutics (reviewed in [37]). Despite the challenges faced by this approach with respect to toxicity and specificity, numerous kinase inhibitors have been developed, some of which have even made it recently to the clinic [38].

AD was one of the earliest disorders linked to Glycogen Synthase Kinase-3 (GSK-3) dysfunction. Thus, the discovery of small molecule GSK-3 inhibitors has attracted significant attention for the therapeutic intervention in AD and related disorders [39]. For many years, the mood stabilizing drug lithium has been considered as the reference GSK-3 inhibitor and it has been generally assumed that a significant proportion of the therapeutic action of lithium in bipolar disorders results from the inhibition of GSK-3 and thus a few observational, pilot studies have attempted to address the clinical effect of lithium in patients with AD, with mixed results due in part to low number of subjects and low compliance (reviewed in [40]). Interestingly, a recent randomized, double-blind, placebo-controlled study on 45 people with amnesic mild cognitive impairment (aMCI) treated with lithium for 12 months has been reported [41]. Lithium treatment was associated with a significant decrease in CSF concentrations of pTau and with a better performance in cognitive tasks, strongly

suggesting that lithium (most likely through GSK-3 inhibition) might have disease-modifying properties in AD and perhaps other tauopathies.

The GSK-3 inhibitor tideglusib [42] has been recently tested in a double-blind, placebo-controlled, randomized, escalating dose phase II clinical trial on 30 mild-moderate AD patients for up to 20 weeks. Although the primary objective was to evaluate the safety and tolerability, some efficacy measures were assessed as secondary objectives. Tideglusib oral administration was well tolerated and showed encouraging positive effects in Mini-Mental Status Examination (MMSE), Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog+), word fluency, Geriatric Depression Scale (GDS), and a final Global Clinical Assessment (GCA) without statistical significance in this small sample [43]. However, in a more recent 26-week Phase IIb trial (named as ARGO) to evaluate the efficacy of tideglusib versus placebo plus standard of care for the treatment of over 300 mild-to-moderate AD, tideglusib failed to meet primary cognitive endpoint and two of the secondary endpoints, according to the Spanish company Noscira. Further analysis of the data (including biomarkers) is much needed to determine why clinical benefit was not observed.

In the meantime the same compound received the orphan drug status by the FDA and EMA and was assessed in a placebo-controlled, year-long Phase II efficacy trial on Progressive Supranuclear Palsy (PSP), a pure tauopathy. The trial tested oral treatment with tideglusib versus placebo in 146 patients with possible or probable mild to moderate PSP (Golbe stages 1–4) for 52 weeks at 24 sites in Europe and the U.S. Although the drug failed to meet the pre-specified clinical endpoints, trial investigators have reported at a late-breaking abstract session during the Alzheimer's Association International Conference held in Vancouver that a MRI sub-study showed a significant slowdown of the brain atrophy rate in tideglusib-treated patients [44].

As an alternative to kinase inhibition, activation of phosphatases has also been proposed as a strategy for reducing tau phosphorylation, especially in the case of protein phosphatase 2A (PP2A), which seems to be the main brain phosphatase involved in tau phosphorylation [45]. Thus, oral treatment of tau transgenic mice with the PP2A activator sodium selenate reduces tau hyperphosphorylation, abrogates NFT formation, improves contextual memory and motor performance, and prevents neurodegeneration [46]. Activating PP2A has its own challenges though, as it has broad substrate specificity and a number of regulatory subunits, which make it difficult to target the right pool of PP2A to the right extent. The activity of PP2A is regulated by several post-translational modifications, including phosphorylation and methylation, but also by two non-competitive inhibitor proteins, termed I1PP2A and I2PP2A [47]. Recently, a novel etiopathogenic mechanism of AD mediated by the cleavage of I2PP2A has been suggested [48], opening the possibility of new therapeutic approaches for AD.

4. Microtubule stabilization

Based on the notion that tau detachment from microtubules results in loss of its normal microtubule stabilizing function, most likely leading to axonal transport impairment and eventually to synaptic dysfunction, some microtubule-stabilizing agents have also been proposed as a therapeutic alternative. Among those, some anti-mitotic compounds such as paclitaxel or epothilone have been used in tau transgenic animals (reviewed in [49]). The most advanced of these agents is davunetide (NAP), an eight-aminoacid peptide derived from the activity-dependent neuroprotective protein (ADNP) [50], which has shown to be able to decrease tau phosphorylation and A β levels in animal models [51,52]. Although its mechanism of action is not entirely clear,

davunetide seems to counteract tau effects on microtubules by maintaining and stabilizing the microtubular network [53].

AL-108 (Allon Therapeutics Inc.), an intranasal formulation of davunetide, has recently moved into early clinical development for both AD and PSP [54]. A placebo-controlled, ascending-dose study that explored the safety, tolerability and efficacy of 12 weeks of treatment with AL-108 in 144 subjects with amnesic mild cognitive impairment has been recently carried out. Analyses of efficacy data failed to detect a statistically significant difference between the treatment groups on the composite cognitive memory score impairment [55]. Adding up to the disappointment, the company announced on December 19, 2012 press release that its pivotal phase II/III randomized double-blind, placebo-controlled study to evaluate the safety and efficacy of davunetide as a treatment for PSP failed to demonstrate efficacy in this population in any of the primary and secondary outcomes

5. Inhibiting tau aggregation

Another potential point of therapeutic intervention is the aggregation process itself in which a variety of different tau species are formed: monomers, oligomers, prefilaments, truncated, granules, fibrils, insoluble aggregates. As discussed above, some sort of hyperphosphorylated, misfolded soluble tau intermediates are the actual (or the most) toxic tau species, so that inhibiting early aggregation steps might be critical for a successful pharmacological intervention aimed at preventing neurodegeneration (see [56] for a review). Several chemical families able to inhibit tau aggregation have been identified through *in vitro* and or cell-based screening assays, including, N-phenylamines, rhodanines, phenylthiazol-hydrazides, anthraquinones, aminothienopyridazines and others [57]. Although many of these compounds are effective *in vitro* and considerable recent medicinal chemistry efforts have improved drug-like properties such as toxicity, bioavailability, and pharmacokinetics, evidence of efficacy for inhibiting tau aggregation *in vivo* is still limited [58].

Noteworthy, the non-neuroleptic phenothiazine methylene blue (chloride methylthioninium, Rember™, TauRx Pharmaceuticals Ltd.) has reached the clinic and actually completed a Phase II trial in AD patients [59]. This compound and its derivatives have been used to treat malaria for quite some time [56]. The 24-week monotherapy trial did not meet efficacy endpoints with respect to altering cognition and claims of efficacy after post hoc subgroup analysis or neuroimaging [60] have generally been met with scepticism. Unfortunately, other than press releases or a few abstracts in conferences, no peer-review data on this or similar trials have been published. A new stabilized, reduced form of the drug called LMTX™ (leuco-methylthioninium), which is no longer blue and renders the drug more bioavailable and less toxic at higher doses, is currently being used in several phase II and III clinical trials for mild-moderate AD and fronto-temporal dementia (FTD). Publication of the results is eagerly awaited.

6. Reducing tau levels

It has been over twenty years that increased intracellular tau levels were described in brains from AD patients as compared to age-matched non-demented controls [58] and it has thus been suggested that increased tau levels might be toxic for neurons. On the other hand, several tau-deficient mouse strains have been generated [61–67] and while initially reported to lack an overt phenotype, behavioral changes and motor deficits have been reported recently in some strains [68]. Indeed, a reduction in the amount of tau has protective effects in cultured neurons [24,69] and in animal models of neurodegeneration [25,70]. Taken together, all this evidence point out to the reduction of tau levels

as a potential therapeutic approach for AD and other tauopathies [71], as recently supported by data on inducible transgenic mouse models [72,73].

Another potential therapeutic approach worth discussing is the regulation of tau gene expression, especially considering that tau mRNA appears to be also increased in AD brains [74]. Nevertheless, very little is known about the precise molecular mechanisms controlling tau expression, although some specific factors affecting this process have been described, including fibroblast growth factor (FGF) [75], dual-specificity tyrosine-phosphorylation regulated kinase 1A (Dyrk1A) [76], and the mi-R34 family of microRNAs [77]. On the other hand, although no mutations within the tau gene have been identified in AD patients, a genetic association has been shown between some haplotypes at the MAPT locus with gene level expression and an increased risk for AD [78]. However, whether these polymorphisms are associated to overall tau expression or rather to changes in splicing in different brain regions is still a matter of debate [79,80].

Reduction of tau levels might be achieved not only by reducing its synthesis, but also by increasing its clearance. As neurons age, their survival depends on eliminating a growing burden of damaged, potentially toxic proteins and organelles—a capability that declines owing to aging and disease factors. Two proteolytic systems are mostly responsible for protein quality control in neurons: the ubiquitin–proteasome system (UPS) and the autophagy–lysosome system. While the UPS has been considered for decades as the critical regulator in the degradation of various aggregate-prone proteins, autophagy has more recently been shown to be an important pathway implicated in neuronal health and disease [81,82]. These pathways have been suggested to normally keep tau levels at a low level which may prevent aggregation [83], opening the door to consider pharmacological methods that target these degradation systems for the treatment and prevention of AD and perhaps other tauopathies.

7. Decreasing extracellular tau

Development of tau pathology is associated with progressive neuronal loss and cognitive decline. In the brains of AD patients, tau pathology spreads following a predictable, anatomically defined progression pattern, which has suggested that the actual interneuronal transfer of a toxic factor must be involved in the

progression of neurodegenerative tauopathies [84,85]. Although traditionally thought of as an exclusively intracellular protein and its presence in the extracellular milieu (or in CSF) would be a consequence of neuronal death, the occurrence of extracellular tau having a functional role has recently emerged from *in vitro* [86–91] and *in vivo* [92–94] studies. Released tau seems to exist in a relatively dephosphorylated state, compared to that of intracellular tau [91].

Interestingly, tau released by cortical neurons in physiological conditions is regulated by neuronal activity, as release is enhanced by glutamate receptor stimulation induced by the agonist S-AMPA, a process also modulated by phosphorylation [91]. Hence, would be interesting to know whether AMPA receptor antagonists indeed decrease tau secretion. On the other hand, it has been recently suggested that extracellular tau might induce a receptor-activated increase in intracellular calcium, including through M1/M3 muscarinic receptor stimulation [95], a process dependent on tau phosphorylation [96], which in turn will lead to endocytosis of extracellular tau, thus contributing to spreading of tau pathology [97,98]. Another potential approach to reduce tau secretion would be lowering intracellular calcium, since calcium-chelating factors prevent tau release (Simón et al., in preparation).

The precise molecular mechanisms involved in tau release from neurons to the extracellular space have not yet been fully elucidated. The presence of extracellular tau in exosomes [99] has also been recently reported and non-vesicle associated tau is constitutively secreted at a low level by an unconventional secretion process that cannot be blocked by inhibitors of the conventional secretory pathway [90]. Furthermore, while extracellular tau seems to be mostly full length, cleavage of tau at 421 has been shown to enhance its secretion [89]. In any event, the relationship between tau release under physiological conditions, and the spreading of tau pathology in AD and other tauopathies remains to be better understood (Table 1).

8. Immunotherapy targeting tau

Immunotherapy for various neurodegenerative diseases have recently emerged as a promising approach for the clearance of pathological proteins in these disorders [100]. The disappointing outcome of several amyloid-based pharmacological approaches, including anti-amyloid immunotherapy, in late stage clinical trials

Table 1

Selected tau-based investigational drugs in clinical development for the treatment of AD and related disorders.

Drug	Mechanism of action	Development phase	Indication	ClinicalTrials.gov Identifier	Sponsor
Lithium, Valproate	GSK-3 inhibition	1	AD	NCT00088387	NINDS
Lithium carbonate	GSK-3 inhibition	2	AD	NCT01055392	University of Sao Paulo
Lithium carbonate	GSK-3 inhibition	1	PSP	NCT00703677	Westat
Lithium carbonate	GSK-3 inhibition	2	CBD	NCT00703677	Westat
Lithium carbonate	GSK-3 inhibition				
Valproic acid	GSK-3 inhibition	0	Cognitively intact elderly subjects	NCT01729598	University of Kentucky
Valproic acid	GSK-3 inhibition	2	PSP	NCT00385710	Nantes University Hospital
Tideglusib (NP-12)	GSK-3 inhibitor	2	AD	NCT00948259	Noscira S.A.
Tideglusib (NP-12)	GSK-3 inhibitor	2	PSP	NCT01049399	Noscira S.A.
Tideglusib (NP-12)	GSK-3 inhibitor	2	AD	NCT01350362	Noscira S.A.
Davunetide (AL-108)	MT stabilizer	2	PSP, CBD, FTDP-17, PNA	NCT01056965	UCSF
Davunetide (AL-108)	MT stabilizer	2	MCI	NCT00422981	Allon Therapeutics
Epothilone D (BMS-241027)	MT stabilizer	1	AD	NCT01492374	Bristol-Myers Squibb
Methylene blue (Rember)	Anti-aggregation	2	AD	n/a ^a	Tau Rx Therapeutics
Leucomethylene blue (LCMX)	Anti-aggregation	2	AD	TRx-237-005 ^a	Tau Rx Therapeutics
Leucomethylene blue (LCMX)	Anti-aggregation	2	AD	TRx-237-015 ^a	Tau Rx Therapeutics
Leucomethylene blue (LCMX)	Anti-aggregation	3	FTD	n/a ^a	Tau Rx Therapeutics
AADvac1	Active immunization	1	AD	NCT01850238	Axon Neuroscience

AD = Alzheimer's disease. PSP = progressive supranuclear palsy. CBD = corticobasal degeneration. FTD = frontotemporal dementia. FTDP-17 = frontotemporal dementia with Parkinsonism linked to chromosome 17. PNA = progressive nonfluent aphasia. MCI = mild cognitive impairment. n/a = non available.

^a Not found on ClinicalTrials.gov. Identifier provided in the company's website.

during the last few years has spurred increased interest in novel tau-based therapies and various vaccination approaches have been recently assessed in preclinical models for tau-antibodies-induced tau clearance, indicating that this may be a viable option for clearing tau deposits in these diseases.

The immunotherapy approach is based on eliciting anti-tau antibodies able to clear tau molecules that negatively affect neuronal viability thus resulting in clearance of tau pathological species and eventually neuronal function improvement [101]. Feasibility of this approach was first demonstrated when P301L tau transgenic mice were immunized with a 30-aminoacid tau phospho-peptide spanning aminoacids 379–408, including phospho-Ser at positions 396 and 404 [102]. This resulted in a specific antibody response and reduced brain tau pathology, and better performance on motor tasks. Previously, vaccination of wild type mice with recombinant full-length human tau protein (unphosphorylated) had led to encephalomyelitis accompanied of axonal damage, inflammation and behavioral deficits [103], pointing out to a neurotoxic potential of tau immunization. However, the same authors later reported a similar experiment by injecting a mixture of three different tau phospho-peptides (Tau195–213[p202–205]; Tau207–220[p212–214]; and Tau224–238[p231]) to transgenic mice overexpressing a double-mutant human tau protein (K257T/P301S) [104]. In contrast with the previous experiment with full-length unphosphorylated human tau protein, but consistent with the data by Asuni et al. [102], the new immunization protocol led to a 40% decrease in NFT burden in brain and spinal cord in the absence of any encephalitogenicity, neurological deficits or axonal damage, thus suggesting the therapeutic potential of this approach.

Several additional studies have been recently reported following passive immunization approaches in different animal models using various tau peptides as immunogens. The first one uses the same 30-mer peptide described above [102] in hTau/PS1 mice, which express all six wild-type isoforms of human tau on a mouse tau knockout background [34] plus human mutant (M146L) presenilin 1 (PS1) [105]. These mice develop tau pathology primarily in the cortex and hippocampus with very little in areas important for motor activity. In this case, tau immunotherapy not only decreased tau phosphorylation but also the cognitive decline in immunized animals. Another recent report has used a twelve amino acid peptide (395–406 of the human tau sequence), comprising phosphorylated Ser396/Ser404 of the PHF-1 epitope, to immunize pR5 mice expressing P301L mutant tau [106]. In this case, vaccinated animals showed reduced tau phosphorylation and slower progression of NFT pathology. In a similar study, heterozygous THY-Tau22 mice were immunized with a tau peptide containing phospho-Ser422, which elicited a specific antibody response and a decrease in insoluble tau in the brain [107].

Although the active immunization approach has certain advantages, it may have autoimmune side effects that can be avoided with passive immunization. Thus, several passive immunotherapy approaches targeting tau with specific antibodies have also been recently reported. The first study investigated the effects of injecting tau antibodies PHF1, which recognizes an epitope containing phospho-Ser396,404, or MC1 (a conformation-dependent antibody) in two different transgenic models: JPNL3 and P301S. Immunized animals showed significantly decreased tau pathology and delayed onset of motor function decline [108]. These results were corroborated in a different study performed with antibody PHF1 in JPNL3 transgenic animals [109] and more recently in P301L transgenic mice [110]. In the latter study, the beneficial effects observed with PHF1 and MC1, were not achieved with DA31, a high affinity, non-phospho-specific tau antibody that maps in the amino acid region 150–190 of tau [110]. A different passive approach has proposed to use chaperon-like antibodies targeting misfolded tau [111]. These authors have developed a

monoclonal antibody with high binding constant toward a peptide within the tubulin binding domain of the tau molecule (residues 300–312), able to completely abolish the pathological microtubule assembly promoted by misfolded tau.

Taken together, all these data suggest that targeting abnormally phosphorylated tau epitopes (or perhaps specific pathological conformers) seem to elicit antibodies responses that in turn are able to facilitate tau clearance. As new immunotherapy approaches targeting tau are being actively pursued in different animal models [96], a number of key questions however remain still to be answered, especially in some fundamental areas: the exact nature of the toxic species to be targeted, safety issues associated to potential unwanted immune response, and the mechanism of action by which antibodies clear tau molecules.

Safety is of particular concern to the light of the anti-amyloid immunotherapy failure of the AN-1792 clinical trial [112,113] and special care must be placed in designing the immunogens to avoid unwanted adverse immune responses, considering that injection of recombinant human tau protein led to autoimmune, neurotoxic responses in wild type mice [99], as already discussed. The choice of immunogen and the role of phosphosrylation seems to be a critical factor since the use of phosphopeptides found in late stage NFT might be problematic as its therapeutic value might be questioned [114]. Mounting evidence indicates that some soluble, oligomeric (pre-filament, immature filaments) tau species, rather than the tangles, are indeed the pathogenic ones [115–117], reminiscent of what has happened in recent years in the amyloid field regarding plaques and intermediate A β oligomers, [118]. The demonstration of a link between tau oligomers and brain pathology in animal models (reviewed in [119]) highlights the importance of precisely identifying the tau species to be targeted by immunotherapy.

Another critical point is the mechanism by which immunotherapy clear tau from the brain. It has been suggested that anti-tau antibodies reach the brain due to a compromised blood brain barrier (BBB) in transgenic animals or diseased human brain [120]. Tau is normally a cytosolic protein, and NFT are formed intracellularly, so once within the brain the antibodies must be able to get into the cell in order to clear tau molecules effectively, perhaps through receptor-mediated endocytosis, as it has been just shown to happen in neuronal cultures and brain slices [121,122].

9. Future developments

Drug discovery and development efforts for AD in the last two decades have primarily focused on targets defined by the amyloid cascade hypothesis, so far with disappointing results. In contrast, tau-based strategies have received little attention until recently despite that the presence of extensive tau pathology is central to the disease. However, as discussed in this review, a number of different strategies targeting several aspects of the tau pathogenesis are being actively pursued, broadening our range of potentially useful therapeutic tools to treat AD and other tauopathies. In fact, several compounds have already reached phase II clinical trials and some proof-of-concept studies are currently ongoing or planned.

There is also a significant effort being made at the preclinical level to validate a number of tau-related targets and develop novel ways to the treatment of these conditions. In the specific case of the immunotherapies, as the different strategies are being pursued, it is of paramount importance to always keep in mind the safety immunogens and antibodies being tested. Clinical trials with amyloid-based vaccines have highlighted some of the safety associated with immunotherapy approach and a better understanding of the immune responses associated to these strategies will also be very helpful in designing tau-based vaccines as well.

Future clinical trials will reveal the safety and efficacy of the various preclinical treatment strategies being tested in translational laboratories across the world.

Some of the known drug discovery and development challenges will also be faced by tau-based therapies: lack of good predictive animal models, lack of good validated biomarkers of disease progression, clinical trial design, early diagnosis and treatment, definition of target population, difficulties in demonstrating disease modifying effects, etc. Despite some open questions and challenges that still remain ahead, the data reviewed here will likely encourage further efforts on tau-based therapies for AD and other tauopathies in the coming years. We must however acknowledge the complexity of the protein aggregation and misfolding processes as well as the immune response in these pathologies so we can better design and develop pathological state-specific strategies. Whether tau-based therapeutics (alone or in combination) will be successful for treating AD and other neurodegenerative disorders, only time will tell, but certainly the next few years will bring us considerable efforts in this area and will serve to further validate tau-based therapeutic approaches to fight tau-related dementias.

References

- [1] Drechsel DN, Hyman AA, Cobb MH, Kirschner MW. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Mol Biol Cell* 1992;3(10):1141–54.
- [2] LoPresti P, Szuchet S, Papasozomenos SC, Zinkowski RP, Binder LI. Functional implications for the microtubule-associated protein tau: Localization in oligodendrocytes. *Proc Natl Acad Sci USA* 1995;92(22):10369–73.
- [3] Ebner A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: Implications for Alzheimer's disease. *J Cell Biol* 1998;143(3):777–94.
- [4] Neve RL, Harris P, Kosik KS, Kurnit DM, Donlon TA. Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2. *Brain Res* 1986;387(3):271–80.
- [5] Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J* 1989;8(2):393–9.
- [6] Lee G, Neve RL, Kosik KS. The microtubule binding domain of tau protein. *Neuron* 1989;2(6):1615–24.
- [7] Goedert M, Jakes R. Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerization. *EMBO J* 1990;9(13):4225–30.
- [8] Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol* 1985;101(4):1371–8.
- [9] Kosik KS. Tau protein and Alzheimer's disease. *Curr Opin Cell Biol* 1990;2(1):101–4.
- [10] Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA* 1986;83(13):4913–7.
- [11] Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med* 2009;15(3):112–9.
- [12] Lee VM, Goedert M, Trojanowski J. Neurodegenerative disorders. *Annu Rev Neurosci* 2001;24:1121–59.
- [13] Hernandez F, Avila J. Tauopathies. *Cell Mol Life Sci* 2007;64(17):2219–33.
- [14] Ludolph AC, Kassubek J, Landwehrmeyer BG, Mandelkow E, Mandelkow EM, Burn DJ, et al. Tauopathies with parkinsonism clinical spectrum: neuropathological basis, biological markers, and treatment options. *Eur J Neurol* 2009;16(3):297–309.
- [15] Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *Proc Natl Acad Sci USA* 1994;91(12):5562–6.
- [16] Alonso AC, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med* 1996;2(7):783–7.
- [17] Gustke N, Steiner B, Mandelkow EM, Biernat J, Meyer HE, Goedert M, Mandelkow E. The Alzheimer-like phosphorylation of tau protein reduces microtubule binding and involves Ser-Pro and Thr-Pro motifs. *FEBS Lett* 1992;307(2):199–205.
- [18] Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem* 1986;261(13):6084–9.
- [19] Ivy GO, Kitani K, Ihara Y. Anomalous accumulation of tau and ubiquitin immunoreactivities in rat brain caused by protease inhibition and by normal aging: a clue to PHF pathogenesis. *Brain Res* 1989;498(2):360–5.
- [20] Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, et al. Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *J Neurosci* 2007;27(14):3650–62.
- [21] Iqbal K, Liu F, Gong CX, Alonso Adel C, Grundke-Iqbal I. Mechanisms of tau-induced neurodegeneration. *Acta Neuropathol* 2009;118(1):53–69.
- [22] Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* 1992;42(3 Pt 1):631–9.
- [23] Goedert M, Jakes R. Mutations causing neurodegenerative tauopathies. *Biochim Biophys Acta* 2005;1739(2–3):240–50.
- [24] Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to β -amyloid-induced neurotoxicity. *Proc Natl Acad Sci USA* 2002;99(9):6364–9.
- [25] Roberson ED, Scearce-Levie K, Palop JJ, et al. Reducing endogenous tau ameliorates amyloid β -induced deficits in an Alzheimer's disease mouse model. *Science* 2007;316(5825):750–4.
- [26] Marx J. A new take on tau. *Science* 2007;316(5830):1416–7.
- [27] Medina M. Recent developments in tau-based therapeutics for neurodegenerative diseases. *Recent Pat CNS Drug Discov* 2011;6(1):20–30.
- [28] Jaworski T, Kügler S, Van Leuven F. Modeling of tau-mediated synaptic and neuronal degeneration in Alzheimer's disease. *Int J Alzheimers Dis* 2010;2010.
- [29] Spillantini MG, Goedert M. Tau pathology and neurodegeneration. *Lancet Neurol* 2013;12(6):609–22.
- [30] Haass C, Selkoe DJ. Protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 2007;8(2):101–12.
- [31] Iqbal K, Grundke-Iqbal I. Developing pharmacological therapies for Alzheimer disease. *Cell Mol Life Sci* 2007;64(17):2234–44.
- [32] Brunden KR, Trojanowski JQ, Lee VM. Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. *Nat Rev Drug Discov* 2009;8(10):783–93.
- [33] Morsch R, Simon W, Coleman PD. Neurons may live for decades with neurofibrillary tangles. *J Neuropathol Exp Neurol* 1999;58(2):188–97.
- [34] Andorfer C, Acker CM, Kress Y, Hof PR, Duff K, Davies P. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J Neurosci* 2005;25(22):5446–54.
- [35] de Calignon A, Spire-Jones TL, Pitstick R, Carlson GA, Hyman BT. Tangle-bearing neurons survive despite disruption of membrane integrity in a mouse model of tauopathy. *J Neuropathol Exp Neurol* 2009;68(7):757–61.
- [36] Zhang X, Hernandez I, Rei D, Mair W, Laha JK, Cornwell ME, et al. Diaminotriazoles modify Tau phosphorylation and improve the tauopathy in mouse models. *J Biol Chem* 2013;288(30):22042–56.
- [37] Schneider A, Mandelkow E. Tau-based treatment strategies in neurodegenerative diseases. *Neurotherapeutics* 2008;5(3):443–57.
- [38] Medina M, Castro A. Glycogen synthase kinase-3 (GSK-3) inhibitors reach the clinic. *Curr Opin Drug Discov Dev* 2008;11(4):533–43.
- [39] Medina M, Avila J. Glycogen synthase kinase-3 (GSK-3) inhibitors for the treatment of Alzheimer's disease. *Curr Pharm Des* 2010;16(25):2790–8.
- [40] Medina M, Garrido JJ, Wandosell FG. Modulation of GSK-3 as a therapeutic strategy on tau pathologies. *Front Mol Neurosci* 2011;4:24.
- [41] Forlenza OV, Diniz BS, Radanovic M, Santos FS, Talib LL, Gattaz WF. Disease-modifying properties of long-term lithium treatment for amnesic mild cognitive impairment: randomised controlled trial. *Br J Psychiatry* 2011;198(5):351–6.
- [42] Domínguez JM, Fuertes A, Orozco L, del Monte-Millán M, Delgado E, Medina M. Evidence for irreversible inhibition of glycogen synthase kinase-3 β by tideglusib. *J Biol Chem* 2012;287(2):893–904.
- [43] del Ser T, Steinwachs KC, Gertz HJ, Andrés MV, Gómez-Carrillo B, Medina M, Vericat JA, Redondo P, Fleet D, León T. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. *J Alzheimers Dis* 2013;33(1):205–15.
- [44] Press release: http://www.noscira.com/media/docs/Nota_Prensa20072012_en.pdf.
- [45] Gong CX, Iqbal K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Curr Med Chem* 2008;15(23):2321–8.
- [46] van Eersel J, Ke YD, Liu X, Delerue F, Kril JJ, Götz J, Ittner LM. Sodium selenate mitigates tau pathology: neurodegeneration, and functional deficits in Alzheimer's disease models. *Proc Natl Acad Sci USA* 2010;107(31):13888–93.
- [47] Tanimukai H, Kudo T, Tanaka T, Grundke-Iqbal I, Iqbal K, Takeda M. Novel therapeutic strategies for neurodegenerative disease. *Psychogeriatrics* 2009;9(2):103–9.
- [48] Wang X, Blanchard J, Kohlbrenner E, Clement N, Linden RM, Radu A, et al. The carboxy-terminal fragment of inhibitor-2 of protein phosphatase-2A induces Alzheimer disease pathology and cognitive impairment. *FASEB J* 2010;24(11):4420–5321.
- [49] Golde TE, Petrucelli L, Targeting Lewis J. Abeta and tau in Alzheimer's disease: an early interim report. *Exp Neurol* 2010;223(2):252–66.
- [50] Bassan M, Zamostiano R, Davidson A, Pinhasov A, Giladi E, Perl O, et al. Complete sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide. *J Neurochem* 1999;72(3):1283–93.
- [51] Matsuoka Y, Jouroukhin Y, Gray AJ, Ma L, Hirata-Fukae C, Li HF, Feng L, et al. A neuronal microtubule-interacting agent: NAPVSIPQ, reduces tau pathology

- and enhances cognitive function in a mouse model of Alzheimer's disease. *J Pharmacol Exp Ther* 2008;325(1):146–53.
- [52] Shiryayev N, Jouroukhin Y, Giladi E, Polyzoidou E, Grigoriadis NC, Rosenmann H, Gozes I. NAP protects memory: increases soluble tau and reduces tau hyperphosphorylation in a tauopathy model. *Neurobiol Dis* 2009;34(2):381–8.
 - [53] Oz S, Ivashko-Pachima Y, Gozes I. The ADNP derived peptide, NAP modulates the tubulin pool: implication for neurotrophic and neuroprotective activities. *PLoS ONE* 2012;7(12):e51458.
 - [54] Gozes I. Tau pathology and future therapeutics. *Curr Alzheimer Res* 2010;7(8):685–96.
 - [55] Morimoto BH, Schmechel D, Hirman J, Blackwell A, Keith J, Gold M. AL-108-211 Study. A double-blind, placebo-controlled, ascending-dose, randomized study to evaluate the safety, tolerability and effects on cognition of AL-108 after 12 weeks of intranasal administration in subjects with mild cognitive impairment. *Dement Geriatr Cogn Disord* 2013;35(5–6):325–36.
 - [56] Takashima A. Tau aggregation is a therapeutic target for Alzheimer's disease. *Curr Alzheimer Res* 2010;7(8):665–9.
 - [57] Bulic B, Pickhardt M, Schmidt B, Mandelkow EM, Waldmann H, Mandelkow E. Development of tau aggregation inhibitors for Alzheimer's disease. *Angew Chem Int Ed Engl* 2009;48(10):1740–52.
 - [58] Bulic B, Pickhardt M, Mandelkow E. Progress and developments in tau aggregation inhibitors for Alzheimer disease. *J Med Chem* 2013;56(11):4135–55.
 - [59] Wischik CM, Benthall P, Wischik DJ, Seng KM. Tau aggregation inhibitor (TAI) therapy with Rember™ arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks. *Alz Dement* 2008;4(Suppl. 1):T167.
 - [60] Buchholz K, Schirmer RH, Eubel JK, Akoachere MB, Dandekar T, Becker K, et al. Interactions of methylene blue with human disulfide reductases and their orthologues from *Plasmodium falciparum*. *Antimicrob Agents Chemother* 2008;52(1):183–91.
 - [61] Wischik C, Staff R. Challenges in the conduct of disease-modifying trials in AD: practical experience from a phase 2 trial of Tau-aggregation inhibitor therapy. *J Nutr Health Aging* 2009;13(4):367–9.
 - [62] Khatoun S, Grundke-Iqbal I, Iqbal K. Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. *J Neurochem* 1992;59(August (2)):750–3.
 - [63] Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature* 1994;369(6480):488–91.
 - [64] Takei Y, Teng J, Harada A, Hirokawa N. Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. *J Cell Biol* 2000;150(5):989–1000.
 - [65] Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci* 2001;114(6):1179–87.
 - [66] Tucker KL, Meyer M, Barde YA. Neurotrophins are required for nerve growth during development. *Nat Neurosci* 2001;4(1):29–37.
 - [67] Ittner LM, Ke YD, Delerue F, et al. Dendritic function of tau mediates amyloid- β toxicity in Alzheimer's disease mouse models. *Cell* 2010;142(3):387–97.
 - [68] Ke YD, Suchowerska AK, van der Hoven J, De Silva DM, Wu CW, van Eersel J, Ittner A, Ittner LM. Lessons from tau-deficient mice. *Int J Alzheimers Dis* 2012;2012:873270.
 - [69] Caceres A, Kosik KS. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature* 1990;343(6257):461–3.
 - [70] Dawson HN, Cantillana V, Jansen M, Wang H, Vitek MP, Wilcock DM, Lynch JR, Laskowitz DT. Loss of tau elicits axonal degeneration in a mouse model of Alzheimer's disease. *Neuroscience* 2010;169(1):516–31.
 - [71] Götz J, Xia D, Leinenga G, Chew YL, Nicholas H. What renders TAU toxic. *Front Neurol* 2013;4:72.
 - [72] Van der Jeugd A, Hochgräfe K, Ahmed T, Decker JM, Sydow A, Hofmann A, et al. Cognitive defects are reversible in inducible mice expressing propagating full-length human Tau. *Acta Neuropathol* 2012;123(6):787–805.
 - [73] Hochgräfe K, Sydow A, Mandelkow EM. Regulatable transgenic mouse models of Alzheimer disease: onset, reversibility and spreading of Tau pathology. *FEBS J* 2013;280(18):4371–81.
 - [74] Barton AJ, Harrison PJ, Najlerahim A, Heffernan J, McDonald B, Robinson JR, et al. Increased tau messenger RNA in Alzheimer's disease hippocampus. *Am J Pathol* 1990;137(3):497–502.
 - [75] Tatebayashi Y, Iqbal K, Grundke-Iqbal I. Dynamic regulation of expression and phosphorylation of tau by fibroblast growth factor-2 in neural progenitor cells from adult rat hippocampus. *J Neurosci* 1999;19(13):5245–54.
 - [76] Qian W, Jin N, Shi J, Yin X, Jin X, Wang S, et al. Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1A) enhances tau expression. *J Alzheimers Dis* 2013;37(3):529–38.
 - [77] Dickson JR, Kruse C, Montagna DR, Finsen B, Wolfe MS. Alternative polyadenylation and miR-34 family members regulate tau expression. *J Neurochem* 2013;127(6):739–49.
 - [78] Myers AJ, Pittman AM, Zhao AS, Rohrer K, Kaleem M, Marlowe L, et al. The MAPT H1c risk haplotype is associated with increased expression of tau and especially of 4 repeat containing transcripts. *Neurobiol Dis* 2007;25(3):561–70.
 - [79] Tratzuni D, Wray S, Vandrovicova J, Ramasamy A, Walker R, Smith C, et al. MAPT expression and splicing is differentially regulated by brain region: relation to genotype and implication for tauopathies. *Hum Mol Genet* 2012;21(18):4094–103.
 - [80] Majounie E, Cross W, Newsday V, Dillman A, Vandrovicova J, Morris CM, et al. Variation in tau isoform expression in different brain regions and disease states. *Neurobiol Aging* 2013;34(7). 1922.e7–1922.e12.
 - [81] Ihara Y, Morishima-Kawashima M, Nixon R. The ubiquitin-proteasome system and the autophagic-lysosomal system in Alzheimer disease. *Cold Spring Harb Perspect Med* 2012;2(8).
 - [82] Lee MJ, Lee JH, Rubinstein DC. Tau degradation: the ubiquitin-proteasome system versus the autophagy-lysosome system. *Prog Neurobiol* 2013;105:49–59.
 - [83] Wang Y, Mandelkow E. Degradation of tau protein by autophagy and proteasomal pathways. *Biochem Soc Trans* 2012;40(4):644–52.
 - [84] Saper CB, Wainer B, German DC. Axonal and transneuronal transport in the transmission of neurological disease: potential role in system degenerations, including Alzheimer's disease. *Neuroscience* 1987;23:389–98.
 - [85] Su JH, Deng G, Cotman CW. Transneuronal degeneration in the spread of Alzheimer's disease pathology: immunohistochemical evidence for the transmission of tau hyperphosphorylation. *Neurobiol Dis* 1997;4:365–75.
 - [86] Kim W, Lee S, Jung C, Ahmed A, Lee G, Hall GF. Interneuronal transfer of human tau between Lamprey central neurons in situ. *J Alzheimers Dis* 2010;19:647–64.
 - [87] Karch CM, Jeng AT, Goate AM. Extracellular Tau levels are influenced by variability in Tau that is associated with tauopathies. *J Biol Chem* 2012;287:42751–62.
 - [88] Shi Y, Kirwan P, Smith J, MacLean G, Orkin SH, Livesey FJ. A human stem cell model of early Alzheimer's disease pathology in Down syndrome. *Sci Transl Med* 2012;4:124ra129.
 - [89] Plouffe V, Mohamed NV, Rivest-McGraw J, Bertrand J, Lauzon M, Leclerc N. Hyperphosphorylation and cleavage at D421 enhance tau secretion. *PLoS ONE* 2012;7:e36873.
 - [90] Chai X, Dage JL, Citron M. Constitutive secretion of tau protein by an unconventional mechanism. *Neurobiol Dis* 2012;48:356–66.
 - [91] Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep* 2013;14(4):389–94.
 - [92] Marklund N, Blennow K, Zetterberg H, Ronne-Engstrom E, Enblad P, Hillered L. Monitoring of brain interstitial total tau and beta amyloid proteins by microdialysis in patients with traumatic brain injury. *J Neurosurg* 2009;110:1227–37.
 - [93] Yamada K, Cirrito JR, Stewart FR, Jiang H, Finn MB, Holmes BB, et al. In vivo microdialysis reveals age-dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. *J Neurosci* 2011;31:13110–17.
 - [94] Magnoni S, Esparza TJ, Conte V, Carbonara M, Carabba G, Holtzman DM, et al. Tau elevations in the brain extracellular space correlate with reduced amyloid-beta levels and predict adverse clinical outcomes after severe traumatic brain injury. *Brain* 2012;135:1268–80.
 - [95] Gomez-Ramos A, Diaz-Hernandez M, Rubio A, Miras-Portugal MT, Avila J. Extracellular tau promotes intracellular calcium increase through M1 and M3 muscarinic receptors in neuronal cells. *Mol Cell Neurosci* 2008;37:673–81.
 - [96] Diaz-Hernandez M, Gomez-Ramos A, Rubio A, Gomez-Villafuertes R, Naranjo JR, Miras-Portugal MT, Avila J. Tissue-nonspecific alkaline phosphatase promotes the neurotoxicity effect of extracellular tau. *J Biol Chem* 2010;285:32539–48.
 - [97] Simón D, García-García E, Royo F, Falcón-Pérez JM, Avila J. Proteostasis of tau. Tau overexpression results in its secretion via membrane vesicles. *FEBS Lett* 2012;586(1):47–54.
 - [98] Simón D, Hernández F, Avila J. The involvement of cholinergic neurons in the spreading of tau pathology. *Front Neurol* 2013;4:74.
 - [99] Saman S, Kim W, Raya M, Visnick Y, Miro S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem* 2012;287:3842–9.
 - [100] Gu J, Sigurdsson EM. Immunotherapy for tauopathies. *J Mol Neurosci* 2011;45(3):690–5.
 - [101] Sigurdsson EM. Tau-focused immunotherapy for Alzheimer's disease and related tauopathies. *Curr Alzheimer Res* 2009;6(5):446–50.
 - [102] Asuni AA, Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *J Neurosci* 2007;27(34):9115–29.
 - [103] Rosenmann H, Grigoriadis N, Karussis D, Boimel M, Touloumi O, Ovadia H, et al. Tauopathy-like abnormalities and neurologic deficits in mice immunized with neuronal tau protein. *Arch Neurol* 2006;63(10):1459–67.
 - [104] Boimel M, Grigoriadis N, Loubopoulos A, Haber E, Abramsky O, Rosenmann H. Efficacy safety of immunization with phosphorylated tau against neurofibrillary and tangles in mice. *Exp Neurol* 2010;224(2):472–85.
 - [105] Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau prevents cognitive decline in a new tangle mouse model. *J Neurosci* 2010 Dec 8;30(49):16559–66.
 - [106] Bi M, Ittner A, Ke YD, Götz J, Ittner LM. Tau-targeted immunization impedes progression of neurofibrillary histopathology in aged P301L tau transgenic mice. *PLoS ONE* 2011;6(12):e26860.
 - [107] Troquier L, Caillierez R, Burnouf S, Fernandez-Gomez FJ, Grosjean ME, Zommer N, et al. Targeting phospho-Ser422 by active Tau Immunotherapy in the THY22 mouse model: a suitable therapeutic approach. *Curr Alzheimer Res* 2012;9(4):397–405.

- [108] Chai X, Wu S, Murray TK, Kinley R, Cella CV, Sims H, et al. Passive immunization with anti-Tau antibodies in two transgenic models: reduction of Tau pathology and delay of disease progression. *J Biol Chem* 2011;286(39):34457–67.
- [109] Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *J Neurochem* 2011;118(4):658–67.
- [110] d'Abramo C, Acker CM, Jimenez HT, Davies P. Tau passive immunotherapy in mutant P301L mice: antibody affinity versus specificity. *PLoS ONE* 2013; 8(4):e62402.
- [111] Zilka N, Kontseikova E, Novak M. Chaperone-like antibodies targeting misfolded tau protein: New vistas in the immunotherapy of neurodegenerative foldopathies. *J Alzheimers Dis* 2008;15:169–79.
- [112] Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003;61(1):46–54.
- [113] Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* 2005;64(9):1553–62.
- [114] Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* 2007;53(3):337–51.
- [115] Bretteville A, Planel E. Tau aggregates: toxic, inert, or protective species? *J Alzheimers Dis* 2008;14(4):431–6.
- [116] Congdon EE, Duff KE. Is tau aggregation toxic or protective? *J Alzheimers Dis* 2008;14(4):453–7.
- [117] Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, et al. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci Rep* 2012;2:700.
- [118] Walsh DM, Selkoe DJ. Abeta oligomers – a decade of discovery. *J Neurochem* 2007;101(5):1172–84.
- [119] Kaye R, Jackson GR. Prefilament tau species as potential targets for immunotherapy for Alzheimer disease and related disorders. *Curr Opin Immunol* 2009;21(3):359–63.
- [120] Sigurdsson EM. Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies. *J Alzheimers Dis* 2008;15(2): 157–68.
- [121] Gu J, Congdon EE, Sigurdsson EM. Two novel tau antibodies targeting the 396/404 region are primarily taken up by neurons and reduce tau pathology. *J Biol Chem* 2013;288:33081–95.
- [122] Congdon EE, Gu J, Sait HB, Sigurdsson EM. Antibody uptake into neurons occurs primarily via clathrin dependent Fcγ receptor endocytosis, and is a prerequisite for acute tau clearance. *J Biol Chem* 2013;288(49): 35452–65.