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COMMENTARY

"Tau immunotherapy: Hopes and hindrances"

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurological disorder having two major pathological hallmarks: the extracellular senile plaques and intracellular neurofibrillary tangles composed of amyloid beta protein and hyperphosphorylated tau respectively. Removal of protein deposits from AD brains are the newer attempts for treating AD. The major developments in this direction have been the amyloid and tau based therapeutics. While senile plaque removal employing monoclonal antibodies (mAbs) restore brain function in mouse models of AD, tau has been recently introduced as the major neurodegenerative factor mediating neural cell death. So, several research groups have focused on tau therapy. So far, the outcome of tau immunotherapy has been promising and clearance of hyperphosphorylated tau has been shown to restore the brain function in animal models. But the point is which phosphorylated tau is the most critical form to be removed from the brain, especially because removal of physiologic tau can cause neurodegenerative consequence. Recently, we have shown that phosphorylated tau at Thr231 in the *cis* conformation is a very early driver of neurodegeneration and *cis* mAb treatment efficiently restores brain structure and function in TBI models. Because of efficient therapeutic effects in mice model of TBI and considering *cis* pT231-tau accumulation in AD brains, it could be a very good candidate for tau immunotherapy upon several tauopathy disorders including AD.

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The way between amyloid-beta and tau

Over the last ten years, researchers have invested significant effort in developing immunotherapies that are capable of targeting a variety of proteins and endogenous peptides. AD is believed to result from the deposit of amyloid-β (Aβ) in the form of amyloid plaques; as such, the earlier approaches that were developed to treat AD specifically aimed to remove these plaques. However, these therapies have recently evolved to target additional pathological aggregates associated with AD and many other neurological disorders.

The results of early clinical trials found that the clearance of amyloid plaques did not significantly reduce the progression of dementia and, as such, researchers concluded that there was a need to identify and study alternative prophylactic therapies.[1,2] Later research findings following Phase III Aβ antibody trials supported this finding,[2] and a more recent Phase II trial of an Aβ antibody was reported to exhibit a broad reactivity with various forms of Aβ.[3] However, not all research findings are in agreement. An alternative Phase I trial of various Aβ antibodies concluded that there was a possibility that the Aβ antibody could reduce the progression of AD; however, the initial promising findings were dissipated to some extent as a result of later follow-up studies on the same subjects.[3] The researchers concluded that there was a need to conduct larger trials to review the use of Aβ antibodies to treat AD. In many regards, it is somewhat understandable that removing Aβ may be insufficient to halt the progression of AD after clinical symptoms have manifest because there is no strong correlation between the degree of dementia and Aβ plaque burden.

Schenk et al. developed an active immunization following a study in which APP transgenic mice were vaccinated with Aβ1-42 (AN-1792).[4] In addition, a passive immunization approach has been employed that involved the use of monoclonal antibodies to reduce Aβ, and this method was found to reduce fibrillization *in vitro*.[5] The active immunization strategies that have more recently emerged include vanutide cridificar,[6] CAD106,[7] and AD02, the latter of which is a synthetic peptide that acts by imitating the Aβ peptide's N-terminus structure.[8] However, none of the methods that have been tested to date have been proven to result in significant clinical improvements in the symptoms associated with AD.[9] That said, some studies have yielded some interesting results. For example, one research study found that administering repeated subcutaneous injections of ACI-24 to AD transgenic mice resulted in the generation of high titers of anti-Aβ antibodies and a reduction in the concentration of soluble Aβ1-42 and insoluble Aβ1-40 and Aβ1-42.[10,11] In addition, the use of ACI-

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24 has also been found to enhance novel object recognition without eliciting a proinflammatory response.[11] Coimmunization involving a combination of Aβ1-42 DNA and protein has been found to induce Th2-type Aβ-specific antibodies while concurrently avoiding T-cell-mediated autoimmune responses and suppressing unsolicited inflammatory reactions.[12]

However, these treatment strategies are not without their issues. In one study, the administration of an active immunization using AN-1792 (full-length Aβ1-42) generated autoimmune responses, and 6% of the patient population developed meningoencephalitis as a result of T-cell infiltration.[13] A number of clinical trials involving a range of different antibodies, including BAN2401 (recognizing protofibrils),[14] crenezumab (aggregated species),[15] gantenerumab (fibrils),[16–18] and solanezumab (Aβ mid-domain)[19,20] are currently ongoing.

Recently, tau pathology is also of great interest to researchers who are seeking methods of treating AD and other tauopathies. Research has found that there is a stronger correlation between pathological tau and memory loss than that associated with Aβ deposition.[21] As such, there is a possibility that targeting tau may represent a more effective method of treating AD than removing Aβ if a patient is exhibiting clear signs of cognitive impairments. However, Aβ-targeting therapies remain significant as a prophylactic measure, and many clinical trials are currently evaluating this approach in hereditary cases of AD,[7] and in patients who are in the early stages of the sporadic form of AD.[4] It is highly likely that combination treatment approaches will emerge that target tau, Aβ, and other characteristics AD as a means of preventing or impeding its progression.

Tau immunotherapies

Another significant pathological factor of AD is Tau malfunction followed by the formation of NFTs.[22,23] Since there is a stronger correlation between tau pathology and the severity of dementia than Aβ pathology, there is a possibility that more positive clinical outcomes could be secured by focusing on the removal of tau as opposed to Aβ aggregates at the point the disease has progressed to the point that the patient exhibits cognitive impairments.[23] In addition, research has found that the evaluation of tau protein can provide a reliable indication of the extent to which AD has progressed in a subject suffering from the disorder. Although the evidence that is available at present isn't sufficient to resolve the tau-amyloid debate, the findings do indicate that further research into treatments that specifically target tau are warranted in the quest to identify more effective diagnostic and treatment strategies. Although researchers have proven that tau antibodies interact both extra- and intracellularly with the protein, the extent to which each site is significant for tau clearance is yet to be clearly defined.

Active and passive vaccines are two widely accepted immunotherapy strategies for the treatment of AD. Active immunization involves administering a pathogenic agent via an injection, while passive immunization involves administering a specific antibody to target a given antigen. The main objective of vaccinations that incorporate tau epitopes is to provoke an immune response against a set of pathological conformers of phosphorylated tau without simultaneously invoking an autoimmune reaction against the physiological varieties of this pervasive intracellular protein. Historically, active tau immunotherapy was first described in 2006,[24] followed by passive approach in 2010; however numerous other preclinical and clinical programs has been reported during the recent years with different characteristic based on clearance of tau pathological forms.[25]

Active tau immunotherapy

Currently, there are at least two available agents for generating active tau immunotherapy in clinical trials for AD (Table 1).

1) ACI-35 is a liposome-based vaccine that contains 16 copies of a synthetic tau fragment phosphorylated at S396 and S404. It currently is in Phase I clinical in the USA for the treatment of AD. ACI-35 elicits an immune response that specifically targets certain pathological conformers of phosphorylated tau while also avoiding invoking autoimmune B cell or T cell reaction against physiological types of this intracellular protein. Previous studies have found that administering ACI-35 via injection to tau P301L transgenic mice slightly reduced hyperphosphorylated pathological tau (64 kDa) and tau pathology by immunohistochemical characterization.[26] In addition, ACI-35 was reported to reduce three of the four clinical parameters that were tested: It extended the subjects' lifespan, increased body weight retention, and delayed the onset of a clasping motor phenotype in mouse.[27] However, the rotarod test indicated that it did not improve endurance. The trial is complete and results are pending yet (ISRCTN13033912). A further study is in process that aims to compare the safety and effects of an ACI-35 with a placebo when administered to patients with mild-to-moderate AD in Finland and the United Kingdom.
2) AADvac-1 is an axon peptide 108 conjugated to KLH that is formed of a synthetic peptide that originates from amino acids 294–305 of the tau sequence. It has been used as an additional means of immunotherapy for AD.[28] It currently is Phase 2 clinical trials in the USA for the treatment of AD.[29] Since 2013, the use of AADvac-1 has been tested by three different trials. Of these, the results of two trials indicated that AADvac-1 offers excellent immunogenicity and has a positive safety profile.[28,30] However, most importantly, the results also revealed that active immunization successfully eliminated the major signs of neurofibrillary pathology and resulted in a significant improvement in the clinical presentation of the transgenic rat population. The third trial was a 24-month, double-blinded, multi-center, Phase 2 randomized study that specifically focused on assessing the efficacy and safety of AADvac1 that was administered to a population that consisted of people with mild Alzheimer's disease and a placebo-controlled parallel group.[29]

The advantages and disadvantages of active tau immunotherapy

Studies on a tau transgenic model concluded that active tau immunization, with either P301Ltau or human wild-type tau, successfully reduced tau pathology and inflammation. As such, it represents a promising form of treatment for tauopathy

Table 1. Anti-tau therapeutic agents in clinical trials for treating Alzheimer's disease.

Therapy Type	Name	Synonyms	Company	Mechanism of action	U.S. FDA Status	ClinicalTrials .gov Identifier	Status
Immunotherapy (active)	AADvac-1	Axon peptide 108 conjugated to KLH	Axon Neuroscience SE	Immunostimulants; Tau protein modulators	Alzheimer's Disease (Phase 2)	NCT01850238 NCT02031198 NCT02579252	Phase 1 (completed) Phase 1 (completed) Phase 2 (recruiting participants)
	ACI-35		AC Immune SA, Janssen	Immunostimulants	Alzheimer's Disease (Phase 1)	Not available in ClinicalTrials.gov but in isrctn.com it was ISRCTN13033912	Phase 1b (completed)
Immunotherapy (passive)	C2N 8E12	ABBV-8E12	AbbVie, C2N Diagnostics, LLC	Tau protein inhibitors	Alzheimer's Disease (Phase 2)	NCT02880956	Phase 2 (recruiting participants)
	RG7345	RO6926496	Roche	Undefined mechanism	Alzheimer's Disease (Discontinued)		
	RO 7105705	RG 6100	AC Immune SA, Genentech, Hoffmann-La Roche	Tau protein inhibitors	Alzheimer's Disease (Phase 1)	NCT02820896	Phase 1 (active, not recruiting participants)
Small Molecule	LMTM	TRx0237, LMT-X, Methylene Blue, Tau aggregation inhibitor (TAI)	TauRx Therapeutics Ltd	Tau protein inhibitors	Alzheimer's Disease (Phase 3)	NCT01626391 NCT02245568 NCT01689233 NCT01689246	Phase 2 (terminated) Phase 3 (enrolling participant) Phase 3 (completed) Phase 3 (completed)
	Epothilone D	BMS-241027	Bristol-Myers Squibb	Tau protein inhibitors; Tubulin modulators	Alzheimer's Disease (Discontinued)	NCT01492374	Phase 1 (completed)
	Rember TM	Methylene Blue, methylthioninium (MT), TRx-0014, Tau aggregation inhibitor (TAI)	TauRx Therapeutics Ltd	Tau protein modulator	Alzheimer's Disease (Discontinued)	NCT00515333 NCT00684944	Phase 2 (completed) Phase 2 (completed)
	TPI 287		Cortice Biosciences	Microtubule protein modulators; Tubulin polymerisation inhibitors	Alzheimer's Disease (Phase 1),	NCT01966666	Phase 1 (active, not recruiting participants)
	Tideglusib	NP031112, Nypta®, Zentylor™, Glycogen synthase kinase 3 inhibitor, NP12	Zeltia Group	Tau protein modulators	Alzheimer's Disease (Discontinued)	NCT00948259 NCT01350362	Phase & 2 (completed) Phase 2 (completed)

disorders, including AD.[31] However, while a general understanding of how tau epitopes to target has developed,[32,33] there remains a solid need to assess multiple mouse models and to more precisely determine dose-response relationships for the antibodies that can effectively treat AD. However, the use of active tau immunotherapy is not without issues. Phosphorylation acts as the main physiological mechanism by which the tau structure and function are regulated. As such, one significant concern that is associated with active tau immunotherapy is that phospho-tau peptides may invoke an immune response to the physiological tau species.[34] Clinical trials with Aβ[13] or neuronal apoptosis have determined that active tau immunization is associated with a risk of inducing encephalitis. The results of these trials were aligned with those of an earlier study, which found that immunizing female C57BL/6 mice with full-length recombinant tau resulted in NFT-like changes, neurological deficits, an inflammatory infiltrate, and gliosis.[24] This research concluded that tau pathology could be initiated in response to the administration of tau to non-transgenic animals in the context of severe innate immune activation.[35]

A study on E257T/P301S-tau Tg mice and wild-type mice found that there is also a distinct risk of deleterious effects when phosphorylated tau is used as an epitope. During the study, immunizations that consisted of a combination of three phospho-tau peptides were repetitively administered to the mice. The subjects exhibited a reduction in tau pathology and neurofibrillary tangle burden by Gallyas staining, and a reduction in the phosphorylated forms of tau, as detected by immunostaining with the AT8 and AT180 antibodies. Furthermore, the immunized subjects exhibited an increase in lectin-positive microglial staining in comparison to the control subjects.

In addition, there are challenges that are associated with active tau immunization. First, the mechanism by which the immunization acts is heavily dependent on the immune response of the subject and this differs from patient to patient. As the immune response involved is complex and variable among individuals, there is a requirement for Phases 2 and 3 trials that more explicitly examine how immune responses vary and develop deeper insights into the relationship between the response and the dose and route of immunotherapy, regimen, and adjuvants. Second, there is a risk that the subject may develop tolerance in response to repeated immunizations over time. This needs to be monitored and assessed in more depth, particularly when a self-antigen is involved.

Passive tau immunotherapy

This section examines passive immunization with various antibodies. Four passive immunotherapies are currently in use as anti-tau agent: BMS-986168 (IPN007), RG7345 (RO6926496), C2N 8E12 (ABBV-8E12), and RO 7105705 (RG 6100) (Table 1). Each of these is reviewed in more depth below.

1) BMS-986168 is a humanized IgG4 monoclonal antibody that targets extracellular, N-terminally fragmented forms of tau (eTau). However, there is a risk that it can increase the production of Aβ and, thereby, cause the pathology to spread.[36] One study found that the use of an active vaccination to target the pS422 tau epitope reduced the amount of insoluble phosphorylated tau and improved the behavioral performance in a transgenic tauopathy mouse model. Since 2014, four trials (Phase 1–2) have been implemented for this antibody; however, the findings have yet to be reported.
2) RG7345 is a humanized monoclonal antibody that targets phospho-tau (pS422). Studies have found that the use of an active vaccination to target the pS422 tau epitope reduces the levels of insoluble phosphorylated tau and enhances behavioral performance in a transgenic tauopathy mouse model. Since 2014, one Phase 1 trial study has assessed the pharmacokinetics, tolerability, and safety of RO6926496 in healthy male participants.
3) C2N-8E12 is a humanized antibody that recognizes an extracellular, aggregated form of pathological tau. C2N-8E12 is different to some of the other anti-tau antibodies in that its mechanism of action does not depend on the uptake into neurons. There is currently a Phase 2 trial study in progress that is evaluating the efficacy and safety of the administration of C2N-8E12 to subjects with AD. This commenced in 2016.
4) RO 7105705 is a monoclonal antibody, targeting misfolded tau proteins. The first Phase 1 trial just started in 2016, to compare the antibody to placebo on safety, tolerability, pharmacokinetics, and preliminary activity outcomes. The study has been expected to run until May 2017.

The advantages and disadvantages of passive tau immunotherapy

Given the issues associated with an active immunization approach, as described above, it is reasonable to assume that a passive immunization approach with anti-phospho-tau directed mAbs could represent a safer treatment option. Two trials that examined the use of passive immunization as the targeting strategy found that tau-related pathology and motor deficits can be reduced if the antibody is administered prior to the onset of tau pathology.[37,38] A further study that involved the serial intracerebroventricular administration of anti-tau antibodies to P301S tau Tg mice aged six months of age over a three-month period found that the subjects exhibited a reduction in pathology and contextual fear conditioning deficits.[39] While this research proved that immunization with anti-tau antibodies at a time when the pathology was already present could improve behavior, the researchers concluded that the intraventricular route employed in this research represented a major disadvantage. In addition, the only study that has been conducted to date that demonstrated an improvement in pathology after its onset was unable to prove that the long-term survival rate of the immunized animals was better than that of the controls.[40] The researchers involved in this study compared MCI (detects a pathological tau conformation), DA31 (a pan-tau antibody), and PHF1 (detects pSer396/404) in P301L Tg tau mice, which have an onset of pathology at about three months of age. While the mice that were immunized with MCI exhibited a reduction in tau-related pathology immunohistochemically and biochemically between 7 and 10 months of age, there were no differences in the survival rate between the subjects injected with MC1 or PHF1 between 6 to 14 months of

age versus the control Tg mice.[40] Previous studies have found PHF1 can reduce tau-related pathology if the subjects are treated before the onset of disease.[37] In combination, the results of the existing trials indicate that, although immunotherapy that specifically targets tau is promising, there is an underlying toxicity risk. As such, more research needs to be conducted that more clearly identifies the tau form to be administered and the optimal time at which immunotherapy should be initiated.[41] It is also of note that not all phospho-specific tau antibodies (passive immunization) are effective at preventing the development of tau pathology in animal models; in fact, some phospho-specific tau antibodies have been found to intensify pathology.[41]

Small molecules in tau target therapy

While five different agents have previously been presented as small molecule agents in tau therapy that targets AD, three of these, including Epothilone D, Rember TM, and Tideglusib have been discontinued for FDA approval (Table 1). The two that remain in use are described below.

1) TRx 0237 (LMTX^{TM}), which is a purified form of Methylene Blue, is a second-generation tau protein aggregation inhibitor. At present, no Phase 1 trials on TRx0237 have been conducted. A four-week Phase 2 safety study in which TRx0237 was administered to patients with mild-to-moderate Alzheimer's disease at a dose of 250 mg/day was initiated in September 2012, was terminated the following April with administrative reasons cited. To date, three Phase 3 studies on TRx0237 have been conducted. However, the study by Gauthier et al. was the only one to specifically assess AD treatment, and the results of this trial were negative, indicating that the use of TRx0237 small molecules in tau target therapy to treat subjects with mild-to-moderate AD was not beneficial.[42]
2) TPI 287 is a microtubule-stabilizing and tubulin-binding drug that is a synthetic derivative of the taxane diterpenoid drugs that are administered to patients with cancer. At present, two trials are listed on clinicaltrials.gov that involve the use of TPI 287 as an anti-tau agent. The University of California, San Francisco, initiated a Phase 1 trial of TPI 287 in in 2013. Their research involves 33 patients who are suffering from mild-to-moderate AD and seeks to identify the maximal tolerated dose of the TPI 287 drug and the effects of drug exposure in plasma and CSF. This trial, like a further joint study that is being conducted by the University of Alabama, is ongoing and will continue until March 2017.

Tau immunotherapy has been promising

Previous research has found a link between tau hyperphosphorylation and neurodegeneration with phosphorylation in more than 20 sites in the brains of subjects suffering from AD.[43–45] Anyhow, it is not really clear which phosphorylated state is the most pathogenic and critical to remove from the brain.[46,47] Especially, AD progression takes quite a while to happen,[48] sometimes over a decade, makes it hard to track the pathogenicity and target the most critical p-tau epitope driving neurodegeneration. Moreover, AD brain patients go through atrophy as the disease develops,[49] which means the right timing for immunotherapy (apparently before brain shrinkage) is a determinant factor. Taking these together, early diagnosis and therapy is a very critical factor for immunotherapy which has to be done before brain atrophy.

On the other hand, the inappropriate tau clearance may cause some abnormalities in the axonal structure and function.[50] Thus, it is of crucial importance to target the early pathogenic p-tau epitope. So far, most of known abnormally phosphorylated states are detectable only at very late stages of hyperphosphorylation which seems that clearance is too late to retrieve neuronal function.[25,51] While there have been extensive attempts to neutralize tau toxicity employing mAbs removing different p-tau epitopes, only by targeting specific epitopes like pSer202, pSer413, pThr231 and pSer422 a decrease in insoluble/soluble tau in the brain was observable and the brain loss of function has been stopped in those mice models.[52]

Furthermore, treatment with a phospho-tau peptide (containing the phosphorylated PHF-1 epitopes Ser 396 and Ser 404) in animal models prior to the onset of pathology has proven successful in preventing development of tau aggregates in the Tg P301L mouse tau model.[53] Phosphorylation at these specific epitopes has been found to increase the fibrillogenic character of tau and enhance the formation of paired helical filaments.[54,55] It is also of note that studies that specifically target pSer409 have not found the expected significant improvement in the subjects' condition.[25,56] A further study that employed an Ab to target the Thr231 epitope found that it reduced p-tau AT8-immunoreactivity in the hippocampal fraction of the tg4510 animals.[30] As such, although it may be possible to present some generalizations concerning which tau epitopes to target via tau immunotherapy,[32,33] there is a requirement for further studies that assess various mouse models and evaluate which dose-response relationships for antibodies are the most effective.

Cis pT231-tau is the major pathogenic factor in tauopathy

There are more than 80 phosphorylation sites on longest human tau isoform.[57] Most of the sites are being phosphorylated under physiological conditions but hyperphosphorylation would cause pathogenicity and neurodegeneration. AD process takes more than a decade to happen[48] but so far, neuroscientists have been able to detect the very late stages p-tau epitopes.[43] Tauopathy starts from one spot in the brain and then spreads into neighboring areas; causes comprehensive neurodegeneration and brain atrophy. Thus, early diagnosis and treatment is indeed of crucial importance for efficient therapy. It has not been clear which phosphorylation event is the most pathogenic one for driving tauopathy so that makes tau immunotherapy less impressive.[57] We have recently shown that phosphorylated tau at Thr231 could be exist in the two distinct cis and trans conformation whose conversion is being mediated by Pin1 isomerase[32,33] and have demonstrated that cis but not trans conformation is extremely neurotoxic.[58] We have gone on to generate conformation specific cis and trans monoclonal antibodies (mAbs) that pass through blood brain barrier (BBB)

after Traumatic Brain Injury (TBI) and could be taken up by neurons. We have introduced *cis* pT231-tau as central mediator in TBI and neurodegeneration, leading to CTE, which is a risk factor for AD, and that cis mAb efficiently cleans cis p-tau and restores brain structure and function upon TBI which sounds like an excellent therapeutic. We have shown that various stresses including hypoxia culturing, nutrition depletion and serum starvation in the cultured neurons and also trauma in mice brain would induce prominent *cis*, but not *trans* pT231-tau accumulation in neurons. We have demonstrated that the more *cis* p-tau reflects the more cell death in cultured neurons. Interestingly, optional removal of *cis*, but not trans, pT231-tau using our cis mAb brings back the phenomena and suppresses neural cell death. Also, *cis* p-tau causes microtubule destruction resulting in mitochondrial transport deficiency while *cis* mAb treatment restores the axonal transport. We have shown that *cis* p-tau causes axonal conductivity impairment while *cis* pT231-tau clearance using *cis* mAb repairs the abnormality in mouse brain. While *cis* pT231-tau causes brain atrophy, *cis* mAb treatment restores the brain size. Also, *cis* p-tau causes abnormal risk-taking behavior and *cis* mAb application improves the cognitive decline. Notably, we have previously shown that there is a prominent *cis*, but not *trans* pT231-tau accumulation in MCI and AD brain patients employing *cis & trans* polyclonal antibodies[32,33] and monoclonal antibodies (unpublished data) which makes the *cis* mAb a reasonable therapeutic for AD therapy.

Cis pT231-tau is the early monomeric pathogen p-tau epitope

Hyperphosphorylated tau goes through microtubule dissociation, dimerization and NFT formation; the process of which takes a long time whereby has not been possible to track the aggregation thus far. We have demonstrated that *cis* pT231-tau is not only a monomeric p-tau epitope but is the driver of tau aggregation. We have examined the aggregation process using sarkosyl extraction and have found that *cis* pT231-tau is detectable 24 hours of neural stress but later on appeared in tau aggregates in neurons. We have detected colocalized *cis* pT231-tau with AT100, AT180, AT8, PHF-1 and Alz50, the late stages p-tau epitopes, but also appeared early upon neural cell stresses. Also it was colocalized with T22, a marker of tau oligomers. Importantly, optional *cis* pT231-tau removal using *cis* mAb could stop tau aggregation *in vitro* and *in vivo*.[58] On the other hand, we have shown that *cis* pT231-tau has a prion nature, could be spread into neighboring brain areas as well as CSF. It has been shown that *cis* p-tau is observable in AD and MCI but not normal CSF samples[32,33] demonstrating of a diagnostic AD marker at early stages long time ahead of aggregation and pathogenicity. Thus, targeting *cis* pT231-tau using mAb sounds like an excellent therapeutic strategy.

Conclusion

Although Aβ targeting has shown promising results in preclinical studies tau seems a better therapeutic target.[23,59] As mentioned earlier, extensive works have been carried out to neutralize p-tau toxicity but it has remained unclear which p-tau epitope is the central mediator in tauopathy disorders including AD.[30,31] Since we have clearly introduced *cis* pT231-tau as central mediator in TBI and neurodegeneration and its elimination using our *cis* mAb restores the phenomena,[58] and also considering its accumulation upon MCI and AD, it seems a very good candidate for AD therapy as well. Especially, we have shown that *cis* mAb improves ultrastructure as well as brain size upon TBI in mice models and recovers neuronal physiology in Ab-treated TBI mice. Anyhow, that *cis* mAb is a mouse antibody and is not applicable to human at the present form and needs to be humanized. The biggest concern is when modifying the Ab, it may not pass through BBB and may not get in to neurons anymore. Also, it still may have immune response which will be other major problem. Anyhow, there are several feasible ways to stop probable side effects. For example, loading the Ab into vesicles may suppress immunogenic response[60] and intranasal administration may enhance drug delivery into brain.[61] Taking all these together, *cis* pT231-tau could be a very good candidate for tau immunotherapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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