Chapter 8

Passive Immunotherapy in Alzheimer's Disease

Philip J. Dolan and Wagner Zago

Additional information is available at the end of the chapter

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Abstract

The development of therapeutics for the treatment of Alzheimer's disease (AD) has been challenged with a myriad of obstacles: an evolving and incomplete understanding of disease etiology and progression, challenges with early diagnosis, multifactorial genetic and environmental factors that contribute to patient variability, and the cost of conducting lengthy clinical trials. One approach that has garnered a significant amount of attention and resources for its potential as a disease modifying approach is passive immunotherapy directed at clearing amyloid-β (Aβ) species, a pathological hallmark of Alzheimer's disease. While passive immunotherapeutic trials directed at Aβ have not yet demonstrated clinical benefit, they have prompted important advances in the application and understanding of biomarkers, patient selection, novel functional readouts, and safety monitoring. Application of these lessons has enabled more recent clinical trials to incorporate better trial designs and refine inclusion criteria to optimize patient population enrollment. In addition, new passive immunotherapy targets emerging in the clinic have emerged, as well as novel technologies to enhance future antibody therapeutics. Taken together, the advances in research and clinical science have prepared the passive immunotherapy field to advance emerging promising disease modifying treatments in AD.

Keywords: amyloid-β, tau, passive, immunotherapy, Alzheimer's disease

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disease that clinically presents as a gradual onset of dementia, beginning with mild cognitive and functional deficits, leading eventually to an inability to carry out everyday tasks. Alzheimer's disease and other dementias have a reported worldwide prevalence of approximately 42 million people, with an age-standardized rate of 761 per 100,000 [1]. Current therapeutics are limited to symptomatic

approaches, such as acetylcholinesterase inhibitors and NMDA receptor (NMDAR) antagonists, which aim to enhance the function of unaffected neurocircuitry but do not target the underlying cause of the disease, thus there is a desperate need for approved disease modifying therapies.

Alzheimer's disease is characterized by the dual pathological hallmarks of extracellular senile plaques and neurofibrillary tangles, composed of the amyloid-β (Aβ) peptide and tau protein, respectively. In addition, the primary familial forms of the disease are caused by mutations that directly affect Aβ homeostasis [2]. Due to both the pathological and genetic link to disease initiation, Aβ has been a prominent target for the development of disease-modifying therapeutics.

One such therapeutic approach is anti-Aβ immunotherapy. Active immunotherapy approaches utilize either the ability of the immune system to raise polyclonal antibodies against a therapeutic composed of an Aβ sequence-derived antigen and adjuvant, while passive immunotherapy approaches treat a patient with monoclonal antibodies with known antigen binding capabilities. While a large amount of research and development has been carried out regarding active immunotherapy towards AD targets [3], this chapter will focus on passive immunotherapy in AD, with the goal of describing what has been learned from past clinical studies, and what lessons may be applied to future efforts.

2. Aβ

2.1. Mechanisms of Aβ pathophysiology

The primary component of senile plaques is Aβ, a small peptide derived from the amyloid precursor protein (APP). In AD, Aβ is formed via sequential cleavage of APP by β-secretase [4] and the presenilin-1 (PS1) subunit of γ-secretase [5], respectively. This results in peptides of varying length, ranging from 38 to 43 amino acids [6], of which $A\beta_{1–42}$ is the most amyloidogenic [7]. A central tenet in the understanding of causative factors of AD is the amyloid cascade hypothesis [8], which holds that the pathological increase of amyloidogenic Aβ in AD is a central initiating event in disease, that precedes and initiates a cascade of events that lead to other pathologies such as the formation of neurofibrillary tangles, inflammation, oxidative stress, neuronal dysfunction, and cell death [9]. While the amyloid cascade hypothesis has been challenged since first proposed [10, 11], there is abundant evidence from *in vitro* and *in vivo* studies confirming the significant role Aβ plays in inducing neurotoxicity, synaptic dysregulation, and pathology.

Degeneration of cultured neurons by treatment with aggregated forms of Aβ has been observed in multiple laboratories, and appears to correlate with extent of aggregation [7, 12]. Strong evidence indicates that soluble aggregated forms of Aβ might exert direct toxicity to neurons [13–15] through a variety of mechanisms, including (but not limited to) disruption of plasma membranes [16], dysregulation of mitochondrial function and dynamics via direct interaction [17], and excitotoxicity [18]. Confirming the centrality of Aβ's role in neurotoxicity,

myriad transgenic mouse models expressing mutant APP or APP/PS1 recapitulate many AD phenotypes, including plaque pathology, synaptic dysfunction, decreased cognition, neuroinflammation, and neuronal loss (reviewed in [19]).

One of the earliest mouse models of Aβ plaque deposition was the PDAPP mouse (Line109). These transgenic mice exhibit high human APP expression (>10-fold higher than endogenous levels), which is accompanied by extracellular Aβ plaque deposition, development of neuritic dystrophy, gliosis, and loss of synaptic and dendritic structures in the hippocampus [20]. The PDAPP mouse model was instrumental to demonstrate that therapies developed to clear Aβ deposits could potentially ameliorate functional deficits. Schenk and colleagues were the first to develop an active immunization approach using aggregated $A\beta_{1–42}$ [21], which resulted in prevention of plaque formation in mice immunized before the development of pathology, and more importantly demonstrated that the induced polyclonal response can promote plaque clearance in aged PDAPP mice via phagocytosis by resident microglia. This breakthrough was later extended by administering the anti-N-terminal Aβ monoclonal antibody (mAb) 3D6 directly to PDAPP mice (passive immunotherapy); antibodies crossed the blood-brain barrier (BBB), localized to pathological features, and induced the opsonization and clearance of senile plaques in a microglia-dependent manner [22]. These preclinical findings validated Aβ-directed passive immunotherapy as a potential therapeutic strategy for AD.

2.2. Aβ passive immunotherapy in the clinic

The first Aβ immunotherapy clinical trial utilized active vaccination with $A\beta_{1–42}$ (AN1792) and was halted during Phase IIa due to the appearance of meningoencephalitis, likely due to the infiltration of T-cells in the brain as a result of the presence of T-cell epitope(s) in the antigen, which contained the full-length $A\beta_{1–42}$ peptide [23]. However, long-term follow-up indicated that patients that developed an immune response displayed modest but significant sparing of function, as assessed by the Disability Assessment for Dementia (DAD) and the Dependence scale [24]; in addition, autopsy of a patient immunized with AN1792 without meningoencephalitis displayed an absence of plaque pathology at autopsy and the presence of Aβ-reactive microglia, indicating that AN1792 was successful at engaging phagocytes to remove plaques [25].

Concerns for safety in active Aβ vaccination trials shifted most development efforts to passive immunotherapy, which carries less risk of an inflammatory response to drug. An overview of clinical Aβ antibody efforts described in the following text is listed in **Table 1**.

2.2.1. First-generation Aβ passive immunotherapies

Bapineuzumab, directed at the N-terminus of Aβ, was the first monoclonal antibody therapy developed to target Aβ in AD. It was first tested in a phase I study in AD patients with single ascending doses ranging from 0.5 to 5 mg/kg administered every 13 weeks to evaluate safety, tolerability, and pharmacokinetics (PK) [26]. A significant safety finding of this study was the presence of vasogenic edema (VE) in the highest-dose cohort: 3/10 patients

Name	Epitope	Most recent clinical phase	References
First-generation Aβ passive immunotherapeutics			
Bapineuzumab	1–6	PhIII (terminated)	[22]
Solanezumab	16–26	PhIII	[33, 41]
Ponezumab	35–40	PhIIa (terminated)	[32, 42]
Second-generation Aβ passive immunotherapeutics			
Crenezumab	16–26 (aggregate-selective)	PhIII	[41]
Gantenerumab	3–11, 18–27	PhIII	[34]
BAN-2401	Protofibrils	PhII	[36]
Aducanumab	N-terminus	PhIII	[37, 38]

Table 1. Past and current Aβ antibody therapeutics.

displayed these abnormalities, two of whom were asymptomatic. Due to the observation of VE at 5 mg/kg a dose regimen ranging from 0.15 to 2 mg/kg, administered every 13 weeks for 18 months was selected for the multiple ascending dose phase II trial [27]. In the phase II trial, study completers that received all 6 planned infusions displayed significant improvements in DAD score and the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog), though this effect was not observed in the intent-to-treat population. VE was observed in ~10% of bapineuzumab treated patients (half of whom were asymptomatic), in comparison to 0% of the placebo group; the appearance of VE was dose-dependent and appeared early during the course of treatment. Interestingly, the majority (10/12) of VE cases occurred in carriers of the *APOε4* allele, a risk factor for aggressive AD [28].

Two phase III trials for bapineuzumab were completed to evaluate efficacy in patients with mild to moderate AD who were either *APOε4* carriers or non-carriers in separate trials, with a lower dose regimen in the carrier trial [29]. These trials did not meet the co-primary cognitive and functional endpoints, though CSF phospho-tau, a proposed biomarker of neurodegeneration in AD, did decrease in both studies and positron emission tomography-Pittsburgh B (PET-PIB) imaging revealed less amyloid pathology in the *APOε4* carrier group treated with bapineuzumab compared to placebo. One important finding is that of the subgroup that underwent PET-PIB imaging, 6.5% of *APOε4* carriers and 36.1% of non-carriers did not have detectable PET-PIB signal at trial entry, raising concerns about misdiagnosis and improper subject selection in the trials. While these studies did not succeed in meeting primary endpoints, they did provide information to guide future trials, particularly in understanding MRI abnormalities, such as VE and microhemorrhages.

During the course of the phase III trials, the observation that VE and microhemorrhages correlated with anti-amyloid dose levels was more pronounced in *APOε4* carriers, and were normally transient and asymptomatic [30] led to the formation of an Alzheimer's

Association-led workgroup composed of industry and academic experts to advise the FDA on potential routes to monitor VE and microhemorrhages. The term amyloid-related imaging abnormalities (ARIA) was adopted to address the spectrum of MR imaging abnormalities observed with anti-amyloid therapies, spanning from sulcal effusion and vasogenic edema seen on FLAIR MRIs to hypointensities (hemosiderin deposits) on T2* MRI. The ARIA terminology was further subdivided to ARIA-E (sulcal effusion and edema) and ARIA-H (hemosiderin deposits) [31]. Recommendations from the workgroup included (a) standardization of technical and monitoring practices for MRI, (b) exclusion from trials of patients with preexisting ARIA-H, and (c) monitoring of symptoms potentially associated with ARIA. The adoption of these standards, and the understanding that ARIA is largely a short-lived treatment related effect inherent to many anti-amyloid therapies, opened the possibility of testing higher and more frequent drug administration regimens with appropriate patient safety monitoring.

In parallel with bapineuzumab, two additional anti-Aβ passive immunotherapies underwent contemporaneous clinical trials: Ponezumab, directed at the C-terminus of Aβ, underwent Phase I and IIa trials, but was discontinued after Phase IIa [32]. Solanezumab, directed at an internal epitope of Aβ and hypothesized to function by binding soluble species in the CNS and periphery, failed a phase III trial in mild AD patients [33], and a trial conducted in prodromal patients was discontinued. However, it is currently being tested in genetically-defined Alzheimer's disease populations, with results expected in 2021 (clinicaltrials.gov; Identifier: NCT02008357).

2.2.2. Second-generation Aβ passive immunotherapies

Whereas the first generation of Aβ therapeutic mAbs differed in binding to distinct antibody domains (N-, mid-, and C-terminus), the second generation are intended to primarily bind specific conformations and aggregation states. Gantenerumab, currently in two phase III trials for mild and prodromal AD, binds a discontinuous epitope consisting of the N-terminus and an internal epitope, implying a unique conformational binding specificity (clinicaltrials.gov; Identifiers: NCT01224106, NCT02051608) [34]. Crenezumab, currently in phase II and phase III trials for autosomal dominant AD and prodromal-to-mild AD, respectively, is reported to selectively bind soluble and insoluble aggregates, but not monomers (clinicaltrials.gov; Identifiers: NCT01998841, NCT03114657) [35]. In contrast to other therapeutic mAbs, crenezumab is engineered on an IgG4 backbone to reduce effector function, and microglial-mediated phagocytosis of Aβ deposits is not anticipated. BAN-2401, is in clinical development in a large phase II study in early AD patients; is proposed to selectively bind Aβ protofibrils (clinicaltrials.gov; Identifier: NCT01767311) [36].

A promising antibody candidate from this group that is currently in the clinic is aducanumab. Aducanumab is a human mAb that selectively targets soluble aggregates and fibrils, and binds the N-terminus of Aβ. Preclinical studies demonstrate that the chimeric form of aducanumab peripherally administered to an APP transgenic mouse (a) crosses the BBB and binds to plaques (b) reduces calcium overload in neurons [37], and (c) reduces plaque burden in a dose-dependent manner [38]. An interim report from a double-blind, placebo controlled

phase Ib study revealed a dose-dependent decrease of amyloid PET signal that corresponded with significant slowing of cognitive decline at 52 weeks at the highest dose level, 10 mg/kg [38]. While ARIA was reported at a similar frequency compared with previous trials, adherence to guidelines formalized by the Alzheimer's Association ARIA working group [31] allowed for higher and more frequent dosing, potentially contributing to the positive results seen in these early studies. Aducanumab is currently in phase III trials in prodromal early AD patients, with endpoints and patient populations informed by the successful phase Ib study [39]. Interestingly, enrollment for these phase III clinical trials was recently increased by approximately 15% due to patient variability in the primary functional endpoint [40].

3. Tau

While most passive immunotherapy clinical trials in AD have been directed at Aβ, key discoveries regarding tau function and contribution to disease mechanisms have prompted significant efforts directed towards tau. Hyperphosphorylated and aggregated tau protein are the main component of neurofibrillary tangles (NFTs), which, together with Abeta plaques, are considered a primary hallmark in Alzheimer's disease. Because of its intracellular localization, tau deposits have historically been thought to be unavailable to immunotherapeutic treatments. However, results outlined in this section indicate the potential for targeting tau through a passive immunotherapeutic approach.

3.1. Tau biology and pathophysiology

Since the discovery that NFTs are composed of the microtubule-associated protein tau [43–45], many efforts have been devoted to elucidating molecular mechanisms of tau pathophysiology. Tau is an intracellular microtubule binding protein, which is involved in the regulation of microtubule stability and dynamics. In the brain, tau exists principally as six different isoforms, which vary in the absence or presence of N-terminal acidic repeats and a microtubule repeat; these differences are due to the splicing in or out of exons 2, 3, and 10 [46]. In normal physiological situations, the specific ratio of tau isoforms is developmentally regulated, likely due to the changing needs of microtubule fluidity versus stability throughout development and maturity [47].

Tau is an intrinsically disordered, natively-unfolded protein [48] whose physiological function is tightly regulated by post-translational modifications—principally via phosphorylation, which regulates microtubule binding affinity [49, 50]. In the AD brain, tau aggregates to form hyperphosphorylated NFTs and inclusions, composed of paired-helical and straight filaments [51]. In contrast to the intrinsically disordered nature of monomeric tau in solution, these structures adopt an ordered structure composed of a β-sheet core comprised of central residues, surrounded by a disordered coat comprised of the C- and N-termini of the molecule [52]. In AD, the appearance of tau pathological features positively correlates with dementia and disease progression [53, 54], leading to the hypothesis that the formation of tau pathology is a primary causative agent in the development of AD.

While the stereotypic appearance and progression of tau pathology down the perforant pathway—the neurocircuit from the entorhinal cortex to the hippocampus—has been described [55, 56], the molecular mechanisms underpinning this observation had remained elusive. Neurons in the performant path have long been known to be selectively vulnerable to insult such as hyperactivity [57] and expression of AD-related presenilin mutations [58], but the discovery that, when injected into the brain parenchyma, tau from a mutant mouse could simulate the formation of tau aggregates in a previously healthy animal [59] allowed the possibility that this progression may be mediated by aggregated and misfolded forms of the protein. This was strikingly confirmed in mice with tau expression restricted to the entorhinal cortex: in these mice, tau pathology propagated from the region of expression to distant efferent neurons [60, 61], demonstrating that direct cell-cell contact was not required for propagation, and that the pathological signal could be spread trans-synaptically. The demonstration that tau itself was present in interstitial fluid [62], could be secreted from neurons [63], and passed between cells [64] and neurons [65] provided evidence that tau species themselves could be directly transmitted between neurons *in vivo*, providing a potential mechanistic basis for the propagation of tau pathology. Although tau and Aβ are likely associated with different pathophysiological processes in Alzheimer's disease, the presence of pathogenic extracellular tau species could theoretically also be targeted by immunotherapeutic approaches, in this case by a different mechanism of action: interception/sequestration and prevention of cell-to-cell transmission.

3.2. Tau passive immunotherapy

An overview of preclinical and clinical tau antibody efforts described in the following text is listed in **Table 2**.

Pioneering tau immunotherapy studies demonstrated that immunization with phospho-tau peptides (phosphorylated at Ser396/404) in two different tau transgenic lines raised anti-tau antibodies, which immunohistochemically stained the brains of P301L-tau transgenic mice. In addition, active immunization resulted in reductions in tau pathology. The mice also displayed improved performance in motor tasks [66, 67]. Purified anti-tau antibodies from

Name	Epitope	Most recent development phase	References
MC1	7–9, 313–322	Preclinical	[69, 70]
BIIB092/BMS986168	17–28	PhII (recruiting)	[85, 88]
ABBV-8E12	25–30	PhI open label extension	[81, 82]
Cis mAb	*Cis*-pT231	Preclinical	[74]
RO7105705	pSer409	PhII (recruiting)	[71]
PHF1	pSer396/404	Preclinical	[67, 69, 70]
TOMA	Tau oligomer	Preclinical	[76]

Table 2. Tau clinical and preclinical antibodies discussed in this chapter.

immunized mice were peripherally injected into naïve transgenic mice and localized to neurons in the brain displaying tau pathology, demonstrating their ability to cross the blood-brain barrier (BBB) and localize to their target. In a separate study performed by the same lab, passive administration of the mAb PHF1, directed at the Ser396/404 phosphoepitope, also resulted in reductions in tau pathology in mice compared to isotype control [68]. The findings from this series of studies were proposed to be due to two potential mechanisms: (a) antibody-mediated clearance of extracellular tau deposits and (b) intracellular uptake of tau antibodies. The efficacy of passive immunotherapy using PHF1, as well as the conformational antibody MC1, were also confirmed in independent labs [69, 70], bolstering early evidence of this novel promising therapeutic avenue.

An antibody targeting a different phosphoepitope, pSer409, also shows promise in preclinical models; however, conclusions regarding the mechanism of antibody function were considerably different than those proposed in the initial active and passive studies described in the prior paragraph. In this study, a highly selective mAb was able to bind tau phosphorylated at Ser409 and specifically bind AD brain tissue. The mAb was shown to neutralize oligomer-induced neurotoxicity; however, the neutralization activity of the antibody was reduced in mixed neuron-microglial cultures. Antibody engineered with reduced effector function (REF) maintained neutralization activity in mixed neuron-microglial cultures, while the wild-type anti-pSer409 antibody did not prevent neurotoxicity and in fact promoted the release of pro-inflammatory cytokines from microglia [71]. Both wild-type and REF variants of the antibody prevented the progression of tau pathology in the tau P301L mouse, leading the authors to conclude that phagocytic clearance of tau structures was not a contributing mechanism of action to efficacy in the transgenic mouse model. In addition, the lack of FcR message found in isolated neurons prompted the conclusion that receptor-mediated uptake did not occur. The antibody examined in this report has been developed into a therapeutic candidate, which is currently in clinical development (clinicaltrials.gov; Identifier: NCT03289143).

Additional studies have been conducted to identify and target post-translationally modified forms of tau to explore effects of antibody treatment. One compelling approach targets a unique structural isoform of tau induced by phosphorylation of tau at T231. Phosphorylation of tau at T231 occurs during disease progression; the prolyl isomerase Pin1 normally binds and converts the pT231/Proline motif from a toxic *cis* form to a soluble nontoxic *trans* form [72]. A mAb targeting *cis* but not *trans* pT231-tau detects pathology during mild cognitive impairment (MCI) [73]. In addition to AD, this post-translational signature (as well as others) appears in the brains of traumatic brain injury (TBI) patients. When administered peripherally in a murine TBI model carried out in tau transgenic mice, the *cis*-pT231 tau antibody prevented the spread of tauopathy and cortical LTP deficits, and improved performance in the elevated plus maze, which was correlated to TBI-induced disinhibition behavior in patients [74]. Another effort targeting disease-specific forms of tau is centered around developing antibodies that bind soluble oligomeric tau—hypothesized to be the most toxic form of the molecule [75]—and have minimal binding to monomeric or mature NFTs [76]. Tau oligomer-specific monoclonal antibodies (TOMAs) were dosed via intracerebroventricular (i.c.v.) infusion to tau P301L mice. Strikingly, a single i.c.v. injection reduced

tau oligomers and histopathology, and rescued deficits in rotarod and spontaneous alternation tests. Examination of serum revealed oligomeric tau and antibody/antigen complexes, suggesting peripheral clearance as a mechanism of action [77].

Informed by studies indicating the potential for the propagation of tau pathology across cell membranes [64], as well as the demonstration of trans-synaptic transmission *in vivo* [60, 78], an independent effort to discover tau antibodies that interrupted cell-to-cell transmission yielded phosphorylation-independent antibodies that blocked uptake of tau aggregates to cultured cells [79, 80]. When administered to tau transgenic mice centrally via an Alzet minipump, these antibodies slowed the advance of tau pathology, as measured by immunohistochemical and biochemical means [79]. One of the efficacious antibodies used in this report, HJ8.5, was used in a peripheral administration model to further explore its potential as a therapeutic agent [81]. HJ8.5 is a high affinity anti-N terminal mAb that recognizes residues 25–30, which are present on all splice isoforms of tau. In this study, P301S tau transgenic mice were dosed intraperitoneally over a 3-month period with 10 or 50 mg/kg of HJ8.5. The high dose cohort displayed decreases of insoluble tau, AT8 staining, and thioflavin S staining. In addition, this cohort exhibited improvements in sensorimotor function compared to isotype control and low-dose cohorts. The preclinical efficacy profile, as well as the concordance of *in vivo* data with mechanistic *in vitro* studies, propelled the humanized analogue of this antibody into the clinic (clinicaltrials.gov; Identifier: NCT03391765) [82]. Interestingly, a separate effort focused on discovering antibodies and epitopes important for uptake and transmission determined that while N-terminal antibodies could indeed block uptake of recombinant and AD patient-derived tau, there were other epitopes with potentially more potent function, notably antibodies binding C-terminal to the acidic inserts [83].

A key component of the amyloid cascade hypothesis is that Aβ aggregation induces, either indirectly or directly, fibrillization of tau as well as other disease processes (reviewed in [84]). The finding that extracellular secreted and truncated forms of tau (termed eTau) could regulate Aβ levels demonstrated a potential upstream role of tau in relation to Aβ, complementary to the amyloid cascade hypothesis. In this study, secreted eTau was isolated from iPSC neurons derived from patients with AD; treatment of neurons with eTau displayed increases in secreted Aβ, and these increases could be prevented via application of eTau-binding antibodies such as MC1 and IPN002, which recognizes residues 17–28. Aβ levels were not affected by PHF1 antibody, as the PHF1 epitope is not present in eTau. This finding was recapitulated in transgenic P301L-tau mice; peripheral treatment with IPN002 resulted in reductions in Aβ in the interstitial fluid and cortical tissue [85]. These findings were recently confirmed by a different group using mAbs that target very similar N-terminal tau epitopes; in these studies, behavioral improvements as well as decreases in Aβ were noted in mice transgenic for mutant forms of presenilin, APP, and tau [86, 87]. IPN002 has been developed into a clinical therapeutic and is undergoing clinical trials as BIIB-092/BMS986168 (clinicaltrials.gov; Identifier: NCT03068468) [88].

Though the success of preclinical studies with tau antibodies has provided sufficient rationale to begin exploration in the clinic, a greater understanding of the full range of factors involved in tau toxicity and the mechanisms of action of tau passive immunotherapy are needed. These

mechanisms may be different than those proposed for Aβ immunotherapy. There remain conflicting details from the studies presented here, such as the relative contribution of microglial-mediated phagocytosis, the relative importance of eTau-mediated Aβ production, the extent of trans-synaptic transmission in transgenic mice with widespread expression in the brain, and the optimal epitope to target. Gaining a clearer understanding of these factors continues are a current research focus.

Clinical trials with Aβ immunotherapies have demonstrated the importance of proper clinical diagnosis, patient selection, sensitive cognition tests, and effective biomarkers to monitor efficacy and disease progression. Though some general commonalities may exist in the clinical design of Aβ and tau passive immunotherapy trials, there are substantial differences in the targets and any potential clinical development approaches. In contrast to Aβ, there are a number of non-AD tauopathies such as progressive supranuclear palsy (PSP) [89] and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [90] that may provide alternative clinical development pathways to test novel tau-directed therapeutic approaches. In contrast to AD, these diseases present pathological signatures composed almost uniformly of tau and neurofibrillary tangles; in addition, FTDP-17 is an autosomal dominant disorder, genetically validating the causative role of tau. Diagnosis of these and other tauopathies have historically been made solely based on clinicopathology; due to the difficulty of diagnosis from to the overlap of symptomologies with other neurodegenerative disorders, as well as the lack of clear biomarkers, diagnosis is only confirmed at autopsy [91]. Modern tau PET imaging agents are currently under clinical investigation [92]; while early generations of tau PET tracers displayed nonspecificity and suboptimal binding and PK characteristics, the newest class of tracers display improved specificity, PK properties, and may allow for improved diagnosis in tauopathies as well as an ability to monitor tau pathology in AD clinical trials [93].

4. New targets and technologies

4.1. Targeting the immune system in AD

The vast majority of passive immunotherapeutic approaches in AD have targeted Aβ and tau; this is a natural outcome of the primacy of these proteins as the principal pathological hallmarks of the disease. The association of mutations of APP (and proteins that modulate its generation, such as presenilin-1) to familial AD, and the high degree of correlation between tau pathological development and cognition, strengthen the validity of these two proteins as important causative disease agents. However, new approaches, primarily targeting immunomodulatory proteins, are also currently under development.

The presence of neuroinflammatory processes and signatures in AD has been well established, but the exact role they play in disease etiology, or whether neuroinflammation has a primarily protective or harmful role, has not been clear (reviewed in [94]). Studies examining the complement cascade have helped to understand this duality. The synaptic pruning activity carried out by microglia is regulated by complement [95]. The initiating protein of the

classical complement cascade, C1q, is enriched in the developing mouse CNS and localizes to synapses; genetic ablation of this protein results in misregulated innervation due to increased presence of synapses [96]. While C1q is normally downregulated after development, it is elevated in normal aging [97] and disease, including AD [98]. In a transgenic APP mouse, C1q localizes to synapses, and is required for pathological synapse loss. Treatment of C1q knockout mice with oligomeric Aβ displayed no synaptic loss, indicating that C1q is a required mediator of Aβ-induced toxicity. Interestingly, an anti-C1q antibody rescued Aβ-induced synaptotoxicity *in vivo*, and LTP impairment *in situ*, when compared to isotype control [99]. These data hinted at the promise of C1q immunotherapy to provide protective benefits by neutralizing a key mediator of Aβ-induced microglial overactivation, which results in synaptic loss. The anti-C1q antibody used in this study has been developed into a human therapeutic, and is beginning clinical trials (clinicaltrials.gov; Identifier: NCT03010046) [100].

The mounting evidence of involvement of the adaptive immune system in restraining the advance of AD pathology has opened the possibility of directing passive immunotherapies to the periphery, which considerably eases the challenge of achieving sufficient drug exposure in the CNS to affect pathology. Microglia resident in the brain are known to be recruited to sites of injury such as senile plaques, but the finding that peripherally-derived bone marrow stem cells are able to enter the CNS, and differentiate into microglia [101, 102], was the first direct evidence that repopulation and recruitment of microglia from the periphery was an active process. This finding was extended to AD mouse models with the finding that peripherally-recruited microglia are mobilized by Aβ, recruited to the site of senile plaques, and are able to clear plaques via phagocytosis [103]. The protective role of these immune cells in the presence of AD-like pathology was confirmed with the observation that (a) knocking out the chemokine receptor CCR2 in an APP-transgenic mouse resulted in decreased recruitment of monocytes to Aβ plaques [104], and (b) the specific ablation of bone-marrow derived cells via diphtheria-toxin receptor expression resulted in increased Aβ plaques [105]. Furthermore, increasing trafficking of macrophages by inhibiting the normally immunosuppressive regulatory T-cells through pharmacologic or genetic methods results in reduced Aβ pathology [106].

Elucidation of the biology of inhibitory signaling pathways and proteins such as Programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), known as immune checkpoints, led to the development of antibody therapeutics for use in cancer (reviewed in [107]). These therapies function by neutralizing immune checkpoints and activating T-cells, which prompts antitumor activity. The characterization of checkpoint signaling pathways, along with the findings that peripheral immune cells modulate AD-like pathology in a regulatory T-cell (T_{reg})-dependent manner, has prompted examination of the PD1/PD-L1 axis in AD. In a recent study, AD transgenic mice were treated with an anti-PD1 antibody to blockade the PD1/PD-L1 axis. Remarkably, checkpoint blockade in this model resulted in substantial rescue of performance in a behavioral assay of memory and cognition after a single dose, and mice exhibited decreases in Aβ pathology with only two dose administrations [108]. The effect on pathology was observed even in mice with profound amyloid burden. While the findings of a profound effect on functional measures after such a short dose regimen are very exciting, they should be taken with a note of caution. A follow-up study, carried out by three pharmaceutical companies using three transgenic models and numerous

PD-L1 antibodies was attempted to recapitulate these results. Despite peripheral immune activation, in all instances neither reductions in Aβ pathology nor infiltration of peripheral monocytes were detected [109]. Further studies are needed to elucidate the potential of checkpoint modulation.

4.2. Increasing blood-brain barrier (BBB) penetrance for passive immunotherapeutics

A significant barrier in the development of passive immunotherapeutics for AD is the low percentage of circulating antibody that crosses the BBB. Animal studies have indicated that ~0.1–0.5% of IgG enters the CSF from the periphery [110, 111], which is borne out by preclinical [112] and clinical [113, 114] data obtained with antibodies tested for use in AD. This has led to trials with increasing amounts of antibody administered to patients ([82]; clinicaltrials.gov, Identifier NCT03318523) with the hope of delivering sufficient amounts of antibody to the CNS to achieve a clinical effect. There are, however, indications that concentrations of antibodies are higher in brain parenchyma than what is present in CSF. The chimeric form of aducanumab reported brain:plasma AUC ratios when tested in a transgenic APP model of 1.3% [38]. This is in agreement with the finding that the concentration of protein analyte present in the interstitial fluid is approximately 10-fold higher than in the ISF [62, 115]. This could be due to the rapid turnover of CSF volume [116] compared to ISF, longer elimination times of antibodies in brain parenchyma compared with CSF, or increased residence time due to target-mediated binding. Nevertheless, methods and technologies to increase BBB penetrance of biomolecules urgently need to be applied to antibodies and other proteins.

One of the more promising approaches to increase penetrance of protein therapeutics into the brain utilize endogenous receptors that transcytose between the brain and periphery, such as transferrin receptor (TfR) [117], insulin receptor [118], and LDL receptor-related protein 1 (LRP1) [119]. Protein engineering approaches feature fusion of the therapeutic molecule to proteins, ligands, or peptides that bind these receptors and facilitate transcytosis across the BBB (reviewed in [120]). One of the best understood receptor-mediated delivery systems is the use of TfR, though a similar path has been taken in the development of technologies that utilize insulin receptor. Increased brain uptake of transferrin/antibody fusion proteins were detected in rats [121], though the relatively large size (~80 kDa) of full-length transferrin make this impractical for biotherapeutic use. The detection of increased transcytosis of anti-TfR antibodies and antibody fragments [122, 123], and later advances in antibody generation technologies, enabled bispecific antibodies that bind TfR as well as target [124]. As understanding of the transcytotic properties of TfR binding moieties have increased, so has the understanding of how best to incorporate properties to ensure delivery to the brain. For example, reducing TfR affinity improves delivery, as a low affinity anti-TfR moiety will release from the receptor faster than a high affinity moiety [124]. As receptor-binding fusions enter the clinic, further questions regarding safety and distribution changes brought about by higher CNS concentrations will need to be continually addressed [125, 126]. Work continues to identify receptors that may be useful for increasing BBB concentrations of antibodies to allow engagement with wider range of drug targets [127, 128].

5. Conclusions and future perspectives

AD provides a monumentally challenging drug development landscape. The uncertainty about disease etiology, variability in patient genetics and disease progression, and difficulties in early diagnosis are all but a noncomprehensive list of hurdles to developing effective drugs. Though development of therapeutics to slow or halt AD disease progression, including passive immunotherapeutics, have not yet yielded clinical benefit, the prospect of applying lessons learned in the clinic towards validated targets such as Aβ and tau provides optimism for future success. In addition, our understanding of the mechanisms of other principal contributing factors to disease progression will provide a variety of new targets to explore. Combined with advances in drug technology to increase the availability of biomolecules in the CNS, these clinical and biological advances offer great promise around future success in treating AD.

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Conflict of interest

PJD and WZ are employees of Prothena Biosciences.

Author details

Philip J. Dolan* and Wagner Zago

*Address all correspondence to: pdolan@prothena.com

Prothena Biosciences, South San Francisco, CA, USA

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