Antibody Therapeutics Targeting A β and Tau

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The astonishing findings that active and passive immunization against amyloid- β (A β) in mouse models of Alzheimer's disease (AD) dramatically decreased amyloid burden led to a rapid initiation of human clinical trials with much enthusiasm. However, methodological issues and adverse effects relating to these clinical trials arose, challenging the effectiveness and safety of these reagents. Efforts are now underway to develop safer immunotherapeutic approaches toward A β and the treatment of individuals at risk for AD before or in the earliest stages of cognitive decline with new hopes. Furthermore, several studies have shown tau as a potential immunotherapeutic target for the treatment of tauopathy-related diseases including frontotemporal lobar dementia (FTLD). Both active and passive immunization targeting tau in mouse models of tauopathy effectively decreased tau pathology while improving cognitive performance. These preclinical studies have highlighted tau as an alternative target with much anticipation of clinical trials to be undertaken.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common form of dementia and is characterized by widespread neurodegeneration that progresses throughout the neocortex and limbic system. More than five million people in the United States alone suffer from AD, and this number is expected to quadruple by 2050 because of the increasing aging population. AD is the sixth leading cause of death in the United States, with an economic burden exceeding \$1 trillion annually and with no definitive method for diagnosis or treatment.

Neuropathologically, the AD brain contains an extracellular buildup of amyloid plaques, which are composed of amyloid- β peptide (A β), and intraneuronal accumulation of intra-

cellular neurofibrillary tangles (NFTs), which are composed primarily of aggregated forms of hyperphosphorylated tau protein. The cleavage of amyloid precursor protein (APP) by β - and γ -secretases generates the A β peptide. Several factors cause A β to aggregate and form amyloid plaques. Rare forms of dominantly inherited AD are caused by mutations in the APP or the presenilin (*PSEN1* and *PSEN2*) genes. *PSEN1* and *PSEN2* proteins are part of the γ -secretase complex that cleaves APP. Together, these gene mutations strongly implicate A β aggregation as causative to the disease process (Sisodia and St George-Hyslop 2002; see also Johnson et al. 2017). These early observations led to the "amyloid cascade hypothesis," which states that the accumulation of amyloid plaques results from an imbalance among the produc-

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tion, aggregation, and clearance of A β , initiating the disease process (Hardy and Allsop 1991; Hardy and Selkoe 2002). The accumulation of A β aggregates further facilitates the formation of intracellular NFTs, which progresses disease. These findings have led to therapeutic strategies that include inhibitors or modulators of β - and γ -secretases to target A β production or to promote A β clearance (Holtzman et al. 2011; Ghosh et al. 2012).

MOUSE MODELS OF A β AMYLOIDOSIS

Following the observation that A β aggregation and plaque formation are associated with the development of AD, immunization against the plaque-forming A β protein was first tested in animals in 1999. Active immunization with a synthetic human A β 42 protein in PDAPP mice, a transgenic mouse model of AD, developed and maintained a high titer of anti-A β antibody (Schenk et al. 1999). Strikingly, immunization of young animals halted the development of A β -related neuropathological hallmarks of AD, including A β plaques, dystrophic neuritis, and gliosis. Furthermore, immunization of aged animals after the start of plaque deposition also revealed a significant decrease in amyloid burden, neuritic dystrophy, and gliosis. Shortly after these findings, Janus et al. (2000) and Morgan et al. (2000) showed that active immunization in AD mouse models before the development of plaque pathology decreased the development of memory loss and other cognitive deficits in APP transgenic mice. However, the neuroprotective effects on memory did not always corroborate with a significant decrease in amyloid-plaque pathology.

The overwhelming success of active immunization in mouse models of amyloidosis was immediately followed by studies of passive immunization by intraperitoneal injection of monoclonal antibodies against A β . Peripheral administration of monoclonal antibodies against A β peptide in PDAPP mice reduced cortical A β levels and effectively decreased A β plaque pathology (Bard et al. 2000). In addition, treatment with anti-A β antibodies in 13-month-old PDAPP mice, which showed some

early signs of plaque deposition, significantly cleared preexisting amyloid plaques. In another study by DeMattos et al. (2001), chronic parenteral treatment with anti-A β monoclonal antibody suppressed A β pathology in young PDAPP mice and strongly increased plasma A β levels by as much as 1000-fold over basal levels. Furthermore, subchronic and acute passive immunization of PDAPP mice aged 24 months improved their memory and behavioral performance without any apparent effect on plaque pathology (Dodart et al. 2002).

The dissociation between the neuroprotective effects on memory deficit and A β pathology was evident for both active and passive immunization studies. Additional studies in mouse models of AD have further illustrated that effects on behavior do not always correlate with effects on A β plaque pathology (Hartman et al. 2005; Oddo et al. 2006; Chen et al. 2007). Collectively, these studies suggest that other mechanisms, in addition to A β plaque formation itself, may underlie the memory and cognitive deficiency in APP transgenic mouse models of A β amyloidosis (Brody and Holtzman 2008). Conceivably, active or passive immunization targets toxic, soluble A β species (or species that can be solubilized) and sequesters or facilitates clearance of these toxic species from the central and peripheral nervous systems, thus decreasing cognitive and memory deficits (Klein et al. 2001; Selkoe 2002). Indeed, in studies of human AD pathology, the amyloid burden does not show a strong correlation with the severity of dementia, whereas a stronger correlation is shown between the level of biochemically soluble A β and the severity of cognitive impairment (Lue et al. 1999; Wang et al. 1999; Esparza et al. 2013). Furthermore, in vitro and in vivo studies in mice expressing human APP have illustrated that soluble, oligomeric forms of A β are not only neurotoxic but also impair memory (Lambert et al. 1998; Walsh et al. 2002; Lesné et al. 2006; Shankar et al. 2008). A major challenge for the field is to determine whether amyloid-plaque clearance itself or sequestration-clearance/neutralization of soluble oligomeric A β species prevents or slows neurodegenerative processes in human AD.

MECHANISMS OF ANTIBODY-MEDIATED A β CLEARANCE

Understanding the mechanism that accounts for the clearance of A β or neutralization of its toxicity is critical to develop a safer and more effective immunotherapeutic. A number of hypotheses have arisen that are largely dependent on whether antibodies effectively enter the central nervous system (the “CNS hypothesis”) and have their effects there or whether the antibody present in the periphery is sufficient to result in beneficial effects (the “peripheral sink hypothesis”) (Fig. 1). It is possible that more than one mechanism is important. All antibodies present in the periphery enter the CNS to some extent. Approximately 0.1%–0.2% of the concentration of an IgG in plasma is present in the cere-

brospinal fluid (CSF). Initial studies in immunized PDAPP mice illustrated that anti-A β antibodies effectively entered the CNS and decorated A β plaques. Immunohistological analyses presented evidence for a microglia-dependent phagocytosis of A β , as activated microglia cells were associated with the remaining plaques that were immunoreactive for A β (Bard et al. 2000). Moreover, ex vivo assay using sections of PDAPP mouse brains and topical antibody treatment induced a microglia phagocytic clearance of A β plaques that were Fc-dependent. Despite these findings, anti-A β antibodies that lack effector function have still been shown to have efficacy (Bacskai et al. 2002; Adolfsson et al. 2012). This phenomenon suggests that more than one mechanism of local clearance may be at play in addition to FcR γ -mediated microglial

Figure 1. Schematic of potential mechanisms for antibody-mediated clearance of amyloid- β . Antibodies within the central nervous system (CNS) potentially clear amyloid deposits in a microglia-dependent phagocytosis mechanism (“CNS hypothesis”). Local clearance in the CNS may also occur in a non-Fc γ mediated mechanism. Alternatively, antibodies within the periphery sequester amyloid- β (A β), altering the dynamics of A β into the CNS and lowering amyloid burden (“peripheral sink hypothesis”). It is possible that more than one mechanism is important.

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clearance, such as direct dissolution of plaques or alteration of the equilibrium between plaques and soluble A β species.

The initial findings that soluble A β is cleared from the CNS into the periphery across the blood–brain barrier by active transport, together with a continuous influx of A β from the periphery into the CNS, are the initial bases for the “peripheral sink hypothesis” (Fig. 1) (Shibata et al. 2000; DeMattos et al. 2001; Dodart et al. 2002; Deane et al. 2003; Sagare et al. 2007). It is plausible that passive immunization sequesters soluble A β within the periphery, lowering free A β concentrations in the blood and resulting in a net efflux of A β from the CNS (DeMattos et al. 2001; Dodart et al. 2002). Indeed, passive immunization with anti-A β monoclonal antibody m266 effectively reduced amyloid pathology in AD mouse models without directly binding to A β plaques, yet a massive increase of A β levels was detected in the plasma. Therefore, disrupting the A β equilibrium between the CNS and periphery may have played a role in decreasing the amyloid burden. Moreover, active immunization with a nonfibrillogenic A β peptide that elicited an attenuated IgM antibody response that is less permeable to the blood–brain barrier improved cognitive performance and effectively lowered the A β burden (Sigurdsson et al. 2004). Further analysis revealed that plasma levels of IgM correlated inversely with the amyloid burden. In another study, immunization of transgenic mice bearing two vasculotropic mutations within APP that exhibit diminished A β transport across the blood–brain barrier failed to promote A β clearance into the plasma, supporting the “peripheral sink hypothesis” (Vasilevko et al. 2007). These studies argue that peripheral sequestration of A β may also be operative. An antibody such as m266 is also likely to locally sequester monomeric A β in the brain, where it could also directly sequester soluble A β , altering the equilibrium between soluble and aggregated species.

ACTIVE IMMUNIZATION TARGETING A β

The first immunotherapeutic to target amyloid burden in AD patients, AN1792, entered phase I

clinical trial in 2000. The initial trial included 24 patients who were treated with a single dose using a mixture of full-length fibrillar A β protein, along with a saponin adjuvant to prime the immune system. No adverse effects were reported, and patients showed good tolerance. The trial quickly transitioned to a multiple dosing study that involved more than 70 patients with mild to moderate sporadic AD. Although AN1792 was well tolerated and elicited a good immunological response, one patient developed dizziness, drowsiness, fever, and unstable gait following a fifth injection with a reformulated preparation containing polysorbate 80. The patient remained unstable until she died from pulmonary embolism 1 year after the last injection and well after a phase II study had been initiated. Postmortem examination confirmed the patient had developed T-lymphocyte predominant meningoencephalitis, a symptom not associated with AD (Nicoll et al. 2003). The exact cause of meningoencephalitis remains unknown; however, cytotoxic T cells surrounding cerebral vessels suggest an excessive Th1-mediated response.

The phase I study was too small to determine possible efficacy, and a phase II study of active immunization with a mixture of full-length fibrillar A β protein and polysorbate 80 was initiated and included 372 mild to moderate AD patients. The study was abruptly halted in January 2002 after four actively immunized patients developed signs that were consistent with meningoencephalitis (Senior 2002). An additional study identified that a total of 6% (18/300) of actively immunized patients developed meningoencephalitis, with no obvious relationship between the number of injections and the severity of postvaccination meningoencephalitis (Orgogozo et al. 2003). In addition, there was no consistent correlation between A β antibody titers and the development of encephalitis.

Although the AN1792 trial was terminated before the therapeutic benefits on cognitive decline could be evaluated, insight into the potential of immunotherapies was gained by neuropathological analysis of the first deceased participants in the phase I trial. Postmortem

analyses revealed that the brains of patients treated with AN1792 showed characteristics that resembled those in the brains of aged PDAPP mice following A β immunization (Nicoll et al. 2003). Extensive areas of the cerebral cortex displayed low A β plaque density, with decreased plaque-associated dystrophic neurites. Furthermore, both astrocyte and microglia clusters were significantly decreased, and A β immunoreactivity was associated with microglia cells, implicating active phagocytosis as a plausible mechanism of clearance. However, NFTs, neurofibrillary tangles, and cerebral amyloid angiopathy were unaltered and displayed typical AD pathology.

More recent neuropathological studies of the participating patients from the AN1792 immunization trial confirmed that active immunization with full-length fibrillar A β protein can lead to a reduced A β burden (Masliah et al. 2005; Serrano-Pozo et al. 2010). Compared with nontreated AD patients, a significant reduction in total A β deposits with reduced plaque density throughout the cerebral cortex and hippocampus was observed. Furthermore, the decrease in amyloid burden was inversely correlated with anti-A β antibody titers in plasma and CSF. In addition, a significant decrease in A β protein levels from the frontal cortex of actively immunized patients was also evident, which is consistent with a reduction in A β plaques. More recently, quantification of phosphorylated tau and total tau protein in CSF showed reduced levels in immunized patients, suggesting that the neurodegenerative process can be reduced (Gilman et al. 2005; Boche et al. 2010).

Despite the overwhelming evidence that active immunization decreases amyloid burden and related pathology in AD patients, there is little evidence to suggest any major positive effects on cognitive function. Although early reports for a subset of patients treated with AN1792 described modest but significant slowing of cognitive decline, recent long-term follow-up studies have yielded no differences in either progression of dementia or overall survival (Hock et al. 2003). Furthermore, neuropathological studies in a few AN1792 patients who displayed significant complete amyloid-

plaque clearance showed they had still developed severe end-stage dementia at death (Holmes et al. 2008). Importantly, these early studies were not designed to show the effect of treatment on cognition and were terminated very early. In addition, these early studies were performed in individuals with mild to moderate dementia believed to be attributable to AD and took place years after A β deposition had likely reached its maximal extent (Holtzman 2008). Therefore, it is likely that this time point was not optimal to target A β during the course of AD.

Although the AN1792 trial provided little evidence that AN1792 prevented cognitive decline in AD, a second generation of active A β immunization was developed to avoid a Th1-mediated cellular response. CAD106 consists of a small A β fragment (A β 1–6) eliminating residues 15–42, which have been attributed to Th1-lymphocyte activation leading to meningoencephalitis (Furlan et al. 2003; Wiessner et al. 2011). To induce an immune response, A β 1–6 was coupled to a carrier containing 180 copies of the coat protein from bacteriophage Q β . In mouse models of AD, CAD106 induced anti-A β antibodies and reduced amyloid-plaque burden (Wiessner et al. 2011). A phase I clinical trial was initiated in 2012 to assess tolerability, immune response, and potential alterations of biomarkers in mild to moderate AD patients (Winblad et al. 2012). Subjects treated with CAD106 reported minor adverse effects (predominantly nasopharyngitis) but no cases of meningoencephalitis, which ended the AN1792 trial. At 8 weeks after injection, levels of anti-A β antibody in plasma peaked and correlated with an increase in total A β protein in plasma. In the control and CAD106-treated groups, no significant changes were identified in CSF biomarkers. A phase II clinical trial was recently completed that exposed subjects to antibodies at higher doses for longer time points. Data analyses are pending (ClinicalTrials.gov Identifiers: NCT01097096, NCT00956410, NCT00795418, NCT01023685, and NCT00733863).

Affitope and ACC-001 are additional active A β immunotherapies designed to avoid activa-

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tion of the Th1-mediated response. Similar to CAD106, Affitope is composed of a small synthetic peptide of six amino acids from the N-terminus of A β with an aluminum hydroxide adjuvant (Schneeberger et al. 2009). Following a 2-year phase I trial that tested tolerability and immunogenicity, a phase II trial in patients with early AD as diagnosed by episodic memory deficit, hippocampal atrophy, or magnetic resonance imaging (MRI) was recently completed, with data pending (ClinicalTrials.gov Identifier: NCT01117818). ACC-001 is an N-terminal peptide conjugated to a surface-active saponin adjuvant OS-21, an immunostimulatory agent. Currently, ACC-001 is in phase II clinical trial; however, no data have been reported at the time of this review (ClinicalTrials.gov Identifier: NCT01284387).

PASSIVE IMMUNIZATION TARGETING A β

Although active immunization presents a sustainable long-term treatment that may be favorable for AD, especially as a primary or secondary prevention, several concerns have arisen. In particular, active immunization is dependent on immunogenicity, which may not be favorable in elderly patients who may show a decreased immune response. Moreover, the specificities and titer of each generated antibody may vary vastly among individuals, making it difficult to control dosing and adverse effects. Furthermore, adverse effects are difficult to stop because active immunization is long lasting. Passive immunization is an alternative approach to overcome many of these complications and does not rely on an immune response; rather, an externally generated antibody is administered. Therefore, this method of immunization allows strict regulation of antibody titer, permitting a safer control of adverse effects. In addition, passive immunization allows precise targeting of epitopes or conformations, decreasing variability among individuals.

The first passive immunization trial in humans involved Bapineuzumab (Table 1), a humanized version of the mouse anti-A β monoclonal 3D6 antibody targeting the N-terminus of A β 1–5. In mouse models of AD, 3D6 was

shown to bind soluble, oligomeric, and fibrillar A β species, which effectively decreased amyloid burden when administered preventatively (Bard et al. 2000). When given to animals with significant A β burden, 3D6 did not significantly decrease A β burden (DeMattos et al. 2012). A phase II trial revealed that high doses of Bapineuzumab were associated with amyloid-related imaging abnormalities (ARIAs) and intracerebral microhemorrhages, which were observed more frequently in APOE ϵ 4 carriers (Salloway et al. 2009; Rinne et al. 2010). These adverse effects may be caused by certain antibodies, including 3D6, that target aggregated forms of A β and that have intact Fc γ -receptor function, which leads to an increase in vascular A β , inflammation, and damage to the blood–brain barrier (Pfeifer et al. 2002; Wilcock et al. 2004; Racke et al. 2005).

In a phase III trial with Bapineuzumab, a moderate but significant reduction in phosphorylated tau in CSF was reported, specifically in known carriers of APOE ϵ 4 who were treated with Bapineuzumab, which is consistent with phase II results (Salloway et al. 2014). In addition, Pittsburgh compound B (C-PiB) measurements of A β plaques revealed a slight reduction in amyloid burden in APOE ϵ 4 patients treated with Bapineuzumab. However, in small subsets of noncarriers of APOE ϵ 4 studied with biomarkers, there were no significant changes in either biomarkers or amyloid burden. Despite promising results within the APOE ϵ 4 carrier group, the overall phase III trial failed to meet primary end points with respect to changes in cognitive and functional performance. A more recent phase I trial has been completed with a new version of Bapineuzumab (AAB-003) that was reengineered to prevent the adverse effects of ARIAAs and to enable higher sustainable dosing in mild to moderate AD patients (ClinicalTrials.gov Identifier: NCT01193608).

Solanezumab is a humanized version of a murine anti-A β monoclonal antibody m266 that selectively targets the mid-domain of the A β protein (Table 1). Studies in AD mouse models showed that m266 specifically binds soluble A β with little affinity for fibrillar A β

Table 1. Clinical trials

Antibody	Company	Isotype/ binding domain	Targets	ClinicalTrials .gov Identifier	Status
Bapineuzumab	Elan/Pfizer/ Johnson & Johnson	IgG1 N terminus of A β 1–5	Soluble and aggregated A β	NCT00575055	Phase III completed
				NCT00574132	Phase III completed
				NCT01193608	Phase I trial ongoing
Solanezumab	Eli Lilly	IgG1 mid-domain of the A β 16–23	Soluble A β	NCT00905372	Phase III completed
				NCT00904683	Phase III completed
				NCT01148498	Phase II completed
				NCT01127633	Phase III ongoing
				NCT01760005	Phase II/III currently recruiting
Ponezumab	Pfizer	IgG2a C terminus of A β 33–40	Soluble and aggregated A β	NCT01900665	Phase III currently recruiting
				NCT00945672	Phase II completed
				NCT00722046	Phase II completed
Gantenerumab	Roche	IgG1 N terminus and central portion of A β	Aggregated A β	NCT01821118	Phase II currently recruiting
				NCT01760005	Phase II/III currently recruiting
				NCT0122416	Phase III currently recruiting
Crenezumab	Genentech/ Roche	IgG4 mid-domain of the A β 12–23	Monomeric, fibrillar, and soluble oligomers	NCT01723826	Phase II ongoing
				NCT01397578	Phase II currently recruiting
				NCT01343966	Phase II currently recruiting

or amyloid-plaque deposits (DeMattos et al. 2001; Dodart et al. 2002). Passive immunization of aged AD mouse models with m266 reverted memory deficits independent of altering amyloid pathology. In a phase II study, 52 patients displaying mild to moderate AD received monthly injections of Solanezumab for 3 months and showed good tolerance with no significant adverse effects (Farlow et al. 2012). Following a 12-week end point, no significant changes in cognitive performance were measurable between the placebo and Solanezumab groups. In addition, amyloid-imaging studies using PiB did not reveal any significant changes within the group treated with Solanezumab. Biomarker analyses revealed a significant increase in total A β concentrations in the CSF of individuals treated with Solanezumab with no changes in tau protein. Consistent with phase I trials, no significant adverse effects were re-

ported, including no evidence of ARIAs or meningoencephalitis, which had been previously reported for Bapineuzumab and ended the AN1792 trial, respectively (Siemers et al. 2010).

Elevated A β concentration in the CSF of subjects in the group treated with Solanezumab was reminiscent of similar results in AD mouse studies, which led to significant improvements in cognitive and memory impairment. These results prompted two large-scale, double-blind phase III trials, EXPEDITION 1 and EXPEDITION 2 (Doody et al. 2014). The paradigm consisted of monthly injections in more than 1000 mild to moderate AD patients, with primary outcomes measured from baseline to 18 months. Analysis included cognitive function, quality of daily activity, morphological measurements, and biomarkers. Consistent with mouse models of AD, Solanezumab did not alter the amyloid burden in either trial in the

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small subset of subjects in which amyloid imaging was assessed, which was plausibly attributable to its preferential binding to soluble A β and lack of direct binding to fibrillar A β plaques. Both clinical trials, EXPEDITION 1 and EXPEDITION 2, showed no significant differences in whole brain or hippocampal volumes as measured by MRI. Measurement of cognitive performance at the end point for the EXPEDITION I trial revealed no beneficial effects on the progression of dementia in mild to moderate AD patients treated with Solanezumab. Conversely, a prespecified analysis in EXPEDITION 1 revealed a significant reduction in cognitive decline in a subgroup of patients with mild AD. These findings led to a revised analysis for EXPEDITION 2, in which patients with mild AD were considered the primary-analysis population. These analyses revealed significant cognitive and functional benefits in some assessments in patients with mild AD.

Analyses of biomarkers revealed a significant increase in A β in both plasma and CSF 4 months following the administration of Solanezumab, which was sustainable to the end point. An increased A β concentration in the CSF of patients treated with Solanezumab suggests that the dynamic of A β transport was altered, favoring the efflux of A β from the brain and potentially decreasing soluble A β toxicity. No other biomarkers, including tau, showed any significant changes. As in phase II trials, no evidence of ARIAs or meningoencephalitis was reported.

Although both EXPEDITION 1 and EXPEDITION 2 did not meet the overall end point for beneficial effects on cognitive decline in mild to moderate AD, the finding that Solanezumab moderately slowed cognitive decline in mild AD patients has propelled three ongoing trials: a phase III trial in mild AD (EXPEDITION 3); a secondary prevention trial in asymptomatic individuals with cerebral amyloidosis (A4); and an adaptive trial of secondary prevention in the dominantly inherited Alzheimer's network.

Ponezumab, a humanized monoclonal antibody targeting the C-terminus of A β ₄₀, was designed specifically to bind to soluble and aggregated A β but without effector function. Po-

nezumab was uniquely engineered to eliminate binding to Fc γ receptors and complement component 1, minimizing clearance of A β mediated by an immune response (Table 1) (La Porte et al. 2012). Studies in mouse models of AD showed that Ponezumab binds both soluble and fibrillar A β . Passive immunizations led to increased A β levels in plasma and decreased amyloid levels in the brain. Following these studies in mice, a phase I clinical trial was initiated. Reportedly, Ponezumab increased A β ₄₀ levels in plasma and CSF in a dose-dependent fashion and with minimal adverse effects (Burstein et al. 2013; Landen et al. 2013). Similarly, a phase II trial showed increased A β ₄₀ levels in plasma with patients showing good tolerance. However, little to no improvement of cognitive impairment was observed at end point. Ponezumab is not currently being tested in AD, although it is being tested in cerebral amyloid angiopathy, in which A β ₄₀ is the most abundant A β species deposited in the brain (ClinicalTrials.gov Identifiers: NCT00945672, NCT00722046, NCT01821118).

Unlike other passive immunotherapies targeting A β that are humanized versions of murine monoclonal antibodies, Gantenerumab is the first fully humanized IgG1 antibody (Table 1). Gantenerumab displays specific affinity for both the N-terminus and central portions of fibrillar A β , with no affinity for monomeric A β (Novakovic et al. 2013). In studies of AD in mouse models, Gantenerumab decreased amyloid pathology without altering levels of A β in plasma or CSF. Ex vivo assays suggested that Gantenerumab induced phagocytosis of amyloid plaques, which was mediated by microglia (Bohrmann et al. 2012). Subsequently, a phase I clinical trial for Gantenerumab reported a dose-dependent reduction in amyloid burden as measured by amyloid imaging by positron emission tomography (PET) scan with PiB in mild to moderate AD patients (Ostrowitzki et al. 2012). However, higher doses of Gantenerumab were associated with microhemorrhages, which were observed more frequently in APOE ϵ 4 carriers, similar to those seen with high doses of Bapineuzumab (Novakovic et al. 2013).

A more recent phase II adaptive trial involving dominantly inherited AD patients has been

initiated to test tolerability, biomarkers, and amyloid burden assessing Gantenerumab and Solanezumab as well as a potential third agent in the near future (ClinicalTrials.gov Identifier: NCT01760005). In addition, a phase III trial has also been initiated to expand over a 2-year period to determine the efficacy of Gantenerumab in preventing the progression of symptoms in prodromal AD patients (ClinicalTrials.gov Identifier: NCT01224106).

A major concern for certain anti-A β antibodies, particularly those that bind to aggregated A β and have the ability to bind to FcR γ receptors, is inflammation, leading to a breakdown of the blood–brain barrier and the development of amyloid-related imaging abnormalities (ARIAs) that include cerebral microhemorrhages. New strategies have recently been developed to potentially reduce these adverse effects. Crenezumab is a fully humanized IgG4 anti-A β antibody that displays a low affinity for Fc γ receptors, thereby reducing an inflammatory response (Table 1). Crenezumab binds to multiple forms of A β , including monomeric, fibrillar, and soluble oligomers (Adolfsson et al. 2012). In a phase I trial conducted with mild to moderate AD patients, there was a dose-dependent increase in plasma A β levels following a single and multidosing paradigm (Adolfsson et al. 2012). No patients developed ARIAs, including a large subpopulation of APOE ϵ 4 carriers, who have previously been shown to be at higher risk. Two phase II trials have since been initiated, BLAZE (ClinicalTrials.gov Identifier: NCT01397578) and ABBY (ClinicalTrials.gov Identifier: NCT01343966), to evaluate the efficacy of Crenezumab on delaying cognitive and functional decline in mild to moderate AD patients. Preliminary results from these trials that were reported at the International Alzheimer's Disease Conference in Copenhagen, Denmark, in July 2014 revealed some slowing of cognitive decline in subjects with mild AD, similar to results reported for Solanezumab.

FUTURE DIRECTIONS

Despite the overwhelming evidence for immunotherapies targeting A β to prevent amyloid

accumulation and reduce A β toxicity, immunotherapy has presented a low efficacy for preventing cognitive decline in mild to moderate dementia attributable to AD. There are, however, several potential explanations for the results to date. The dissociation between plaque clearance and the continued progression toward severe dementia suggests that plaque removal is not sufficient to halt the neurodegenerative process once a person reaches the stage of moderate dementia attributable to AD. Perhaps, amyloid plaques containing fibrillar, insoluble A β initiate the neurodegenerative process of AD; however, the nonfibrillar, oligomeric forms of A β mediate disease progression. Previous studies have showed dissociations between A β plaque density and severity of dementia. Furthermore, studies have illustrated that soluble, oligomeric A β species, independently of fibrillar A β , blocked long-term potentiation and impaired memory (Walsh et al. 2002; Lesné et al. 2006; Shankar et al. 2008). In accordance with these observations, eliminating or sequestering soluble A β species may therefore increase the efficacy of immunotherapies for AD.

Alternatively, immunization of individuals with mild to moderate AD may have been too late to halt the progression of the disease. AD biomarker studies clearly demonstrate that the neuropathology of AD, particularly the phase of A β deposition, begins ~15–20 years before the onset of clinical symptoms (Holtzman et al. 2011; Bateman et al. 2012; Villemagne et al. 2013). Longitudinal studies on autosomal dominant, familial AD revealed A β 42 levels in CSF indicative of amyloid plaques begin to decline 25 years before the manifestation of symptoms. The decline of A β 42 levels in CSF indicates amyloid accumulation in the brain. As measured by PiB-PET imaging, A β deposition was visible ~15 years before onset of symptoms. These studies demonstrate that the neurodegenerative process of dementia is initiated decades before visible clinical symptoms. Treatment of mild to moderate AD may have surpassed the therapeutic potential of A β immunotherapy, which highlights the importance for earlier intervention.

Attempts to begin treatment at earlier stages before the irreversible degenerative process have

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been initiated in three new trials. The Dominantly Inherited Alzheimer's Network (DIAN) is currently evaluating Solanezumab and Gantenerumab in asymptomatic patients identified as carriers of autosomal dominant genetic mutations for AD. Patients are being assessed for amyloid-plaque burden, CSF biomarkers, and efficacy of preventing cognitive decline.

The Alzheimer's Prevention Initiative (API) is a randomized trial involving a large group of families that are known carriers of a fully penetrant autosomal dominant mutation in *PSEN1*. These individuals are treated with Crenezumab for 5 years and will be evaluated for amyloid burden and CSF biomarkers as well as cognitive decline.

Unlike DIAN and API, Anti-amyloid Treatment in Asymptomatic AD (A4 trial) is beginning to assess individuals 65–85 years of age within the general population who do not carry AD gene mutations. Selection is based on the presence of amyloid burden as measured by PET scan. Participants are required to have no obvious cognitive decline at time of enrollment. Patients will be treated with Solanezumab or a placebo to determine if there is a delay in onset of cognitive decline and will also be assessed for a variety of biomarkers.

FRONTOTEMPORAL LOBAR DEMENTIA AND TAU

Frontotemporal lobar dementia (FTLD) is the second most common form of dementia occurring before the age of 65, yet immunotherapies in clinical trial to battle cognitive decline in FTLD have lagged. Although FTLD and AD are both neurodegenerative diseases that display a progressive decline in cognition, FTLD differs by the absence of A β pathology. In addition, there are different underlying pathologies that account for FTLD. FTLD-tau is characterized by tau-positive inclusions often referred to as Pick bodies. FTLD-tau conditions include Pick's disease, corticobasal degeneration, and progressive supranuclear palsy. Clinically, FTLD is defined by a wide spectrum of syndromes that result from degeneration of the frontal and temporal lobes and that are genetically heterogeneous

(D'Alton and Lewis 2014). There are a number of subcategories of FTLD that are defined by their dominant clinical symptom in addition to their genetic mutations (for review, see Ben-nion Callister and Pickering-Brown 2014).

The first genetic linkage in families with autosomal dominant FTLD was reported to be localized to chromosome 17q21.11, now referred to as FTDP-17 (Wilhelmsen et al. 1994; Baker et al. 1997; Froelich et al. 1997). Histopathological studies of FTDP-17 revealed inclusions that stained positive for the microtubule-associated protein tau (MAPT) (Murrell et al. 1997). The finding that the tau gene MAPT resides within chromosome 17q21.11, in combination with histopathological studies of the FTDP-17 brains that revealed inclusions positive for tau, led to the first novel missense and splice site mutations in MAPT associated with FTLD (Hutton et al. 1998; Spillantini et al. 1998; D'Souza et al. 1999). To date, more than 40 different MAPT mutations have been reported that are either missense, deletions, or that affect alternative splicing of the MAPT transcript and that ultimately lead to the accumulation of tau aggregates.

Tau was initially identified as a microtubule-associated protein and was subsequently found to be the main component of neurofibrillary tangles in several neurodegenerative diseases, referred to as tauopathies (Weingarten et al. 1975; Witman et al. 1976; Joachim et al. 1987; Lee et al. 2001). It is a natively unfolded protein composed of four major regions that include an N-terminal projection region, a proline-rich domain, a microtubule-binding domain (MBD), and a C-terminal region. Tau is expressed from a single gene that is alternatively spliced, yielding six major tau isoforms in the CNS. Each isoform is identified by the number of microtubule-binding repeats together with N-terminal exons. Under nonpathological conditions, half the tau isoforms contain three repeats of MBD (3R-tau), and half contain four MBD (4R-tau) controlled by alternative splicing of exon 10. Mutations that affect alternative splicing, yielding an increase in 4R- over 3R-tau, promote the formation of 4R-tau inclusions. Missense or splice site mutations within exon 10 display

tau aggregates in both neuronal and glial cells, whereas mutations outside of exon 10 restrict tau aggregates to neurons (Zempel and Mandelkow 2014).

Within the CNS, tau is predominately expressed in neurons and maintained at higher concentrations within axons to promote microtubule assembly and stability (Trojanowski et al. 1989). The regulation of tau binding to microtubules is mediated by the phosphorylation of serine and threonine residues at sites immediately adjacent to and within the MBD (Lee et al. 2001). Phosphorylation within or around the MBD alters its conformation, decreasing its affinity for microtubule binding and liberating tau (Zempel and Mandelkow 2014). The accumulation of liberated hyperphosphorylated tau in combination with its intrinsically disordered properties can foster the formation of insoluble-paired helical filaments resulting in tau inclusions.

The formation of tau inclusions was once thought to be cell autonomous, with the formation of filamentous tau inclusions within a single cell independent of neighboring cells. However, several studies (Clavaguera et al. 2009; Frost et al. 2009; Guo and Lee 2011; Lasagna-Reeves et al. 2012; Iba et al. 2013) have now shown the phenomenon of “spreading” tau pathology in a non-cell-autonomous fashion that progresses between cells throughout the brain in a well-defined pattern depending on the specific disease in which it occurs. Neuropathological studies of demented patients postmortem provided the first evidence for a stereotypical spatial and temporal spreading of neurofibrillary tau accumulation in AD (Braak and Braak 1991). The first neurons to develop neurofibrillary tangles during normal aging are within the transentorhinal region (Braak stages I–II). These neurons give rise to the perforant pathway, the major projections to the hippocampus where tau pathology gradually advances (Braak stages III–IV), and finally spreads into the neocortex (Braak stages V–VI) during the progression of AD.

The hierarchical pattern of neurofibrillary tau accumulation in AD, as well as in other human tauopathies encompassing FTLT-tauopathies, is consistent with transmission of tau

fibrils in mouse models of tauopathy. Injection of brain extracts from diseased mutant P301S tau transgenic mice into the cortex or hippocampus of mice expressing human wild-type tau induced the formation of wild-type tau fibrils that spread to distant neurons (Clavaguera et al. 2009). Similarly, injection of tau oligomers isolated from AD brain showed propagation of tau pathology in wild-type mice (Lasagna-Reeves et al. 2012). More recently, injection of preformed synthetic tau fibrils into the hippocampus or striatum of P301S tau transgenic mice induced neurofibrillary inclusions that propagated from the injected site to connected brain regions in a time-dependent manner (Iba et al. 2013). Furthermore, cell culture studies have illustrated that exogenous fibrillar tau is taken up into cells and seeds the aggregation of endogenous soluble tau (Frost et al. 2009; Guo and Lee 2011). Like prions, a recent study illustrated that tau conformations can propagate in vitro and have distinct strain-like features (Sanders et al. 2014). These studies suggest that the propagation of tau fibrils is mediated by a non-cell-autonomous mechanism (Fig. 2).

The route of spreading coincides with synaptic connections between the injection site and affected brain regions, suggesting a synaptic mechanism of tau spreading. Consistent with transsynaptic spreading, selective expression of human tau within the entorhinal cortex leads to tau pathology at the axonal terminals within the dentate gyrus of the hippocampus (de Calignon et al. 2012; Liu et al. 2012). Furthermore, monomeric tau is normally released into the brain’s interstitial fluid (ISF), which is regulated by neuronal activity, yet diseased, aged P301S mice display reduced levels of soluble tau in ISF (Yamada et al. 2011, 2014). Therefore, these studies support the finding that monomeric tau released extracellularly may be in equilibrium with extracellular tau aggregates, which may be the species that propagate.

ACTIVE AND PASSIVE IMMUNIZATION TARGETING TAU

Active immunization against tau has been reported in mouse models of tauopathies. In the

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Figure 2. Schematic depicting tau fibril propagation occurring via a non-cell-autonomous mechanism from unhealthy neurons to neighboring neurons. Immunotherapeutic targeting of tau species potentially blocks tau propagation and seeding.

earliest of studies, P301L transgenic mice were immunized with a 30-amino-acid fragment (tau 379–408) containing two phosphorylation sites (Ser396 and Ser404). The P301L tau mutation is responsible for FTL in patients, and expression of P301L-tau in mice recapitulates the many features of FTL, including tau inclusions and motor impairment (Lewis et al. 2000). Immunization of young P301L mice effectively decreased tau pathology in multiple brain regions, altered the insoluble to soluble tau ratio, and improved motor dysfunction (Asuni et al. 2007). However, measurements of cognition were not evaluated, as it is difficult to discern between memory impairments and motor abnormalities. In a follow-up study, double transgenic mice that expressed human tau isoforms together with the M146L presenilin mutation and that displayed spatial memory deficits before motor impairments were immunized. This study showed that active immunization prevented cognitive impairment in addition to reconfirming decreased tau pathology (Boutajangout et al. 2010). Furthermore, active immunization in aged P301L mice with preexisting pathology reduced phosphorylated

tau and NFT pathology (Bi et al. 2011). In addition, passive immunization with antibodies targeting tau phosphoepitopes at serines 396 and 404 reduced tau pathology and improved motor function in P301L mice as well as in a second transgenic mouse expressing mutant P301S tau (Boutajangout et al. 2011; Chai et al. 2011). These improvements in behavior and pathology were also evident by passive immunization using antibody MC1, which recognizes early tau pathology (Chai et al. 2011).

Although these studies demonstrate that immunization against tau effectively reduced tau pathogenesis, the mechanism that prevented the spread of tau and the tau species responsible for seeding remains unclear. Previous studies have illustrated that tau fibrils that penetrate into cells can induce misfolding of intracellular native tau (i.e., “seeding”). A more recent passive immunization study illustrated that the most effective antibodies at blocking tau-seeding activity were also the most effective at decreasing tau pathology and improving cognition (Yanamandra et al. 2013). Following an effort to identify anti-tau antibodies that selectively block tau-seeding activity in a cellular bi-

osensor assay, infusion of these antibodies into P301S tau transgenic mice dramatically reduced tau pathology. In addition, another recent passive immunization study illustrated that targeting oligomeric tau species effectively protected against tau-dependent pathogenesis in P301L transgenic mice (Castillo-Carranza et al. 2014). Using a tau oligomer-specific monoclonal antibody that does not target monomeric tau, passive immunization decreased oligomeric tau species, reversed motor abnormalities, and decreased tau pathology. Therefore, these studies provide evidence that one target of anti-tau antibodies may be oligomeric tau species, and blocking certain forms of tau oligomers may block seeding and the spread of tau aggregates, thereby suppressing tau pathology and improving behavior (Fig. 2).

There is great interest in translating tau immunotherapy to humans. There are several immunotherapy trials targeting tau that have begun. NCT02031198 by Axon Neurosciences is an active immunization phase I trial assessing safety with AADvac1, a vaccine directed against pathologically modified AD tau protein. There are also two passive immunotherapy approaches that have entered phase I trials. NCT02494024 is a phase I single ascending dose trial of C2N-8E12 in progressive supranuclear palsy (PSP). NCT02460094 is a phase I multiple ascending dose study in PSP.

CONCLUSION

With uncertainty as to whether immunotherapeutics targeting A β will be useful in preventing cognitive decline in already symptomatic individuals with AD, much scientific focus has recently been on targeting tau, the other aggregate-prone protein in AD and FTL, as an alternative. Recent studies have illustrated that tau pathology within specific brain regions and tau levels in CSF (in AD) correlate well with cognitive decline and synapse loss in human disease, speculating that tau pathogenesis may ultimately drive neurodegeneration. Moreover, tau pathology occurs in more than 20 distinct neurodegenerative diseases in conjunction with recent evidence that extracellular tau species

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may lead to transcellular propagation of tau pathology. Thus, newly emerging data further warrant exploration of the potential beneficial effects of immunotherapeutics targeting tau.

CONFLICT OF INTEREST DISCLOSURE

D.M.H. is a cofounder of C2N Diagnostics and is on its scientific advisory board. A pending patent entitled "Antibodies to Tau" has been filed. D.M.H. and G.G. are inventors on this patent. This patent has been licensed by Washington University to C2N Diagnostics. Eli Lilly has licensed patents related to the anti-A β antibody Solanezumab from Washington University. D.M.H. is an inventor on those patents, and both he and Washington University will receive royalties from this license consisting of part of the net sales of Solanezumab, if it is approved as a treatment. D.M.H. also consults for Genentech, AstraZeneca, Eli Lilly, and Neurophage. The D.M.H. laboratory at Washington University receives funding from the National Institutes of Health, C2N Diagnostics, and Eli Lilly.

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