Chapter 2

Active Immunization Against the Amyloid-β Peptide

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Abstract

Alzheimer's disease (AD) has a devastating toll not only on the affected individuals but also on their families, caregivers, and society as a whole. Several therapies have been approved to treat AD, all of which provide modest effect on the symptoms of the illness but without slowing or halting the underlying disease processes. Since the last of these therapies was approved, the largest research effort has been devoted to developing therapies targeting amyloid-β, specifically $A\beta_{42}$, as this protein is thought to initiate the cascade of events that lead to the disease. This chapter focuses on active immunotherapy (vaccines) and specifically on therapies that currently are in clinical development.

Key words Alzheimer's disease, Amyloid-β, Active immunotherapy, Therapeutic vaccine

1 Introduction

Alzheimer's disease (AD) is a serious and invariably fatal neurodegenerative disease and the major cause of dementia in the elderly [1–4]. Progressive deterioration in both cognition and function over time leads to serious clinical outcomes including increased dependence and decreased survival. Besides the direct cost for patient care, indirect costs add incrementally to the burden on society. These are represented by care provided by families and other unpaid caregivers of AD patients, by the impact on caregivers in terms of lost time at work, lost wages and depleted finances, as well as increased caregiver emotional stress and medical needs [5, 6]. Even a small delay in the onset, e.g., by 1 year, of AD dementia would result in a significant reduction in the global burden of the disease. A 1-year decrease in both onset and progression of AD dementia would reduce the 2050 global burden by more than nine million cases with the majority of the reduction among the most severe cases [5]. Therefore, any significant effective treatments that delay, halt, or prevent the progression of disease should decrease costs to patients, caregivers, and society as a whole as well as improve patient and caregiver quality of life.

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The characteristic progressive loss of memory and other cognitive functions, manifest as progressive dementia in AD, develops in parallel with the hallmark neuropathological changes of extracellular proteinaceous lesions (senile plaques) and intraneuronal neurofibrillary tangles, leading ultimately to neuronal death and neurodegeneration. The predominant component of senile plaques is the amyloid-β (Aβ) peptide, particularly the 42-amino acid isoform ($A\beta_{42}$), which is derived from a larger amyloid precursor protein (APP) [7]. The N-terminus of Aβ is cleaved first by the β-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), and then by γ-secretase at the C-terminus. In the brain, $A\beta_{42}$ can form soluble neurotoxic oligomers, fibrillar parenchymal plaques closely associated with neuritic dystrophy and gliosis, and fibrillar (congophilic) amyloid angiopathy [7, 8].

Research over more than 30 years provides evidence that aberrant $A\beta_{42}$ production or clearance, resulting in a chronic dyshomeostasis of $A\beta_{42}$, is a central part in AD pathogenesis. All known genetically linked forms of AD directly affect either the production or the deposition of $A\beta_{42}$, and $A\beta_{42}$clearance appears to be impaired in AD [7–13]. Mutations in the *APP* and the presenilin genes, *PSEN1* and *PSEN2*, result in rare, early-onset, familial forms of AD and increase the accumulation of Aβ [14]. On the other hand, a recently identified allelic variant of APP (A673T), which is a less efficient substrate for BACE-1, was proposed to be protective against the more common sporadic AD in the wider population [15]. Further, in sporadic AD, the genetic risk factor gene allele ApoE ε4, known to be correlated with greater brain amyloid burden [16, 17], increases the risk for development of AD [14].

Multiple lines of evidence implicate Aβ as having a key precipitating role in the pathogenesis of AD. Mainly, the production and/or deposition of toxic forms of Aβ, along with the slowing of Aβ degradation, are viewed as the central and primary events in AD pathogenesis, while neurofibrillary-tangle formation and neuronal cell death occur downstream in this amyloid cascade [7, 8, 18]. Recent in vitro work has demonstrated that Aβ dimers (the major form of soluble oligomers in the human brain) isolated from patients with AD induce both the abnormal phosphorylation of tau that is characteristic of AD and the degeneration of neurites, providing further confirmation of the pivotal role of Aβ in the pathogenesis of AD [19]. However, the work of Braak and colleagues [20] has suggested a refinement of the amyloid cascade hypothesis, in which tauopathy can occur very early, independent of Aβ pathology, progressing in an age-dependent manner. In this model it is likely that the later development of Aβ pathology exacerbates and drives the further development of tauopathy resulting in clinical AD.

2 Therapeutic Approaches

Currently marketed therapies for the treatment of AD include cholinesterase inhibitors and the NMDA receptor antagonist memantine. These drugs only provide modest transient symptomatic effects, aimed at temporary enhancement of impaired neurotransmitter systems to maximize the remaining activity in neuronal populations not affected by the disease [21–23], but do not alter, slow, or halt progression of the disease. The search for a disease-modifying therapy—that affects the underlying disease pathology and has a measurable and long-lasting effect on the progression of disability—has been intense but so far unsuccessful [24, 25].

The pathologic hallmarks of AD—the accumulation of toxic Aβ with the formation of extracellular plaques, the development of intracellular neurofibrillary tangles, and the degeneration of cerebral neurons—provides potential targets for disease-modifying therapies. However, although the large majority of therapies that have been evaluated in the past 15 years have focused on Aβ, anti-tau therapies are beginning to be tested in the clinic (e.g., Axon Neuroscience SE NCT02031198, NCT01850238; AC Immune SA www.acimmune.com). Moreover, next-generation symptomatic approaches which focus on ameliorating the neuropsychiatric and behavioral symptoms associated with AD are also under evaluation (e.g., Pfizer NCT01712074; Lilly NCT00843518; Elan Pharmaceuticals NCT01735630).

Several therapeutic approaches to reduce cerebral amyloid have been explored. While small-molecule approaches aimed at reducing Aβ production by inhibiting or modulating the enzymatic activities of the BACE-1 and γ secretase continue to be explored, this chapter focuses on large-molecule biologic approaches to reduce/prevent accumulation of Aβ.

2.1 Immunotherapeutic Approaches to Amyloid-β Clearance

The concept of immunotherapy as an approach to treat AD was first introduced by Schenk and colleagues [26], who proposed that the immune system could be harnessed to clear toxic Aβ from the brain [27–29]. These approaches involve immune-mediated interventions either by inducing an oligoclonal response through immunization (active immunotherapy) or by administering monoclonal antibodies directed against Aβ (passive immunotherapy) (Fig. 1).

Passive immunotherapy allows for the precise targeting of Aβ epitopes and obviates the need for patients to mount an antibody response, but requires continuous periodic administration for long-term treatment. Active immunotherapy involves the administration of either full-length Aβ peptides or peptide fragments to activate the patient's immune system in order to produce anti-Aβ antibodies. Moreover, the Aβ peptides or peptide fragments can be

conjugated to a carrier protein and may be administered with an adjuvant in order to help stimulate the immune response. Active immunotherapy can induce an oligoclonal (as opposed to monoclonal) response, with antibodies that differ with respect to their binding affinity for a number of toxic Aβ species. Unlike passive immunotherapy, which has to be readministered at frequent intervals, active immunotherapy has the potential to produce persistent levels of anti-Aβ antibody titers with less frequent administration [27–29].

Immunization with aggregated human $A\beta_{42}$ [26] and passive immunotherapy with antibodies directed against the N-terminus of $A\beta_{42}$ [30, 31] have been evaluated in PDAPP mice, an animal model of the ß-amyloidosis and associated cellular changes of AD [32]. These studies have shown a robust reduction or clearance of brain amyloid and have been widely confirmed in other mouse models by many academic and biopharmaceutical research laboratories worldwide [33].

The proof of principle was first demonstrated in the late 1990s [26]. In this study, immunization with intact $A\beta_{1–42}$ resulted in an antibody response that was predominantly directed against an immunodominant epitope located at or near the N-terminus of $A\beta_{1–42}$. In young adult PDAPP mice, immunization generated robust titers of anti-$A\beta_{1–42}$ antibodies and almost entirely prevented the development of AD-like amyloid plaques, neuritic dystrophy,

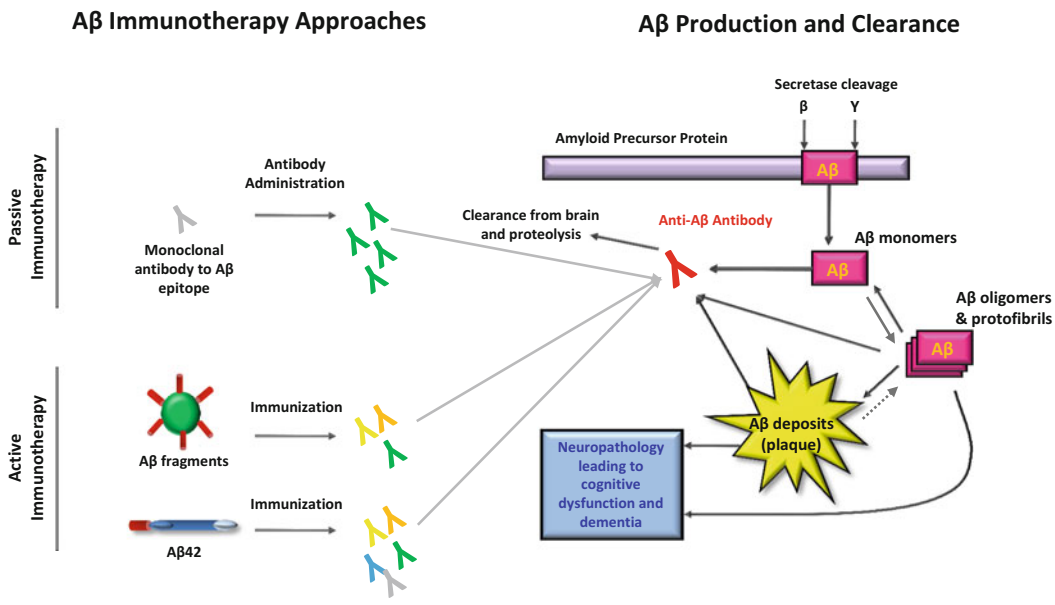

Fig. 1 Passive and active immunotherapeutic approaches to Aβ clearance. Anti-Aβ immunotherapy compounds under development utilize anti-beta-amyloid antibodies, generated through either passive or active immunotherapy approaches (*left*), to target Aβ and promote its clearance from the brain (*right*), with the goal of reversing the neuropathology that leads to cognitive dysfunction

and gliosis. Furthermore, immunization of older PDAPP mice, which had already developed amyloid plaques, markedly reduced the extent of plaques and the progression of the AD-like neuropathology. Therefore, the efficacy of immunization with the synthetic $A\beta_{1-42}$ in the PDAPP model of AD (confirmed in other APP transgenic mouse lines) provided the initial evidence that this approach is a potentially disease-modifying therapeutic strategy for patients with AD [26].

The precise mode of action of Aβ immunization is not known, but based on further experiments performed in PDAPP and other transgenic mice, the effect is clearly mediated by anti-Aβ antibodies that are highly specific towards Aβ epitopes and do not bind other brain or systemic proteins. Further experiments with peripheral antibody administration in PDAPP mice showed that these antibodies can enter the central nervous system (~0.3 %), bind to amyloid plaques, significantly reduce both plaque and neuritic burdens and gliosis, and prevent loss of synaptophysin, a classical marker of synaptic integrity [30]. Antibodies directed at the N-terminus of the $A\beta_{42}$ peptide are thought to act in multiple ways, including direct capture and neutralization of soluble Aβ monomers and oligomers as well as disruption and clearance of parenchymal and vascular Aβ deposits by either direct dissolution of fibrillar material or Fc-mediated phagocytosis (principally via microglia) of amyloid deposits [30, 34–36].

2.2 AN1792 Clinical Experience

Following on the promising preclinical results, AN1792, a synthetic beta-amyloid 1–42 peptide, was the first active amyloid immunotherapy tested in clinical trials [37, 38]. Immunization of subjects with mild-to-moderate AD with AN1792 resulted in an antibody response that was predominantly raised against the dominant epitope located at or near the N-terminus of $A\beta_{1-42}$ [39] in ~53 % (Phase 1; [37]) and 19.7–20 % (Phase 2; (39)) of immunized subjects. However, the AN1792 clinical program had to be halted due to the occurrence of meningoencephalitis in approximately 6 % of subjects in the Phase 2 trial who were immunized with the active product [40]. Most patients who experienced this adverse event developed progressive confusion, lethargy, and headache. Yet other patients reported signs and symptoms such as fever, nausea, vomiting, seizures, and focal neurologic signs. Recovery was reported in 12 of the 18 patients, while 6 patients were noted to have persistent sequelae at the conclusion of the trial. No additional cases of meningoencephalitis were reported over a 4.6-year follow-up study of subjects previously enrolled in the Phase 2 trial [41]. Further investigations indicated the AN1792-associated meningoencephalitis as an event caused by an Aβ-directed proinflammatory cytotoxic T-cell response to a major T-cell antigenic epitope within the carboxyl portion of $A\beta_{1-42}$ [42]. Neuropathologic examination of one case of meningoencephalitis revealed a

perivascular T-cell infiltrate with a lack of B lymphocytes, as well as microglial activation and multinucleated giant cells [43].

Nevertheless, results from these initial studies suggested the potential of immunotherapy for the treatment of AD. Results from these early immunotherapy trials with AN1792 showed potential benefit on certain cognitive and functional outcome measures [37, 38, 41] and a significant reduction in t-tau protein levels in the CSF [38] but a paradoxical greater atrophy rate of certain brain regions [44]. Further, observations on approximately a dozen subjects of the AN1792 trials (Phase 1 and 2) who have come to autopsy indicate that this active immunotherapeutic approach results in removal of amyloid plaques from brains of AD subjects [43, 45–48] and an amelioration of plaque-associated neuritic and glial abnormalities [49]. However, in this small group of subjects who died, brain amyloid removal apparently did not result in improved survival or in an improvement in the time to severe dementia [47]. Whether the effects of immunotherapy on AD pathology and neurofibrillary dysfunction will ultimately translate to clinical benefit and a delayed disability is being evaluated with next-generation immunotherapy programs.

3 Clinical Programs with Amyloid-β Immunotherapy

Several next-generation Aβ active immunotherapies are currently under evaluation (Table 1). These newer Aβ active immunotherapies seek to avoid the T-cell response observed with AN1792, and are designed to elicit a strong B-cell response and carrier-induced T-cell response without activating an Aβ-specific proinflammatory T-cell response. These therapeutic vaccines are typically constructed with short Aβ peptides, fragmented peptides, or peptide mimetics conjugated with a carrier backbone and administered with an adjuvant, the latter two of which are used to bolster the natural immune response [50, 51].

3.1 ACC-001

Vanutide cridificar (ACC-001) is a conjugate of multiple copies of $Aβ_{-7}$ peptide linked to a nontoxic variant of diphtheria toxin (CRM197) which is administered intramuscularly with or without the adjuvant QS-21 [52]. QS-21, a naturally occurring saponin (triterpene glycoside) molecule purified from the South American tree *Quillaja saponaria Molina*, is an adjuvant known to promote both humoral and cellular immune response against a number of antigens in various species. Preclinical data indicate that vanutide cridificar generates N-terminal anti-beta-amyloid antibodies without inducing a beta-amyloid-directed T-cell response and that it reverses cognitive impairment in murine models of AD [53]. Vanutide cridificar phase 2 clinical trials in mild-to-moderate AD (NCT01284387 [US]; NCT00479557 [EU]; NCT00955409

Table 1
List of anti-Aβ active immunotherapy compounds that have reached clinical development

Compound	Sponsor	Phase of development	Epitope/carrier/adjuvant	Route of administration	Population
ACC-001	Pfizer Inc. and Janssen R & D	2	$A\beta_{1-7}$/nontoxic diphtheria toxin (CRM197)/QS-21	i.m.	Mild-to-moderate AD Early AD
AD-02	Affiris	2	$A\beta_{1-6}$ mimetic/KLH/aluminum	s.c.	Mild-to-moderate AD Early AD
ACI-24	AC Immune	1/2	Tetra-palmitoylated $A\beta_{1-15}$/reconstituted in liposome	s.c.	Mild-to-moderate AD
CAD-106	Novartis	2	$A\beta_{1-6}$/bacteriophage Qβ coat protein	i.m./s.c.	Mild-to-moderate AD
Lu AF20513	Lundbeck	1	$A\beta_{1-12}$ + 2 foreign T-helper epitopes (P30/P2) from tetanus toxoid	Not known	Mild AD
UB-311	United Biomedical	2	2-UBITh® synthetic peptide coupled to $A\beta_{1-14}$/CpG oligonucleotide	i.m.	Mild-to-moderate AD
V950	Merck	1 (discontinued)	Multivalent Aβ peptide/ISOCOMATRIX™	i.m.	Mild-to-moderate AD

[EU extension]; NCT00498602 [US]; NCT00960531 [US extension]; NCT00752232 [Japan]; NCT00959192 [Japan]; NCT01238991 [Japan extension]) and early AD (NCT01227564) have been completed.

Data from a study in Japanese patients with mild-to-moderate AD (NCT00752232; [54]) demonstrated that repeated i.m. administration of vanutide cridificar at three different dose levels (3, 10, and 30 μg) with QS-21 (50 μg) at 3-month intervals up to 1 year elicited high antibody titers and sustained anti-Aβ IgG responses, but only after the second immunization and with no difference between the doses. The addition of QS-21 was essential to stimulate high titer responses. Vanutide cridificar at all doses with or without QS-21 was generally safe and well tolerated. Contrary to that reported from other trials evaluating anti-amyloid therapies in AD [55], no ARIA-E or ARIA-H was observed in this study. No significant differences between vanutide cridificar and

placebo were observed in cognitive evaluations, but this may be due to the small sample size and interpatient variability [54].

The completed Phase 2 ACCTION study (NCT01284387; [56]) is among the first AD studies to use amyloid PET imaging as an enrichment strategy to increase diagnostic certainty after observations that a fraction of clinically diagnosed AD patients do not have pathological amyloid burden by in vivo PET imaging [57]. This study evaluated the effect of ACC-001 with 50 μg QS-21 adjuvant on brain fibrillar amyloid burden as measured by amyloid imaging using ^{18}F-AV-45 (florbetapir) positron emission tomography (PET) in mild-to-moderate AD patients [58]. Exploratory endpoints included safety, immunogenicity, and cognitive and functional efficacy. 125 subjects aged 50–89 with baseline mini mental status examination (MMSE) scores of 18–26 were randomized in a 1:1:1 ratio to receive 3 μg or 10 μg of ACC with QS-21, or placebo, stratified by APOEε4 status. ACC-001 with QS-21 was given by six intramuscular injections over 18 months at weeks 0, 4, 12, 26, 52, and 78, with follow-up through week 104. The primary endpoint of change in PET global cortical average (GCA) standardized value uptake ratio (SUVr) was not statistically significantly different between the two ACC-001 with QS-21 treatment groups compared to placebo, but the changes were numerically consistent with a dose response. ACC-001 was immunogenic with anti-Aβ IgG titers modestly higher in the 10 μg group than the 3 μg group, but the proportion of responders (defined as a titer ≥300 U/mL) was similar in both groups. The only safety signal noted with ACC-001 + QS-21 was a 5.8 % incidence of asymptomatic amyloid-related imaging abnormalities with edema/effusion (ARIA-E), not seen with placebo, and an increase in injection reactions (7.7 % vs. 47.7 %), the majority of which were mild and transient. The plasma Aβ levels increased in parallel with peak anti-Aβ titers after each injection. In the subset with CSF assessments, CSF p-tau changes from baseline in both active treatment groups were not statistically different from placebo but were numerically consistent with a dose response. Volumetric brain MRI showed incrementally greater treatment-related decrease in brain volume which was statistically significant in the 10 μg group ($p = 0.023$) compared with placebo. Decline in CDR-SB was typical for the study patient population. A baseline imbalance may have accounted for a somewhat slower decline in the placebo arm. Given the small size of this trial and the small biomarker effects, a lack in clinical efficacy outcomes was expected [58].

3.2 AD01–04

The AFFITOPE family of vaccines is designed to target aggregated Aβ, the purported toxic species in the genesis of AD [59], by using peptide mimics of the N-terminus of Aβ conjugated to keyhole limpet hemocyanin [60]. It is hypothesized that this approach may have a favorable safety profile since the vaccine lacks the common

T cell epitope that is associated with a pro-inflammatory TH1 response [42] and their controlled specificity allows the production of anti-Aβ antibodies while preventing cross-reactivity with the amyloid precursor protein. The first generation of these vaccines (AD01, AD02) administered with an adjuvant (Alum) was shown to elicit antibody titers to a similar degree as the control $Aβ_{1-6}$ KLH + alum conjugate vaccine in Tg2576 mice. These elicited antibodies have higher reactivity to oligomers and fibrils vs. monomers, recognized Aβ deposits in mouse and human brain sections, and reduced brain amyloid levels in Tg2576 mice without inducing CAA and microhemorrhages [61].

Three Phase 1 clinical trials with AD01 (NCT00495417, NCT00711139, NCT01225809), three Phase 1 trials with AD02 (NCT00633841, NCT00711321, NCT01093664), and a Phase 1 trial with AD03 (NCT01309763) in mild-to-moderate AD patients have been completed. A Phase 2 trial with AD02 in patients with early AD (NCT01117818) has been completed and a Phase 2 (NCT02008513) to evaluate continued administration with AD02 was terminated. The data from Phase 1 studies showed a favorable safety profile with AD02 and AD01 at 1 year [62]. No data is available from the completed Phase 1 study with AD03.

In the double-blind, placebo-controlled, randomized, multicenter, AD02 trial with early AD patients, two dose levels of AD02 were evaluated in combination with one of the two adjuvant formulations vs. placebo (placebo formulation 1, placebo formulation 2, 25 μg AD02 + formulation 1, 25 μg AD02 + formulation 2, 75 μg AD02 + formulation 2). 333 subjects with early AD aged 50–80 years were enrolled and received four monthly injections of the study drug followed by two booster immunizations at months 9 and 15. Surprisingly, only the placebo formulation 2 group showed clinical stabilization and reduced hippocampal atrophy. Affiris, the company developing these compounds, has renamed placebo formulation 2 as "AD04" but no further information is currently available (06 Jun2014: http://www.alzforum.org/news/research-news/surprise-placebo-not-av-vaccine-said-slow-alzheimers; 4 June 2014 PressConference:http://webtv.braintrust.at/affiris/2014-06-04/).

3.3 ACI–24

ACI-24 is a liposome-based vaccine in which two terminal palmitoylated lysine residues are covalently linked at each end of $Aβ_{1–15}$ to anchor the peptide into the liposome [63]. Administration of ACI-24 in double-transgenic APPxPS-1 mice elicited antibody responses mainly of the IgG isotype (IgG1, IgG2b, IgG3) that are either associated with non-inflammatory TH2 or T-cell-independent responses. Further, ACI-24 immunization did not result in significant increases of inflammatory cytokines (IL-1β, IL-6, IFN-γ, or TNF-α) or microglial activation/astrogliosis. APPxPS-1 mice treated with six inoculations of ACI-24 over 3 months showed improvements over control-treated mice in a

hippocampal-dependent novel object recognition test. ACI-24 is currently being evaluated in a Phase 1/2, double-blind, randomized, placebo-controlled trial in patients with mild-to-moderate AD (EudraCT 2008-006257-40). Enrolled subjects must be 40–90 years of age, have an MMSE between 18 and 28, and have evidence of brain amyloid burden by amyloid PET imaging. The main objectives of this study are to evaluate the safety, tolerability, immunogenicity, and efficacy of ACI-24 in a 52-week period. Assessments of cognition, function, and fluid/imaging biomarkers are performed.

3.4 CAD106

CAD106 is composed of multiple copies of $A\beta_{1-6}$ conjugated to a carrier, viruslike particle (VLP), derived from *Escherichia coli* RNA bacteriophage Qβ [64, 65]. Preclinical data [64] showed that CAD106 induced Aβ antibody titers which reduced brain amyloid accumulation in two APP transgenic mouse lines without any increase in microhemorrhages or inflammatory reactions. CAD106 elicited production of antibodies of different IgG subclasses and thus has the potential for different effector functions. Antibody production was similarly elicited by CAD106 in rhesus monkeys and these antibodies were shown to protect from Aβ toxicity in vitro. A case of meningitis was observed in one of the 77 monkeys that were treated with CAD106 with no relation to titers and no occurrence of encephalitis [65].

One Phase 1 study (NCT00411580), two Phase 2 studies (NCT00733863, NCT007795418), and their corresponding extension studies (NCT00956410, NCT01023685) have completed [65]. The Phase 1 study evaluated safety, tolerability, and immunogenicity of CAD106 administered subcutaneously over 52 weeks. This study included 58 patients with mild-to-moderate AD in two cohorts: 50 μg CAD106 or placebo administered at weeks 0, 6, and 18 (cohort 1); or 150 μg CAD106 or placebo at weeks 0, 2, and 6 (cohort 2). Most AEs were mild, with injection-site erythema as the most frequent effect (4 % in cohort I; 64 % in cohort II), while serious AEs were considered unrelated to study medication. CAD106 was associated with an antibody response in 67 % of treated patients in cohort 1 and 82 % patients in cohort 2. These results are consistent with CAD106 only eliciting B-cell and Qβ-related T-cell responses.

In two 52-week, Phase 2a, studies in 58 patients with mild AD, 150 μg CAD106 was administered subcutaneously at weeks 0, 6, and 12 (study 1), or either subcutaneously or intramuscularly at weeks 0, 2, and 6 (study 2). The results of study 1 showed antibody response in 20/22 patients. Because the results indicated that the week 2 injection did not enhance antibody response, a 0/6/12-week regimen was selected for further study. In addition, a Phase 2 study investigating repeated administration of CAD106 intramuscularly has completed (NCT01097096). This study evaluated CAD106 at two doses (150 μg or 450 μg) or placebo at a

7:1 randomization ratio in mild AD patients (MMSE 20–26). Subjects received up to seven injections of CAD106 or placebo over 60 weeks with a follow-up at 78 weeks. One hundred twenty-one patients were enrolled with 106 receiving CAD106 and 15 receiving placebo. Two-thirds of the CAD106-treated patients were classified as strong serological responders. CAD106 was generally safe and well tolerated with four cases of asymptomatic ARIA (3 ARIA-H and 1 ARIA-E) reported. In biomarker substudies, strong serological responders demonstrated reduced brain amyloid load on Florbetapir PET and decreased P-Tau levels in CSF as compared to controls [67]. A large Phase 2/3 prevention trial in persons at risk of developing AD due to APOEε4 homozygote status is planned (NCT02565511).

3.5 Lu AF20513

Lu AF20513 is a therapeutic vaccine constructed of three copies of the B-cell epitope of $A\beta_{42}$ ($A\beta_{1-12}$) attached to P30 and P2 T-helper epitopes from tetanus toxoid (TT), which replaces the T-helper epitopes of $A\beta_{42}$. This construct is intended to reduce the potential for proinflammatory responses and to improve the ability of the elderly to mount an effective immune response by stimulation of pre-existing memory T-helper cells from previous exposure to the TT vaccine [68]. Co-administration of Lu AF20513 with an adjuvant (either CFA/IFA or Quil-A, which has a human use version, QS-21) in an AD transgenic mouse model, Tg2576, induced robust anti-Aβ IgG titers, which are functionally potent based on in vitro assay results. Treatment with Lu AF21503 reduced brain amyloid plaque burden as well as soluble $A\beta_{40}$ and $A\beta_{42}$ in Tg2576 mice brain. Finally, Lu AF21503 reduced glial activation without increasing cerebral amyloid angiopathy or microhemorrhages. Currently, a Phase 1 open-label, dose-escalation, multiple immunization study (NCT02388152; EudraCT 2014-001797-34) is being conducted to evaluate the safety, tolerability, and immunogenicity of Lu AF21503 in patients with mild AD.

3.6 UB-311

The UB-311 immunotherapeutic vaccine consists of the $A\beta_{1-14}$ peptide coupled to the UBITh® helper T-cell epitope. UB-311 is designed for minimization of inflammatory reactivity through the use of a proprietary vaccine delivery system that biases T-helper type 2 regulatory responses in preference to T-helper type 1 proinflammatory responses [69]. A Phase 1 open-label clinical trial in mild-to-moderate AD patients (NCT00965588) to evaluate safety, tolerability, and immunogenicity of intramuscularly administered UB-311 at weeks 0, 4, and 12 has been completed. In addition, an observational extension study (NCT01189084) to monitor long-term immunogenicity in subjects enrolled in the original Phase 1 therapeutic trial has also completed. While no data has been posted or published, the company website (United Biomedical, Inc.) stated that UB-311 was safe and well tolerated in the Phase 1 study and that a Phase 2 study is being initiated.

3.7 V950

V950 is a multivalent Aβ compound [70]. Preclinical studies have shown that administration of V950 results in the production of anti-Aβ antibodies in the serum, and CSF that recognizes pyroglutamate-modified and other N-terminally truncated Aβ fragments [70]. A Phase 1 study of V950 in patients with AD has been completed and results are available (www.clinicaltrials.gov; NCT00464334). This study evaluated safety, tolerability, and immunogenicity of i.m. administered V950 formulated with aluminum adjuvant with or without ISCOMATRIX at 0, 2, and 6 months. Four dose levels of V950 (placebo to 0.5, 0.5, 5, or 50 mg) were tested in combination with four dose levels of ISCOMATRIX (0, 16, 47, 94 μg). Subjects were on average 74.2 (± 8.85) years old and 45/86 were female. Anti-Aβ antibody titers measured 1 month post the third immunization ranged from less than baseline or only approximately 2.7-fold higher than baseline. No additional studies have been initiated.

3.8 DNA Amyloid-β Immunotherapy

While still in preclinical evaluations, DNA Aβ vaccines represent the next generation of immunotherapies for AD [71–73]. Since its introduction in the early 1990s as a way to deliver immunogens via genetically engineered DNA, investigators have made much progress on optimizing this platform for eliciting higher antibody responses which are more consistent and sustained [74]. Progress in other disease areas (infectious diseases, HIV, and oncology) has recently led to development of DNA Aβ vaccines for AD. The two main approaches include utilizing viral vectors (either live attenuated or non-live) or naked DNA plasmids and in-tandem fusion of one or multiple copies of the full-length $A\beta_{42}$ (e.g., [75, 76]) or N-terminal Aβ peptides without the T-helper epitope (e.g., [77–79]). The shorter N-terminal peptide DNA vaccines also typically include fusions with a sequence for an immune modulator, such as PADRE (pan human leukocyte antigen DR-binding peptide) that provides a non-self T-helper cell epitope. Immunization with these constructs as seen in other disease areas does not translate to high titers in nonhuman primates or in humans [74]. Large efforts to improve antibody production with different dosing regimen, prime-boost strategies, and optimized delivery methods (e.g., electroporation) are under way (e.g., [80–82]; reviewed in [71–73]) before clinical testing is likely to begin.

4 Benefits and Challenges with Active Immunotherapy

Active immunotherapy offers several advantages over the passive approach. It has the potential of generating persistent therapeutic antibody titers over a longer time period, which obviates the need for frequent re-administration that is required of passive immunotherapy. This simpler mode of administration is appealing in light

of the possible need to treat AD early in the disease course and for years thereafter. The antibodies raised with active immunotherapy are likely to be polyclonal responses against different epitopes and IgG subtypes, thus having the potential for greater efficacy against multiple amyloid beta species versus the monoclonal approach with passive immunotherapy. Due to the slow rise to peak titers and the route of administration (intramuscular or subcutaneous), active immunotherapy may also provide a better safety profile compared with monoclonal antibodies, which are typically administered by intravenous infusion that reaches the maximum concentration rapidly post-infusion.

However, as active immunotherapy relies on the patient's own immune response, the extent and nature of anti-Aβ antibody production are likely to vary substantially among individuals. For this reason, some patients may not be able to mount an efficacious antibody titer level, especially in the immunosenescent elderly population [83]. The reduced predictability and control over antibody titers elicited have implications for the number of individuals who would benefit from treatment. Nonresponders would need to be accurately identified and offered other treatment regimens. The time lag to maximum titers also means that it may take a longer time for the onset of therapeutic benefit. Further, if there is an antibody-related safety issue observed once a response is elicited, it would not be easy to turn off an immune system that is already primed to produce antibodies. Finally, the optimum dose regimen needed to achieve the beneficial antibody titers is also an evolving science that will need to be empirically evaluated.

5 Conclusion

The search for the next-generation therapeutics for AD continues despite the lack of success for the last 10 or more years [24, 25, 84, 85]. Active immunotherapy with therapeutic vaccines targeted against the Aβ molecule represents one promising avenue of drug development. Initial experience with AN1792 led to the development of second-generation vaccines that allow for B-cell-generated specific Aβ antibodies that circumvents the T-helper cell-induced proinflammatory responses associated with the safety events observed with AN1792. The optimum titer required to generate a therapeutic benefit is presently not known and will likely relate to the choice of constructs, formulations, and combinations of adjuvant immunomodulators. The dose regimen to obtain such optimum titers is also under evaluation.

Finally, in recognition that AD begins 10–20 years or more before the earliest clinical symptoms appear and prior to dementia onset, there is a growing consensus in the field that intervention at earlier stages of AD may be more impactful [84–87]. To date, most

programs for active immunotherapy against Aβ have evaluated patient populations at the mild or mild-to-moderate AD stage, whereas more recent programs are moving towards intervention at a stage before widespread neurodegeneration has occurred. In fact, active immunotherapy may be especially suited for long-term treatment of predementia AD patients who are younger, more active, and healthier than those who have already progressed to the dementia stage.

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