REVIEW ARTICLE

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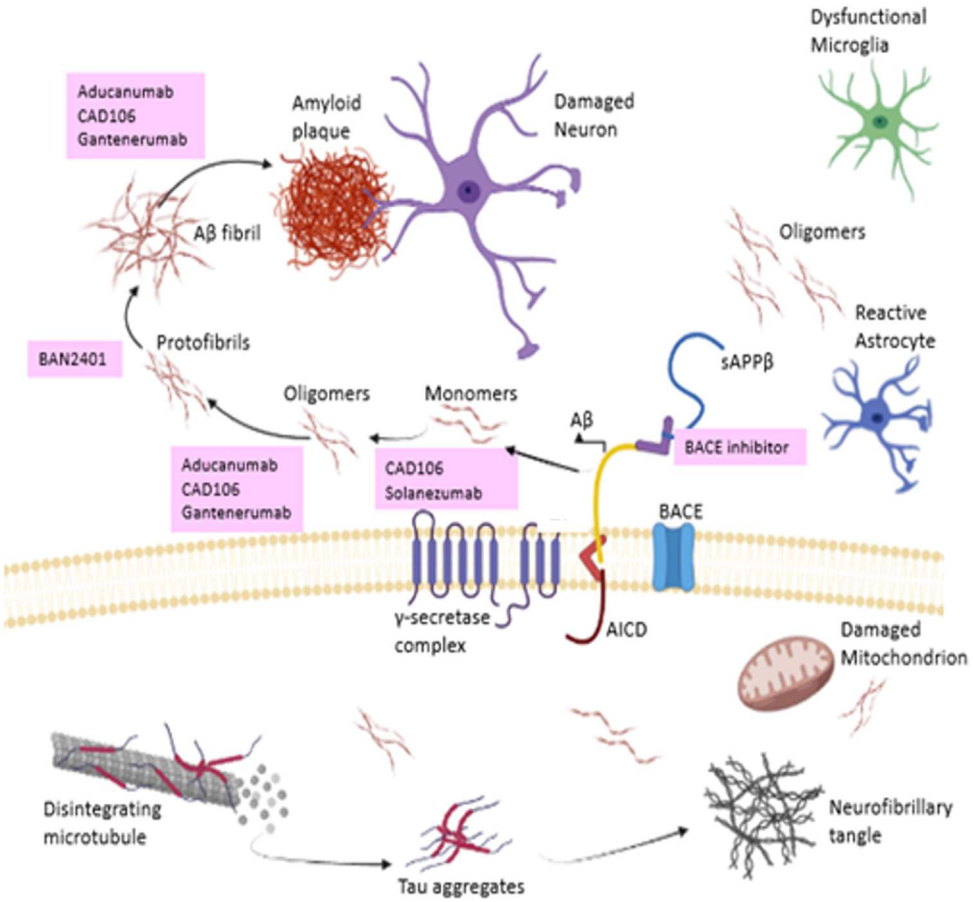

Author: Ciara Downey

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Exploring the Efficacy of Anti-amyloid-β Therapeutics in Treating Alzheimer Disease

Ciara Downey[1]
[1]Biochemistry, National University of Ireland, Galway

ABSTRACT

Alzheimer Disease (AD) is the most prevalent cause of dementia, characterized by initial memory impairment and progressive cognitive decline. The exact cause of AD is not yet completely understood. However, the presence of neurotoxic amyloid-beta (Aβ) peptides in the brain is often cited as the main causative agent in AD pathogenesis. In accordance with the amyloid hypothesis, Aβ accumulation initially occurs 15-20 years prior to the development of clinical symptoms. Current therapies focus on the prodromal and preclinical stages of AD due to past treatment failures involving patients with mild to moderate AD. Passive immunization via exogenous monoclonal antibodies (mAbs) administration has emerged as a promising anti-Aβ treatment in AD. This is reinforced by the recent approval of the mAb, aducanumab. mAbs have differential selectivity in their epitopes, each recognising different conformations of Aβ. In this way, various Aβ accumulative species can be targeted. mAbs directed against Aβ oligomers, the most neurotoxic species, are producing encouraging clinical results. Through understanding the process by which mAbs target the amyloid cascade, therapeutics could be developed to clear Aβ, prevent its aggregation, or reduce its production. This review examines the clinical efficacy evidence from previous clinical trials with anti-Aβ therapeutics, in particular, the mAbs. Future therapies are expected to involve a combined-targeted approach to the multiple mechanisms of the amyloid cascade in a particular stage or disease phenotype. Additional studies of presymptomatic AD will likely join ongoing prevention trials, in which mAbs will continue to serve as the focal point.

Keywords: clinical trials, active immunization, presymptomatic, vaccine, amyloid hypothesis, monoclonal antibodies, passive immunization, amyloid-beta, Alzheimer Disease

INTRODUCTION

AD is one of the biggest biomedical care challenges of this century. Only four decades ago, AD was virtually unknown to the public. A rise in the prevalence of AD is attributed to an aging worldwide population, with approximately 40 million individual AD cases. This figure is expected to double every 20 years until 2050 [1, 2]. Therefore, the provision of effective AD treatments is urgent [3]. Since 2003, no new therapeutics that slow or stop the progression of AD clinical decline have been approved by the American Food and Drug Administration (FDA) [4]. That is until June 7th, 2021, when the FDA granted the mAb aducanumab accelerated approval [5]. For the last 17 years, only symptomatic treatments were available [4]. However, aducanumab marks the first AD treatment associated with slowing the rate of AD progression [6]. AD is accountable for up to 70% of all cases of dementia [7]. Dementia is associated with the age-linked degeneration of the brain's neurocognitive domains, affecting a person's behaviour, thinking, and memory. These effects can occur as initial symptoms of AD, depicted as preclinical AD, and can affect an individual's cognitive function a decade or more before overt brain dysfunction [8].

The cardinal features of AD, namely amyloid plaques and neurofibrillary tangles (NFTs), have been recognised for over a hundred years, having first been described by Alois Alzheimer, the Bavarian psychiatrist that AD was named after [9]. Alzheimer attempted to correlate the clinical symptoms of his patient, Auguste D., to the pathological features he observed in her autopsied brain, specifically nerve cells with dense fibril bundles (NFTs) and 'miliary foci' (amyloid plaques) [10]. Decades later in 1984, George Glenner shifted the focus from AD pathology to genetics through his and Caine Wong's prediction of an AD gene located on chromosome 21. Glenner and Wong derived and analysed cerebrovascular amyloid from patients with Down Syndrome (trisomy 21) and reported the amino acid sequence of Aβ [11]. It was this study that initiated AD's 'amyloid hypothesis,' which was further backed up by three separate studies conducted independently in 1991 [12-14]. The amyloid hypothesis describes how Aβ protein accumulation is the initial event in the AD process [7, 15]. It is the production of Aβ in the brain that leads to the development of AD clinical syndrome [2].

Amyloid plaques are located extracellularly between neurons and consist of fibrils containing Aβ peptides. NFTs are intracellular and consist of tau, a hyperphosphorylated protein. Both these lesions represent the two neuropathological hallmarks of AD that present research is currently focused on [7, 16, 17]. To this day, the presence of Aβ and NFTs are still required for AD pathological diagnosis [18]. The primary amino acid sequence of Aβ was first characterized and purified from extracellular amyloid plaques in 1984 [15]. Aβ is composed of a group of peptides ranging from 37 to 49 amino acid residues in size. Various structural approaches, such as distance geometry, X-ray crystallography, and nuclear magnetic resonance, highlighted the structural conversion from Aβ monomers to oligomers, protofibrils, fibrils, to amyloid plaque deposition. This conversion involves a quick structural state transition, in which the loosely aggregated strands are associated into helical and β-sheet structures [19]. As the oligomeric state is more transient, its structural characterization is more complicated than that of amyloid fibrils [20]. New insights into aggregation and structural pathways may assist in uncovering the mechanisms of amyloid pathogenesis in neurodegenerative diseases. This could ultimately lead to new

therapeutic strategies aimed at preventing the formation of neurotoxic Aβ aggregates [19].

Aβ is a family member of amyloid polypeptides, and following its improper folding, it can inappropriately interact with certain cell types to invoke neurodegenerative diseases, such as Huntington's, Parkinson's, prion disease, and AD [21]. In AD, neurons throughout the brain can become injured and die, causing neuron connections to disassemble and the shrinkage of certain brain regions. In the final AD stages, this process is known as brain atrophy. At an anatomical level AD is defined by brain atrophy, which involves neural loss. To some degree, this atrophy and subsequent brain shrinkage is seen in cognitively healthy aging individuals. However, in individuals who suffer from mild cognitive impairment (MCI), there is an acceleration of this atrophy. This atrophy acceleration is increased further in individuals who progress from MCI to AD [22]. Anomalous neural activity, loss of neurons and synapses, and degeneration of specific populations in the neuronal ensemble are associated with AD patients' cognitive decline [3]. Structural Magnetic Resonance Imaging (MRI) is used to assess brain atrophy, in particular, the hippocampus, and this has been noted as a valid AD biomarker through post-mortem histology [23, 24].

The two main types of AD are late-onset (LOAD), also known as sporadic AD, and early-onset (EOAD) or dominant familial AD. The apolipoprotein E (APOE ε4) allele is linked to abnormal Aβ accumulation and has the strongest genetic risk factor link to LOAD [17]. EOAD occurs in approximately 5-10% of all AD cases and arises from mutations in either the presenilin 1 gene (PSEN-1), the presenilin 2 gene (PSEN-2), or the amyloid precursor protein (APP) encoding gene [25]. These gene products influence the formation of Aβ [26]. The Aβ peptide is generated through the cleavage of APP by γ-secretase and β-secretase. Factors such as alterations in proteolysis, protein folding machinery, amino acid sequence mutations, and protein hydrophobicity can cause Aβ aggregation, which forms the amyloid plaques [27]. Individuals with trisomy 21 have an extra APP gene copy, which results in an increased risk of AD development later in life due to increased amyloid production [7]. From certain EOAD forms, it was discovered that mutations in the APP gene lead to the enhanced production of Aβ, strongly suggesting that amyloidogenic Aβ leads to AD [28]. Protective mutations that reduce cleavage of the APP have been described, which lower the risk of AD development [26]. Through understanding the factors that lead to AD pathogenesis, it becomes vital to address the mechanisms of disease, its diagnosis, and the development of effective anti-Aβ therapeutics against it.

The diagnosis of AD is based upon an individual's medical history and clinical findings, at times verified by brain imaging, such as Positron Emission Tomography (PET) and MRI. Excluding aducanumab, current therapies target symptoms and do not affect AD progression [17]. Only four acetylcholinesterase (AChE) inhibitors and one type of an N-methyl-D-aspartate receptor antagonist have been FDA approved for AD treatment [4]. In the past 18 years, many drugs, including BACE1, γ- and β-secretase inhibitors have been developed to antagonize the aggregation of Aβ, decrease its production, or clear the increased levels of Aβ in the brain, with little to no success (Table 1) [16]. Currently, the most comprehensive anti-Aβ approaches are active and passive immunotherapy [29]. Active immunization stimulates the clearance of Aβ due to an immunological response elicited by administering an Aβ antigen [16]. However, immunologically-based long term adverse

reactions and irregular or absent immune responses may occur, particularly in elderly individuals [29].

As the initiation of Aβ accumulation occurs 15-20 years before clinical symptoms, drugs such as the mAbs are currently in phase III clinical trials in patients with asymptomatic or preclinical stages of AD and patients with a high risk of AD [17]. The approval of aducanumab is conditional and in accordance with prescribing information from the US FDA, only patients with MCI or early AD can be treated using this mAb. This correlates with the populations treated in clinical trials. Consequently, there is no effectiveness or safety data on the initiation of treatment at a later stage of disease progression. Aducanumab's approved status may be contingent upon further clinical benefit results in confirmatory trials [30]. mAbs are produced by cloning immune cells from unique parent cells. These antibodies have a monovalent affinity as they bind to the same epitope.
Either humanized or fully human mAbs are used in treating AD. Humanized antibodies are produced from protein modifications in non-human species that have sequence similarity to the naturally occurring human variants. The fully human mAbs can avoid some of the side effects experienced by humanized antibodies as they are derived from phage display or transgenic mice [31]. Amyloid-related imaging abnormalities (ARIA) are commonly experienced through passive immunization with mAbs [26]. In passive immunization, the toxic Aβ species are cleared directly, with complement activation or through microglia, which are a specialised subtype of macrophages (Fig. 1) [31]. In comparison to active immunization, the adverse events associated with passive immunization can be controlled through stalling treatment. Also, antibody titers are kept consistent [29]. Reasons for past clinical failures associated with mAbs include late intervention, poor-target engagement, and inappropriate trial patient selection [16, 17]. These clinical failures could potentially undermine further development of anti-Aβ therapeutics. However, current ongoing trials will hopefully highlight this critical issue [32].

Aβ and the Amyloid Hypothesis

The association with inherited EOAD resulted in the formation of the amyloid hypothesis [26]. Proteolytic processing is a type of post-translational modification (PTM) in which the target protein's activity is modified from the cleavage of a protease on one or more of its bonds. Secretase enzymes function in the alternative proteolytic processing of APP (reviewed in [33]). It is the proteolytic processing of the ubiquitous glycoprotein APP that generates the Aβ peptide [34]. The metabolism of APP is usually carried out by an extracellular protease α-secretase and an intramembrane protease γ-secretase, creating a soluble protein that can be broken down to aid in neuron repair [2]. This is so-called the non-amyloidogenic pathway, and it co-exists with the pathologic (abnormal) amyloidogenic pathway in which the toxic Aβ peptide is formed [34]. The sequential cleavage of the APP with γ-secretase and β-secretase, also known as β-site APP cleaving enzyme 1 (BACE1), gives rise to Aβ [1, 2]. Cleavage with BACE1 generates an N-terminal fragment, called the soluble amyloid precursor protein-β (sAPPβ), and a membrane-bound fragment C99. C99 in turn is cleaved by a 4-protein enzymatic complex within the membrane, known as the γ-secretase complex. Aβ is released from the cleavage of γ-secretase to a peptide named the amyloid intracellular domain [16]. This Aβ tends to have 'sticky' monomers that clump together to produce extracellular Aβ fibrils highly resistant to proteolysis. Aβ aggregation forms oligomers,

protofibrils, Aβ fibrils, and eventually Aβ plaques (Fig. 1).

The molecular mechanism that drives Aβ aggregation involves the formation of different soluble oligomeric intermediates of various structures and sizes. These oligomeric species play a key role in the amyloid cascade that ultimately develops into AD through various neurotoxic events, such as mitochondrial damage, cell membrane permeabilization, inflammation, oxidative stress, and calcium dysregulation [35]. It is thought that the direct binding of Aβ oligomers to specific neuron receptors disrupts the typical signalling cascade [31]. The most recognised variants of Aβ are Aβ42 and Aβ40, comprising of 42, and 40 amino acid residues, respectively. Aβ42 is the main isoform in amyloid plaques and has the most neurotoxic form [36]. Therefore, it is no surprise that Aβ42 production reduction is one of the three therapeutic strategies used in AD treatment. The other two mechanisms of action (MOAs) employed in anti-amyloid disease-modifying treatments are Prevention of Aβ aggregation, and Aβ clearance [2]. Fig. 1 shows the anti-Aβ drugs that are currently in clinical trials for AD treatment.

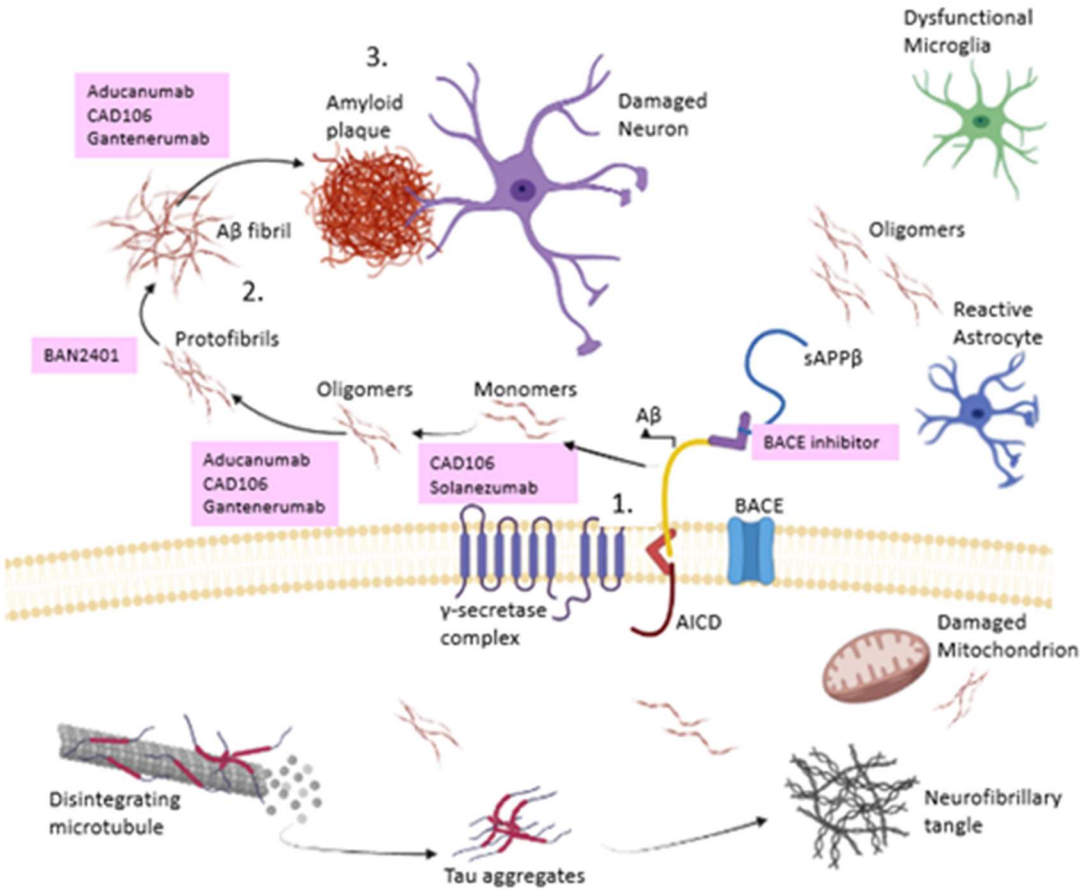

Figure 1 - The different Aβ aggregated targets of the main anti-Aβ drugs. Anti-Aβ drugs (pink boxes) currently in phase III clinical trials for AD treatment. (1). Aβ peptide formation from the cleavage of the APP with BACE1 and γ-secretase. (2). The Aβ peptide self-associates into various aggregated forms; monomers, oligomers, protofibrils, and Aβ fibrils. (3). These aggregative species further mature into amyloid plaques. Abbreviations: AICD: amyloid precursor protein intracellular domain; mAbs: monoclonal antibodies; sAPPβ, soluble amyloid precursor protein-β. Figure created by the Author.

Crosstalk Between Aβ and Tau

The main function of tau proteins is to prevent microtubules from falling apart. Additional roles this microtubule-associated protein has include axonal extension, morphogenesis, and neuronal protein transport [37]. As illustrated above, once a microtubule is disintegrated, tau protein can aggregate from altered PTMs, such as acetylation or hyperphosphorylation, and form NFTs in the brains of individuals with AD [3]. In addition to AD, other neurodegenerative diseases, such as frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), Down syndrome (DS), progressive supranuclear palsy (PSP), and Pick's disease (PiD), show hyperphosphorylated tau aggregation in the brain. Collectively these familial and sporadic neurodegenerative disorders are known as 'tauopathies' [38, 39]. Six different isoforms of human tau are expressed through the alternative splicing of exons 2, 3, and 10 in tau pre-mRNA [40]. In the normal adult brain, equal levels of 3R-tau and 4R-tau are expressed [41]. However, in several tauopathies, there is an altered ratio of 3R/4R-tau expressed through the disruption of tau exon 10 splicing [42-44]. In individuals with FTDP-17, intronic or silence mutations of the tau gene cause the altered ratios of 3R-4R-tau [42]. Tau exon 10 splicing disruption and an altered ratio of 3R/4R-tau is sufficient in inducing dementia and neurodegeneration [37]. Tau NFTs, along with insoluble Aβ fibrils that form the amyloid plaque's core, are considered the main pathological hallmarks of AD [28].

There is compelling evidence that amyloid plaque deposits occur years, if not decades, before the formation of NFTs, brain atrophy, symptomatic changes, and spreading tau pathology [26, 45]. A particular prospective cohort study was conducted on patients with MCI and AD, and it was observed that the deposition of Aβ is prolonged and extended over two decades [45]. Hence, why the prediction of preclinical changes and AD clinical phase onset are vital for facilitating the timing and design of effective therapeutic interventions. In accordance with the amyloid hypothesis, the hyperphosphorylation of tau is considered a downstream Aβ deposition event. However, it is also possible that the pathways of Aβ and tau act in parallel in causing AD and enhancing their collective toxicity [2]. In addition, prevailing studies suggest that due to mutations in tau-coding genes, these NFTs can form independently of the toxic Aβ. The formation of the NFTs is thought to catalyse upstream Aβ toxicity [3, 46], despite these tangles being associated more with memory loss, confusion, and neuron and synaptic decline [3, 47, 48]. Hence, either tau or Aβ oligomers can function in damaging neuronal communication through synaptic damage. Therefore, synaptic loss prevention is a viable strategy to halt memory problems in AD patients [3].

Interestingly, it has been indicated that tau hippocampal deposition in the absence of Aβ may be inadequate in triggering the neurodegenerative process that leads to AD [16]. However, findings from longitudinal studies with inherited or sporadic AD showed an increase in tau cerebrospinal fluid (CSF) biomarker levels during early AD stages, which declined after the manifestation of symptoms [49, 50]. These findings are contradictory to the idea that rising levels of CSF tau in AD patients generally arise from dying neurons [16]. As a result, promising therapeutic approaches directed against tau aggregation must be pursued to prevent a therapeutic vacuum if present anti-Aβ measures fail. Currently, anti-tau therapies are an active area of research, as targeting tau is considered more effective than the clearance of Aβ upon the occurrence of clinical symptoms.

Besides AD treatment, tau-targeting drugs are being examined in other tauopathies, including Pick's disease, FTDP-17, corticobasal degeneration (CBD), and PSP. Thus, treatments have wider applications [51]. Unfortunately, the majority of initial tau therapies based upon tau aggregation, kinase inhibition, or microtubule stabilization have been discontinued due to efficacy failures. However, current anti-tau therapies are immunotherapies and are providing promising results in preclinical studies [16, 51]. Due to the fact that tau and Aβ aggregates have different temporal patterns of progression in the AD brain, three different temporal AD phases have been proposed; (1) Disruption of tau networks occurs in specific regions of the brain in clinically unaffected patients. (2) This tau disruption may trigger brain network changes that are associated with Aβ. (3) The deposition of Aβ initiates the failure of the tau-associated network [16, 52].

Treatment of AD

As of June 2021, only symptomatic therapies were available, and these are exclusively the AChE inhibitors and memantine as an N-methyl-D-aspartate receptor antagonist. One AChE inhibitor, tacrine, is no longer available. However, three more: galantamine, rivastigmine, and donepezil, currently exist on the market [4]. In clinical trials, these symptomatic therapeutics showed improvements on the Mini-Mental State Examination (MMSE) and AD Assessment Scale-cognitive subscale (ADAS-cog), which are questionnaires and neuropsychological assessments used to specify cognitive impairments, respectively [53]. As a result of extensive progress in the understanding of AD pathophysiology, large clinical developments, and huge expenditure on clinical trials, the first disease-modifying treatment for AD, aducanumab, achieved accelerated approval [5]. Prior to this, memantine, in 2003, was the last AD drug to be approved. Suboptimal dosing, improper patient selection, inappropriate intervention times, and outcome measures are all proposed reasons for anti-Aβ drug failures [16, 17].

Drugs that target Aβ are one of the most studied areas in AD research. As seen in Table 1, all the anti-Aβ therapeutic strategies are targeted during the mild to moderate stages of AD. This excludes the terminated trials of Solanezumab and Verubecestat which recruited patients in the prodromal or MCI stage of AD. Generally, the strategies for anti-Aβ drug development fall under one of three main MOA. The three major MOAs employed in anti-amyloid disease-modifying treatments are (1) the reduction of Aβ42 production through the use of γ-secretase inhibitors, BACE1 inhibitors, and modulators of α-secretase. (2) Aβ plaque reduction with drugs that infer with metals or anti-Aβ aggregation agents, and (3) Aβ clearance promotion through passive or active immunotherapy. Passive Aβ immunotherapy with mAbs is currently the most promising and active class [2]. In the past 20 years, most anti-Aβ therapies were tested in LOAD forms of AD. However, some passive anti-Aβ mAbs are being further reviewed in patients with EOAD, prodromal familial AD, and asymptomatic patients [16, 17].

Table 1 - Summary of the anti-amyloid β therapeutic strategies in AD treatment.
* The status of these clinical trials is based upon that reported in ClinicalTrials.gov (https://clinicaltrials.gov). Last accessed: 15th August 2021.

Drug (First released)	Mechanism of Action	CT identifier	Trial Phase	Status	Ref(s)
AN-1792 (2001)	Anti-Aβ vaccine	NCT00021723	II	Terminated	[27, 54, 55]
Tramiprosate/ ALZ-801 (2007)	Aβ aggregation inhibitor (Small molecule)	NCT00314912, NCT00088673, NCT00217763	III	Active, not recruiting	[55-57]
Bapineuzumab (2012)	Anti-Aβ monoclonal antibody	NCT00676143, etc	III	Terminated	[55, 58]
Aducanumab (2012)	Anti-Aβ monoclonal antibody	NCT01677572, NCT02484547, NCT02477800	I/II/III	Terminated	[55, 57, 59]
		NCT04241068	III	Enrolling by invitation	
Solanezumab (2013)	Anti-Aβ monoclonal antibody	NCT01127633, NCT01900665, NCT02760602	III	Terminated	[55, 58, 60]
		NCT02008357	II/III	Active, not recruiting	
		NCT01760005	II/III	Recruiting	
CAD106 (2014)	Anti-Aβ vaccine	NCT02565511	II	Terminated	[55, 61, 62]
Crenezumab (2014)	Anti-Aβ monoclonal antibody	NCT01998841	II/III	Active, not recruiting	[16, 55, 63]
		NCT02353598		Completed	
		NCT02670083, NCT03491150, NCT03114657	III	Terminated	
Gantenerumab (2014)	Anti-Aβ monoclonal antibody	NCT03444870, NCT01760005	II/III	Recruiting	[16, 55, 64, 65]
		NCT03443973	III	Active, not recruiting	
		NCT01224106, NCT02051608	III	Completed	
CNP520 (2015)	β-secretase inhibitor	NCT02565511	II/III	Terminated	[55]
		NCT03131453	II/III	Completed	
Lanabecestat (2018)	β-secretase inhibitor	NCT02245737, etc	II/III	Terminated	[16, 55]
Verubecestat (2018)	β-secretase inhibitor	NCT01739348, NCT01953601	II/III	Terminated	[16, 55]
BAN2401/ Lecanemab (2019)	Anti- Aβ mAb	NCT03887455	III	Recruiting	[55]

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Anti-Amyloid Disease-Modifying Treatments

The Reduction of Aβ Production

In accordance with the amyloid hypothesis, the sequential cleavage of the APP with γ-secretase and BACE1 gives rise to the amyloidogenic pathway. As a result, the inhibition of these secretases has been contemplated as a therapeutic approach against AD. However, γ-secretase also functions in cleaving different transmembrane proteins in addition to APP, for example, the Notch receptor 1 [2]. This receptor functions in controlling cell communication and differentiation and is partly responsible for recent clinical failures of γ-secretase inhibitors namely, avagacestat, tarenflurbil, and semagacestat [60, 66, 67]. Safety concerns regarding the use of γ-secretase inhibitors limited their use for many years until this enzyme was capable of being therapeutically targeted safely [68]. There are currently no γ-secretase inhibitors in clinical trials [2]. However, in early 2021 Rynearson *et al.*, presented promising results for a γ-secretase modulator, namely compound 2, that can safely and efficiently shift where the enzyme cuts the APP fragment at the C-terminal. This produces less toxic Aβ42 [69]. BACE1 inhibition interferes with any Aβ species or Aβ aggregation upstream in the amyloid cascade [16]. The pharmacological inhibition of BACE1 is used as a therapeutic as the drugs reduce Aβ levels in the AD brain [32]. Clinical trials with BACE inhibitors namely, verubecestat and lanabecestat have all recently been discontinued due to ineffectiveness, safety reasons, and lack of efficacy, respectively [2, 70, 71]. A dose-dependent and significant reduction of CSF Aβ42 was observed in all agents. However, these results indicated no functional or cognitive benefit and show how BACE1 inhibitors may be incapable of halting the progression of AD [72].

The Reduction of Aβ Plaque Burden

Aggregation inhibitors prevent the formation of Aβ42 fibres by directly interacting with the Aβ peptide. These inhibitors are considered to be a potential AD therapeutic. However, the oral drug scyllo-inositol (ELND005) was the last Aβ42 inhibitor to be tested in humans over ten years ago. Phase II clinical trials with AD patients provided no evidence of clinical improvements with ELND005 and serve toxic infections associated with this oral agent led to the forced cessation of trials [73]. Currently, the use of peptidomimetics that mimic specific peptides is in development, such as the γ-AApeptides as they partially reverse and inhibit the aggregation of Aβ42. Drugs that interfere with metals are capable of Aβ plaque reduction. This is because AD pathophysiology has strong links with dyshomeostasis and the abnormal accumulation of certain metal ions, for example, zinc, iron, and copper [2]. PBT2 is a metal protein-attenuating drug that has shown promising preclinical data during a three-month phase II AD treatment trial. PBT2 treatment reduced CSF Aβ by 13% and a dose-related improvement in cognitive function was observed in early AD patients [74].

The Promotion of Aβ Clearance

Active and passive immunization are the main immunotherapeutic approaches in AD treatment that are involved in promoting Aβ clearance [2]. During the process of active immunization, phosphorylated tau (ptau) peptides, Aβ peptides, or polymerized British artificial amyloidosis (ABri)- related peptides (pBri) are utilised as immunogens. These immunogens are presented to B cells through the action of antigen-presenting cells. Using ptau and Aβ peptides will produce antibodies

to their respective epitopes. However, both ptau and Aβ epitopes are produced from the use of pBri peptides [75]. In passive immunization, the mAbs to ptau and Aβ epitopes are systemically infused for the penetration of the blood-brain barrier (BBB). As these mAbs cross this BBB, they degrade, clear, or neutralise or disaggregate their specific targets [2]. Once the innate immune system is stimulated by either active or passive immunization, the amelioration of AD pathology is promoted through the function of macrophage [75]. Overall, the promotion of Aβ clearance seems a promising avenue if utilized very early in the progression of AD, prior to any disease symptoms. As a result, these Aβ-targeting strategies are currently in development in preclinical AD trials.

Active Anti-Aβ Immunotherapy

Active immunotherapy offers the long-term production of antibodies from only a short-term administration of drugs. Conversely, the first candidate of active anti-Aβ vaccines for AD patients was marred by an ill-fated clinical trial of AN-1792 in 2002 [27, 29]. This vaccine contained pre-aggregated Aβ42 with the immunological adjuvant QS21 [29]. Initially, in phase I clinical trials with AN-1792, it elicited a promising immunological response. However, one patient became severely unstable and passed away from a pulmonary embolism one year after her last AN-1792 vaccine. Post-mortem analysis indicated the patient developed T cell-mediated meningoencephalitis, an inflammation of the brain's protective membranes [76]. The exact cause of meningoencephalitis remained unclear [27]. Although, it had been suggested that an excessive Th1 response was likely the cause based on evidence from cytotoxic T cells which surrounded the cerebral vessels [27]. This phase I study showed the vaccine cleared most Aβ deposits in the brain but produced no clinical or cognitive benefits due to its small case numbers [54]. Subsequently, an additional phase II active immunization study began. However, around 6% of AN-1792 treated patients developed a similar type of T cell-mediated meningoencephalitis [27]. More recent AN-1792 immunization trials have confirmed a reduction in the Aβ plaque burden from active immunization with fibrillar Aβ proteins [77, 78]. In order to avoid the T cell epitopes located at the C-terminus, second-generation vaccines, such as the Aβ vaccine, ACC-001, and the immunotherapeutic agent, CAD106, have sought to only create anti-Aβ antibodies at the N-terminus [16, 29, 79-81].

Until early 2021, CAD106 is the only active anti-Aβ vaccine in phase III trials [17, 29, 62]. Antibodies elicited from CAD106 react with Aβ monomers and oligomers. Combination trials with CAD106 and a small molecule inhibitor of BACE1, called CNP520 were developed to remove amyloid plaques and reduce Aβ generation, respectively [3]. As of late 2019, CNP520 was discontinued as it was linked to a worsening cognitive score on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) which measures the five cognitive domains of memory, attention, language, executive functions, and spatial. Also, individuals in these trials had an increase in brain atrophy [82]. Other combination trials are hoped to be beneficial in late-stage AD patients as BACE1 inhibition may be insufficient and too late for elderly AD patients [3].

Passive Anti-Aβ Immunotherapy

Early AD intervention is being attempted through secondary prevention trials with mAbs. Secondary prevention trials only recruit preclinical or asymptomatic individuals who have positive AD biomarkers and not just a random selection of asymptomatic individuals

from the general public, as seen in primary prevention trials [83]. Passive immunization with anti-Aβ mAbs may potentially be involved in a preventative way of modifying the course of AD before clinical symptoms and widespread brain damage occurs [32]. Valuable lessons have been gained from previous failed phase III clinical trials with the mAbs solanezumab and bapineuzumab [54, 58]. Nowadays there are strict criteria for inclusion, such as amyloid positivity on biomarkers as part of secondary prevention trials. Furthermore, study designs became more accurate for specific targets, such as Aβ plaque reduction on amyloid PET, and higher dosing was made a requirement to avoid ARIA and APOε4 genotyping [2].

In 1999, Schenk and his colleagues first demonstrated how immunization with the Aβ peptide reduced plaque deposits in mice brains. These mice were genetically modified to experience AD symptoms similar to humans [84]. Three different hypotheses were later developed on the basis that Aβ-specific antibodies reduce AD symptoms (Fig. 2) [17]. It is vital to understand the three MOAs used in anti-amyloid disease-modifying treatments in order to develop more effective and safer immunotherapeutics. The hypotheses that have arisen are dependent on the efficiency of the antibody to enter the central nervous system (CNS) and take effect there or whether the antibody is located in the periphery, which is sufficient enough to give beneficial results [27]. The first hypothesis (1) involves the direct action of anti-Aβ antibodies against Aβ oligomers, protofibrils, fibrils, or Aβ plaques, where the binding destabilised these aggregate species [17]. Secondly, (2) the involvement of microglia-mediated by fragment crystallizable (Fc) receptors which initiates phagocytosis of Aβ and complement activation [85]. Lastly, (3) the 'peripheral sink' hypothesis suggests that amyloid equilibrium through the BBB is changed following free concentrations of Aβ in the blood. The antibodies do not cross the BBB but bind to Aβ circulating in the plasma, thus creating a concentration gradient and net efflux of Aβ from the brain to the blood and plasma [31]. It is still unclear whether the entry of anti-Aβ mAbs through the brain is required.

It has been proposed that past clinical failures with mAbs can be attributed to their poor CNS penetration, as only 0.1% were capable of crossing the BBB [86]. Attempts to improve mAbs penetration into the brain have focused on gene encoding antibody delivery, expression induction, and receptor targeting in the BBB to allow for the induction of active transport of the mAbs into the CNS [29]. The mechanism of action of mAbs is to initially capture the target where the secondary effector function is linked at the Fc domain of the mAbs. A differentiating point in the mAbs is their Fc region, which is invariable. Its interaction with microglial immune cells mediates phagocytosis and degrades the antigen-antibody complex [31].

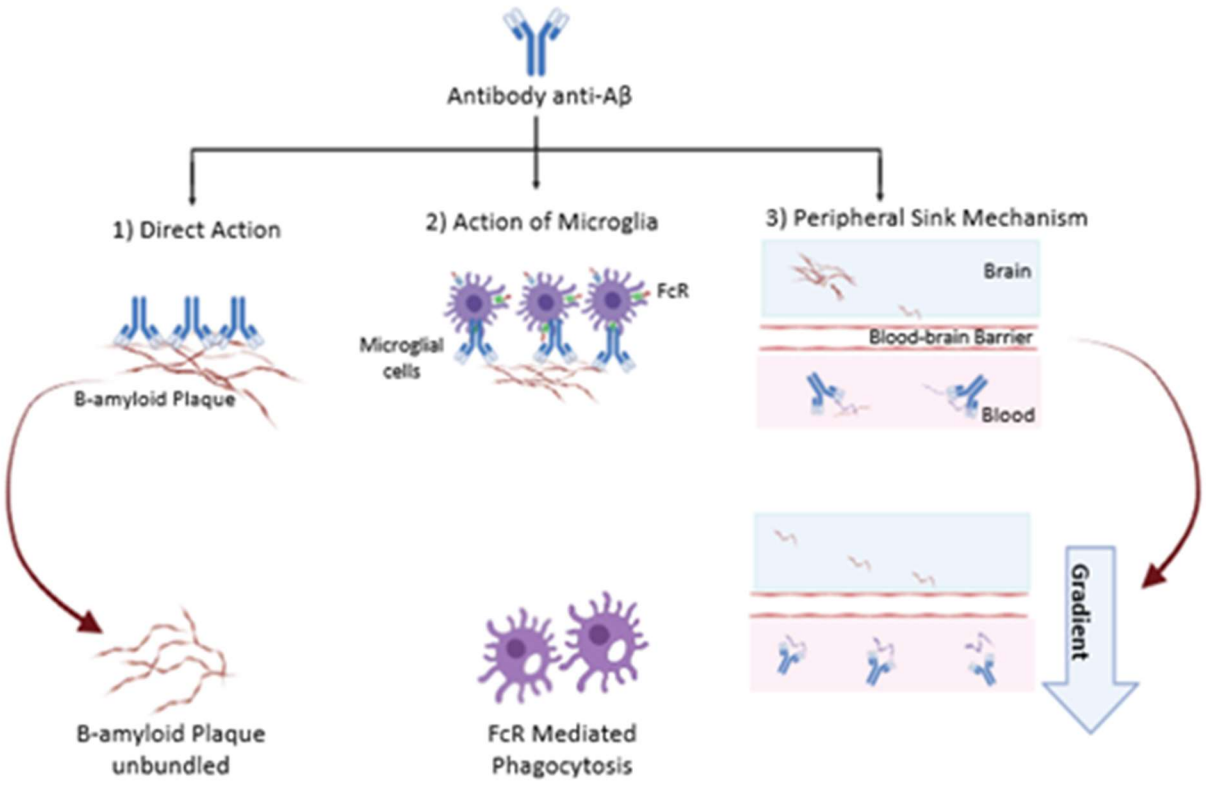

Figure 2 - The three main mechanisms of Aβ removal via specific antibodies. (1) Anti-Aβ antibodies in direct action against the aggregated forms of Aβ. **(2)** Microglia activation initiates the phagocytosis of Aβ. **(3)** Aβ is removed via antibody binding and the generation of a net efflux from the brain to the plasma. Abbreviations: FcR: Fc receptors. The different Aβ aggregated targets of the main anti-Aβ drugs. Figure created by the Author.

Differential Selectivity in Aβ Oligomers

The severity of AD correlates strongly to the level of non-fibrillar, soluble Aβ oligomers. This suggests these soluble Aβ species, rather than the fibrils in amyloid plaques, likely play a vital role in AD pathology. Aβ42 has the strongest intrinsic tendency to self-aggregate compared to the other oligomeric species, even Aβ40, which is produced in a higher abundance [87]. Conversely, Aβ40 has been reported to be unaltered in AD. However, its concentration ratio with Aβ42 (Aβ42/40) is suggested to be more efficient than the concentration of Aβ42 alone in diagnosing individuals with AD [88]. To date, there is a lack of comprehensive reviews on the use of the Aβ42/40 ratio for AD diagnosis [89]. Currently, mAbs in clinical trials are focused on addressing both Aβ42 and Aβ40 isoforms. Various soluble oligomers aggregate during the formation of amyloid, all with different structures, shapes, sizes, and hydrophobicity content [35]. As these variations occur, characterization between different soluble oligomeric aggregates is essential in determining how specific function relates to disease progression. On this basis, recent advances have been made on generating various mAbs capable of recognising the specific epitopes of Aβ in order to hinder its aggregation [35]. These mAbs can

be administered in an injection or infusion [31]. The majority of mAbs in development are human immunoglobulin G (IgG1) derivatives. IgG1s are the same subtype of antibody capable of inducing pro-inflammatory cytokine release including IL-1β, TNF-, and IL-6, hence why specific side effects can occur [26].

Aducanumab, gantenerumab, and BAN2401 are all human or humanized mAbs capable of binding to aggregated forms of Aβ with high affinity. This binding promotes Aβ removal by Fc receptor-mediated phagocytosis. Each of these antibodies shows different selectivity to Aβ soluble oligomers when compared to the insoluble amyloid plaques and fibrils [57]. Aducanumab, gantenerumab, and BAN2401 all bind primarily to insoluble and soluble aggregates [28]. A major drawback in the current clinical trials of passive immunotherapy is the lack of specific targeting in most toxic Aβ oligomers, for example, aducanumab and gantenerumab bind to Aβ fibrillation or aggregates, while crenezumab can recognise different Aβ forms [90-93]. Through the 'peripheral sink' hypothesis, the antibody that targets soluble Aβ may play a role in the preclinical stages of AD. The mAbs should be treated in EOAD in order for their therapeutic effect to be enhanced prior to cognitive or synaptic impairment. Patients who are already experiencing AD symptoms will likely show no significant effect from using these anti-Aβ drug treatments [94].

Current mAbs in Clinical Trials

There are many mAbs trials currently underway in mild and prodromal AD cases, including aducanumab, gantenerumab, and BAN2401 (Table 1). Also, gantenerumab is in studies for at-risk and preclinical individuals [2]. The initial results from the aducanumab and BAN2401 trials suggest that there is a reduction in the cerebral amyloid burden and a declaration of cognitive decline identified based on these treatments in individuals with very mild and prodromal AD [65, 95]. Contrary to this, the initial preliminary clinical trial results of gantenerumab in prodromal AD patients were stopped prematurely due to lack of efficacy [96]. Further analysis was completed and higher gantenerumab doses were suggested for efficacy, and as a result, an extension with more mild AD participants was continued alongside a placebo-controlled double-blind study with individuals with mild AD [97]. The lack of efficiency so far with anti-Aβ mAbs may reinforce the case against the amyloid hypothesis. However, encouraging results with some mAbs equally make completely dismissing this hypothesis difficult [29].

Converging evidence over the last two decades has suggested that the soluble oligomers are the most neurotoxic species of Aβ and are the target of most mAbs [1]. Montoliu-Gaya and Villegas reviewed that targeting the N-terminus region of the Aβ peptide is the most effective method for the mAbs in clearing the neurotoxic aggregated species [98]. The success of N-terminal targeting mAbs is attributed to phagocytosis and microglial activation, which are hypothesised to be the main features of aducanumab, gantenerumab, and BAN2401. Transgenic mouse models demonstrated that the mAbs cross the BBB to bind specifically to Aβ in amyloid plaques [29]. Once mAbs cross the BBB, they can achieve sustained brain concentrations at levels that are efficient in removing or inhibiting Aβ oligomers [57]. However, a major concern with current mAbs is the limitations that exist in using transgenic mouse models. The mice are modified to match the physiological and genetic states in humans. However, differences do exist, thus limiting the translatability of preclinical findings in mice to actual trials in humans with sporadic

AD. Furthermore, in mice that overexpressed APP, anti-Aβ mAbs worsened their neuronal dysfunction [8].

In clinical trials, aducanumab and gantenerumab required around five months of a titration regimen in dosing for peak brain levels to reach a steady state [57]. The administration of the highest intravenous dose of aducanumab (10 mg/kg) monthly was needed to achieve clinical efficacy [2]. In contrast, BAN2401 is expected to achieve constant peak brain levels in approximately two and a half months, based on 10 mg/kg dosing twice monthly. Considering that no titration period is required, BAN2401 may have an earlier onset of clinical efficiency than aducanumab [57]. The clinical efficacy of some monoclonal anti-Aβ mAbs, specifically gantenerumab, solanezumab, and crenezumab, are being evaluated under two preventative studies, Alzheimer's Prevention Initiative (API) and Dominantly Inherited Alzheimer Network (DIAN-TU) in asymptomatic subjects. Both API and DIAN are very sensitive composite scales to cognitive decline. Much higher doses of the mAbs are being used than in previous trials in patients with sporadic AD [32]. If the studies from API and DIAN reject the hypothesis, it should be concluded that the amyloid hypothesis is invalid in sporadic and genetic forms. If the hypothesis is supported, it may be concluded that the two forms have critically different mechanisms [17]. These two trials have the potential to indirectly influence the clinical results of other mAbs currently in clinical trials, such as aducanumab and BAN2401, in individuals with sporadic AD [32].

Aducanumab - Recent Approval

Aducanumab was co-developed by Biogen and Eisai under a licence from the biopharmaceutical company Neurimmune for AD treatment [99]. On June 7th 2021, the USA FDA conditionally approved the EMBARK (NCT04241068) trial of aducanumab [5]. Currently, Aducanumab is under regulatory review in Europe and Japan, and multinational phase 3b clinical studies are ongoing to test its long-term safety and tolerability [59, 100]. ENGAGE (NCT02484547) and EMERGE (NCT02477800) identical phase III clinical trials began simultaneously in autumn 2015 to provide evidence of Aducanumab's efficacy and safety. Individuals included in these trials had mild AD or MCI, a Clinical Dementia Rating-Sum of Boxes (CDR-SB) score of 0.5, MMSE score of ≥24, and positive amyloid PET scans. However, the development of aducanumab was discontinued in March 2019 as Biogen revealed that this mAb had failed its futility analysis in both the ENGAGE and EMERGE trials [101, 102]. This failure was added to the list of pre-existing failures in therapeutics aimed at alerting cognitive decline and removing Aβ plaques [103]. These failures include the humanised mAb, solanezumab, which failed to affect cognitive decline, and the BACE-1 inhibitor, verubecestat, which did reduce Aβ brain levels by 90%, but failed to change AD trajectory [104, 105].

It came as a shock to the AD community when, in October 2019, Biogen filed a Biologics License Application (BLA) request to the US FDA for the approval of aducanumab following a post futility analysis of the EMERGE, as results were 'trending positive' [106]. Some researchers in the area of AD criticise this statement, as this effect could have been as a result of a 6% worsening in placebo groups, not the higher dose exposure as claimed by Biogen [101]. Biogen's comparison between EMERGE and ENGAGE was done in a way to be supportive of EMERGE as the positive trial, in order to show the effectiveness of aducanumab so it would be accepted by the FDA [8]. This sparked controversy in the

resurrection of aducanumab. Upon MMSE and CDR-SB exams, individuals taking aducanumab did show signs of slowing AD progression. Although this data remains controversial as the absolute difference determined by CDR-SB between the experimental and placebo groups was 0.4/18, whereas the relative difference was 23% [28, 101]. Additionally, the outcome of this trial may have been skewed through the potential contribution of APOE ε4 subjects in the treatment and control cohorts [103].

Evaluation Measures

Determining AD biomarkers has been of major focus in AD diagnosis given the significance of the amyloid hypothesis, being on reliably measuring Aβ, and to a lesser extent, tau levels in the brain [8]. Evaluation measures of AD are primarily classified into either fluid or imaging biomarkers [107]. CSF is used indirectly as a diagnostic tool in measuring potential biomarkers of AD, which include phosphorylated tau (p-tau), total tau (t-tau), and Aβ levels [3]. Measuring Aβ levels from CSF samples is invasive. However, these findings indicated that reduced Aβ42 CSF concentrations in AD patients correlated with the aggregation of Aβ deposits [8]. Therefore, upon the revision of the 2018 National Institute on Aging and Alzheimer's Association (NIA-AA) diagnostic guidelines, Aβ42 and other amyloidogenic peptides were accepted as candidate AD biomarkers [108]. However, contradictory theories and results exist regarding what concentrations of Aβ and tau are necessary for AD diagnosis in the prodromal stages. For example, the concentrations of CSF Aβ42 have decreased, increased, or have shown no real change as the cognitive functions of AD individuals continue to deteriorate [109-113].

Preventative therapeutic methods can be developed from screening for AD in its early stages. PET and MRI are types of neuroimaging techniques used to show a gradual and progressive cognitive decline in AD. Amyloid-PET is the most reliable imaging diagnostic tool as it distinguishes aggregated Aβ in the brains of AD patients [107]. Florebetapir is a ligand used in amyloid-PET imaging that is efficient in detecting the deposition of amyloid. Once this ligand is absorbed through the BBB, it washes out the grey matter brain tissues that lack amyloid [57]. Only patients showing a clear AD signature from biomarkers or imaging are recruited in AD trials, and treatment is started in patients with early and prodromal AD [114].

Florebetapir PET imaging showed that aducanumab treatment led to the reduction of Aβ plaques in the brain when completed in a time and dose-dependent manner [23]. On this basis, positive biomarker effects on amyloid-PET resulted in aducanumab's reanalysis in October 2019, following its failed futility analysis in March of that year [57]. Nonetheless, previous failures in anti-Aβ therapeutics can partly be attributed to patients who enrolled in AD trials that lacked significant evidence of Aβ brain deposition from biomarkers. Up to 25% of patients with mild AD enrolled in these past trials and they did not have any evidence of Aβ deposition [32]. The new NIA-AA diagnostic criteria defined what the preclinical stages of AD are in order for early pharmacological intervention to take place during secondary prevention trials [2, 32].

Clinical Efficacy of Anti-Aβ Therapeutics

The mAbs that primarily target Aβ aggregates all have potential for clinical efficacy [94]. Currently, the EMBARK study enrols around

2400 patients that previously participated in aducanumab clinical trials, including the PRIME (NCT01677572), ENGAGE, and EMERGE studies. The main objective of this trial is to evaluate the tolerability and safety of monthly 10 mg/kg aducanumab doses after feeder studies are discontinued [59]. However, recurrent failures in clinical trials with mAbs and other anti-Aβ therapeutics have casted doubts about the efficacy and effectiveness of these treatments (Table 1). Aducanumab and BAN4201 showed a slowdown in cognitive decline through positive biomarker effects in reducing cerebral amyloid in patients experiencing mild and prodromal AD [2]. In contrast, gantenerumab lacked clinical efficacy, despite showing significant biomarker effects from reductions in both CSF tau parameters (t-tau and p-tau) and amyloid [57]. Therefore, higher dosing of gantenerumab was suggested, and currently, a placebo-controlled, double-blind trial in mild AD patients is underway [29].

The majority of mAbs in development are IgG derivatives and, as mentioned previously, this contributes to the observed side effects like ARIA [26]. It was in anti-amyloid antibody therapeutics, where safety issues, such as amyloid-related imaging abnormalities with edema (ARIA-E) or with microhaemorrhages (ARIA-H), were first reported [115]. The amyloid burden in the vasculature of patients seems to correlate with the occurrence of these side effects [115]. mAbs are capable of binding to the vascular deposits and recruiting lymphocytes and monocytes to facilitate amyloid clearance. As a result, brain vessel wall function is weakened, causing interstitial fluid to enter brain tissues, as in the case with ARIA-E. The strongly stimulating phagocytosing IgG subtypes are more prone to ARIA-E [26]. ARIA risk increases with the dose of mAbs, suggesting a correlation between imaging abnormality and how effective the amyloid clearance is [94]. Also, previous studies have indicated a close correlation with safety issues to cerebral amyloid clearance on PET imaging. BAN2401 showed the lowest rate of ARIA-E at 10%, consistent with its lower affinity for amyloid plaques than aducanumab and gantenerumab [57]. At each dose of aducanumab and gantenerumab across all genotypes, the rate of ARIA-E was 30% or higher in their respected phase III clinical trials [116, 117].

APOE ε4 carriers have a higher risk of developing AD and represent over 65% of AD sufferers [118]. When treated with mAbs, these individuals have an increased risk of ARIA-E or ARIA-H due to the rapid removal of amyloid plaques from the brain vessels [115]. As carriers have a higher burden of neurotoxic Aβ, they are the optimal group for initial approval of anti-Aβ drugs and efficacy studies. More Aβ oligomers are targeted at higher doses, which supports its efficacy. However, amyloid plaques are also bound, and this off-target effect triggers the significant dose-limiting ARIA-E side effect [118]. Despite the efficacy of aducanumab in APOE ε4 carriers currently being unknown, the rate of ARIA-E was ~42%, compared to ~35% in the overall study population [116]. A higher clinical efficacy with APOE ε4 carriers was seen in trials with BAN2401. However, ARIA-E incidence was increased from ~10% in the overall study population to ~15% in carriers [119]. Amyloid-PET imaging of aducanumab and BAN2401 showed very high efficacy in plaque clearing. However, there is consistent evidence across studies, suggesting that prolonged engagement of the soluble Aβ oligomers at high doses is needed for clinical benefit. Clinical efficiency is not explained by the clearance of plaques alone, and plaque formation may just be a protective mechanism for the Aβ oligomers in limiting their neurotoxicity [120].

Preventing AD

There is currently no cure to halt AD progression. Disease modification represents around 75% of all the current therapeutic approaches in the AD pipeline, including the recently approved mAb aducanumab [5, 32]. Nonpharmacological treatments include lifestyle interventions, such as a healthy diet, mental challenges, exercise, and socialization. For example, taking antioxidants has proven to compensate for the age-related downregulation of α-secretase, which is needed in preventing the formation of Aβ peptide [25]. There is a substantial body of evidence supporting the use of BACE1 inhibitors in AD prevention as it turns off toxic Aβ production [3]. Firstly, BACE1 inhibition had a direct effect in studies with transgenic mice overexpressing human APP in individuals with familial AD mutations [121-123]. Secondly, there was a 40% decrease in the production of Aβ *in vitro* due to rare human mutation at the cleavage site of BACE1, suggesting BACE1 cleavage alone may be beneficial [124, 125]. However, inefficiencies in BBB penetration were noted with the BACE1 inhibitors [26]. Therefore, few BACE1 inhibitors are currently in development due to increases in toxicity in humans [16]. It is hoped that BACE1 inhibition will be more effective in combination with immunotherapy [3]. The secondary mAbs aducanumab, gantenerumab, and BAN2401prevention trials are currently being tested in patients experiencing the preclinical stages of familial AD, early AD, and high-risk asymptomatic patients. Preliminary results from these trials support the amyloid hypothesis as the elevation of Aβ levels is represented in the early stages of AD, demonstrating the feasibility of secondary prevention trials during the preclinical stages of AD [32].

Future Perspectives

The ultimate proof for the amyloid hypothesis would be that intervention in the amyloid cascade would prevent cognitive deterioration and neuronal loss [31]. A single-targeted approach to AD treatment has not produced any effective therapies yet, so future treatments are focusing on combination therapies to try and target the multiple mechanisms of AD, from its molecular biology to its various cellular pathways [36]. Combination therapies are thought to be the future of AD prevention and treatment [3]. The inhibition of BACE1 and γ-secretase to reduce Aβ production and the use of active and passive immunotherapy to enhance Aβ clearance have both failed to demonstrate therapeutic effects in patients with moderate-mild AD [16]. The AD scientific community suggested that this moderate-mild AD stage is too late for the anti-Aβ drugs to work in reversing or halting AD progression. Therefore, current Aβ-directed pharmacological interventions focus on patients in the preclinical stage or are asymptomatic [32].

Approaches that target more than one cellular system in AD are being developed using small molecule drugs as combination therapies that target the vast amyloid cascade. ALZT-OP1 is a combination therapy consisting of cromolyn sodium and ibuprofen. Ibuprofen is a non-steroidal anti-inflammatory (NSAID) drug that has been proven to reduce Aβ42 levels by modulating the activity of the γ-secretase complex. Cromolyn sodium induces microglia transition to a pro-phagocytic state from a pro-inflammatory, inhibiting Aβ accumulation [32]. In combination, this therapy promisingly appears more effective, less costly, and safer than other anti-Aβ therapeutics [26]. ALZ-801 is another small-molecule drug that avoids the limitations experienced with the higher-dosing mAbs [57]. ALZ-801 is a tramiprosate prodrug

capable of regulating the conformational flexibility of the Aβ42 isoform, therefore inhibiting oligomer formation [36]. Tramiprosate showed a dose-dependent decrease in the assessment of hippocampal atrophy by MRI, making it the only drug to date to show a positive result in brain atrophy prevention [126]. The use of ALZ-801 is the next generation in selective anti-Aβ therapeutics as it has an improved product profile and selectivity to oligomers compared to the mAbs. ALZ-801 provides a probable preventative treatment in presymptomatic patients [57].

CONCLUSION

The amyloid hypothesis has been debated for years, despite being supported by amyloid imaging techniques [26]. Initially, it was proposed that insoluble aggregates of Aβ fibrils were the core hallmark of AD. However, the updated hypothesis suggests that soluble oligomers play a more significant role in neurotoxicity [16]. Despite the strong body of evidence that confirms the toxic nature of amyloid, very few anti-amyloid agents have been shown to have cognitive benefits whilst fulfilling the specific criteria for selection [57]. In the past, drugs primarily focused on targeting Aβ accumulation rather than the removal of Aβ via anti-Aβ agents, so ongoing failures may be attributed to selecting the wrong biological target [17]. However, drugs against Aβ have failed in both the advanced and early stages of the disease, casting doubt on the amyloid hypothesis [32].

The inclusion of non-AD patients in previous trials is the reason current trials only recruit patients with positive AD biomarker signatures [26]. Plasma tau is a non-invasive biomarker compared to measuring CSF levels. Nonetheless, it is limited from a substantial overlap in normal aging and AD, so its use as a stand-alone biomarker is not supported [8]. The source of neurodegeneration is thought to be from Aβ pathology acting through tau. However, even in young individuals, tau pathology is observed in autopsy studies before the marked deposition of Aβ [16]. Furthermore, brain imaging of individuals with preclinical AD suggests that the initial emergence of neurodegeneration biomarkers, such as reduced glucose metabolism and hippocampal volume, aren't dependent on Aβ amyloidosis [17].

In light of the promising late-stage trials with gantenerumab, BAN2401, and aducanumab advanced approval, a major challenge in their future development is devising a preventative way to avoid ARIA [5, 26]. Progressive, slow dosing of mAbs is a preservation method against forming the initial vascular burden [26]. Targeting specific epitopes that are absent in the vascular amyloid, such as Aβ42, might reduce the binding of antibodies to the vessels [35]. Finally, improvements of antibody passage through the BBB from modifying targeting Fc receptors can reduce dosage, thereby reducing side effects. This strategy can increase the brain penetrance of mAbs [127]. However, the loss of antibody affinity is experienced. Future research should explore how other mAbs could be made safer to avoid their apparent tendency to deteriorate cognitive function compared to placebo groups [17]. Shortcomings in treatments have led to other techniques, such as focused ultrasound (FUS), which have been indicated to increase drug penetration through the BBB and increase the overall efficacy of mAbs [26].

Despite AD being the most prominent neurodegenerative disease globally, pharmacologic treatments have been extremely limited, with a few approved drugs providing only symptomatic treatments [4]. By managing AD symptoms, patients can be

provided with independence, dignity, and comfort for longer. The AChE inhibitors galantamine and donepezil show the highest efficacy levels for cognition in patients with mild to moderate AD. Whilst the best profile for acceptability is seen with the N-methyl-D-aspartate receptor antagonist memantine [128]. These drugs control or reduce the behavioural and cognitive symptoms associated with the disease [4]. However, these symptomatic treatments are not a cure.

Aducanumab's recent accelerated approval in June 2021 marks a promising chapter for anti-Aβ therapeutics, as it represents the first disease-modifying drug conditionally approved to treat early AD [99]. Biogen's surprise reversal puts hope on the horizon for additional AD therapeutics [5]. Although this mAb is currently only available in the US, its approved status depends on its long-term tolerability and safety results in ongoing clinical trials [30]. As over 35% of patients experienced ARIA-E with aducanumab treatment, there is a need to improve Aβ-targeting precision [116]. Therefore, careful evaluation of the safety and efficacy of anti-amyloid therapeutics is vital, especially for APOE ε4 carriers, in determining the benefit-risk profile [57].

In the face of the repeated failures of anti-Aβ therapeutics, the anti-Aβ mAbs remain the most advanced drugs in development for AD treatment. The plethora of mAb clinical trials has helped develop an understanding of the mechanism of disease, while supporting the amyloid hypothesis. Overall, the tolerability and safety profile of these mAbs has been acceptable, along with promising biomarkers and clinical effects [32]. Serious complications are rare and must always be compared against the alternative consequences of untreated AD [29]. Should confirmation of aducanumab's efficacy prevail, its researchers should be met with a great debt of gratitude from the AD community, not only for producing the first disease-modifying treatment, but also for opening the door to promising anti-Aβ therapeutic advances.

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All figures, or tables, were created by the Author, unless otherwise mentioned in the description provided of said figure.

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