REVIEW ARTICLE

Clinical trials for Alzheimer disease and perspectives

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Abstract
Alzheimer disease (AD) is the most common type of dementia and may account for 60～70% of dementia cases. Senile plaques are a hallmark of AD, and their major constituent is amyloid β-protein (Aβ) 42. Aβ is produced via endoproteolysis by beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), which cleaves β-amyloid precursor protein at the extracellular domain, followed by cleavages by presenilin/γ-secretase. Aβ accumulation in the brain is thought to occur decades before disease onset, and pathological changes such as synaptic dysfunction and tau accumulation gradually proceed. Most candidates for disease-modifying therapy (DMT) for AD have targeted either inhibition of Aβ generation with secretase inhibitors or removal of produced and aggregated Aβ with anti-Aβ antibodies. None of them has been approved for clinical practice. Moreover, application of BACE inhibitors and γ-secretase inhibitors in several clinical studies caused paradoxical cognitive impairment. However, early AD patients treated with aducanumab, a monoclonal anti-Aβ antibody, showed a significant reduction in cognitive decline and its clinical use is awaiting approval by the FDA in 2020. In this review, we present a brief summary of DMTs that target Aβ and perspectives for future AD therapy.

Key words: *Alzheimer disease, amyloid β-protein, γ-secretase inhibitors, BACE inhibitors, anti-Aβ antibodies*

INTRODUCTION

Alzheimer disease (AD) is the most common type of dementia and may account for 60～70% of dementia cases (Burns, 2009). Its typical symptoms are progressive memory decline followed by other higher brain dysfunctions such as visual space cognitive impairment, executive problems, and language disturbance. Many current clinical studies for AD are limited because they enroll patients with preclinical AD who have few if any symptoms of cognitive decline but have deposition of amyloid β-protein (Aβ) in the brain, patients with prodromal AD, or patients with early AD at the latest. Thus, enrolled candidates are in a narrow clinical window. Aβ deposition is estimated with either by amyloid positron emission tomography (PET) or/and a decrease in the level or the ratio of Aβ42 in cerebrospinal fluid (CSF). No established biomarkers in peripheral blood are available to detect Aβ accumulation in the brain. The prevalence of preclinical AD in the healthy aged population is estimated to be up to 20% (Ihara, 2018). Thus, screening participants for each clinical study to identify those who have Aβ deposition in the brain but show few if any cognitive decline is expensive.

Aβ, a 37- to 43-residue hydrophobic peptide, is a constituent of the amyloid plaques that are a hallmark of AD (Selkoe, 2011) (Figure 1). A major constituent of amyloid plaques is Aβ42, which is thought to induce the pathological process of AD and has a much higher tendency to aggregate than Aβ40, a major Aβ species (Selkoe, 2001) (Figure 1). Aβ is produced via endoproteolysis by beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), which cleaves β-amyloid precursor protein (βAPP) at the extracellular domain (Hussain, 1999; Sinha, 1999; Vassar, 1999; Yan, 1999), and by the presenilin (PS)/γ-secretase complex (Francis, 2002; Yu, 2000), which cleaves C-terminal fragment (CTF)β, a membrane remnant of βAPP in the transmembrane domain (De Strooper, 2003; Edbauer, 2003; Kimberly, 2003; Takasugi, 2003) (Figure 1).

Aβ accumulation in the brain is thought to occur decades before disease onset (Sperling, 2014) and causes gradual progression of pathological changes (Aβ hypothesis) (Hardy, 2002). The other hallmark of the AD brain is neurofibrillary tangles consisting of hyperphosphorylated tau. Tau accumulation is followed by Aβ accumulation and proceeds along with disease progression (Braak, 1991). Most candidates for disease-modifying therapy (DMT) for AD have targeted Aβ. DMT that targets Aβ consists of drugs that either inhibit or modify Aβ generation (γ-secretase inhibitors (GSIs), γ-secretase modulators (GSMs), and

BACE inhibitors) or anti-Aβ antibodies that bind to Aβ and promote its clearance from the brain (Figure 2). However, none of them is approved for use in clinical practice. In this review, we present our recent findings on Aβ generation process, a brief summary of DMT targeting Aβ, and perspectives for future AD therapy.

γ-SECRETASE INHIBITORS(GSIs)

LY450139 (semagacestat) is the first GSI to have progressed to phase III clinical trials (Dovey, 2001). GSIs were developed to inhibit the enzymatic activity that produces Aβ (Golde, 2013; Wolfe, 2012) (Figure 2). By reducing Aβ secretion, GSIs were hypothesized to be able to treat AD. GSIs are classified into two types. One type is the transition state analogue (TSA) inhibitor, which targets the catalytic site of PS/γ-secretase (Shearman, 2000). PS/γ-secretase has many substrates other than βAPP, among which Notch is one of the most physiologically important substrates. PS/γ-secretase catalyzes cleavage of the transmembrane domain of Notch, producing the Notch intracellular domain (NICD), an essential molecule for signal transduction (De Strooper, 1999). Because TSA GSIs abolish enzymatic activity, they inhibit production of Aβ as well as NICD, causing severe adverse effects mainly due to the disruption of Notch signaling (Doerfler, 2001; Geling, 2002; Hadland, 2001; Wong, 2004). The other type is non-TSA inhibitor, such as LY450139 (semagacestat) (Siemers, 2005) and BMS-708163 (avagacestat) (Gillman, 2010). They were considered to have less inhibitory effects on NICD generation at a concentration range at which they sufficiently reduce secreted Aβ. Clinical studies were conducted using non-TSA GSIs as DMT for AD, but disappointingly, all of these trials failed. Moreover, semagacestat (Doody, 2013) and avagacestat (Coric, 2015) not only caused adverse effects but also increased cognitive decline or worsened activities of daily living in patients. The observed side effects may be partially attributed to inhibition of Notch signaling. However, what caused the increase in cognitive decline has been unknown.

Recently, the mechanism of action of non-TSA GSIs, including semagacestat and avagacestat, was re-investigated by measuring the levels of γ-byproducts as a new marker (Tagami, 2017) (Chicurel, 2017). γ-Byproducts are small intracellular peptide byproducts released during the successive cleavage leading to Aβ production by PS/γ-secretase (Funamoto, 2020) (Figure 1). Unlike Aβ, these peptides are not secreted and may thus serve as a more direct indicator of PS/γ-secretase activity than secreted Aβ (Tagami, 2017). Surprisingly, semagacestat and avagacestat do not decrease but paradoxically increase the levels of γ-byproducts inside neurons derived from human induced pluripotent stem cells (Tagami, 2017). Along with increased γ-byproduct levels, accumulation of long Aβ species is also found inside neurons following semagacestat or avagacestat treatment (Tagami,

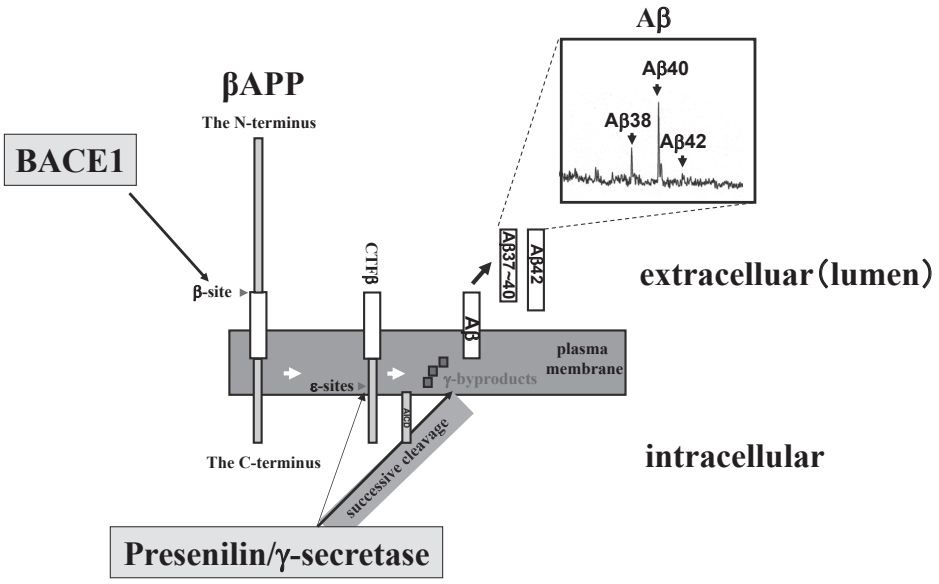

Figure 1. Process of Aβ generation from its precursor, βAPP.
βAPP undergoes extracellular shedding by BACE1. A membrane remnant, CTFβ undergoes ε-cleavage by PS/γ-secretase, releasing AICD in cytosol. Then, successive cleavage by PS/γ-secretase proceeds every three to four amino acids to the N-terminus, producing Aβ species along with γ-byproducts.

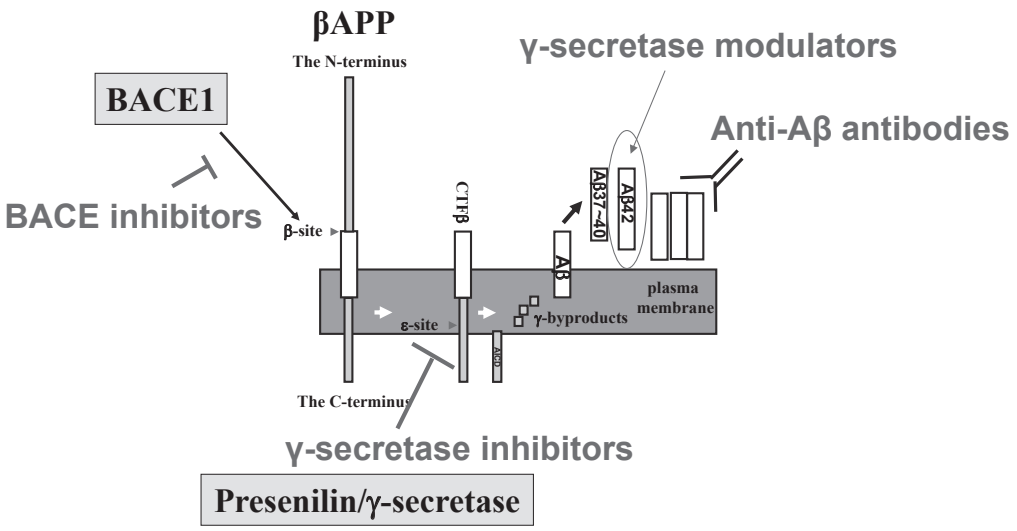

Figure 2. Targets to reduce or modulate Aβ generation or remove Aβ.
DMTs to reduce or modulate Aβ generation or remove Aβ are shown in red.

2017). Intraneuronal Aβ accumulation in transgenic mice expressing multiple familial AD mutations causes synaptic dysfunction and neuronal loss (Oakley, 2006; Oddo, 2003). Moreover, a study demonstrated enhanced Aβ accumulation in the brain of a patient who died after treatment with a high dose of semagacestat for more than a year (Roher, 2014). The surprising results of the effects of semagacestat and avagacestat on PS/γ-secretase may at least in part help explain the aggravation of cognitive decline in patients.

γ-SECRETASE MODULATORS (GSMs)

All the pathological mutations associated with familial AD were identified in either βAPP or PS (Levy-Lahad, 1995; Rogaev, 1995; Sherrington, 1995) and most of them affect cleavage by PS/γ-secretase, thereby increasing pathogenic Aβ42 relative to Aβ40 (Borchelt, 1996; Citron, 1997; Scheuner, 1996). The increase in the Aβ42/Aβ40 ratio (the Aβ42 ratio) is considered to be the primary factor causing familial AD. GSMs are candidates for disease-modifying drugs against AD due to their ability to decrease the Aβ42 ratio, contrary to the effect of familial AD-associated mutations (Wolfe, 2012). Briefly, GSMs decrease longer Aβ species such as Aβ42 and Aβ43 and increase shorter species, including Aβ37-39. GSMs are classified as non-steroidal anti-inflammatory drug such as sulindac sulfide and ibuprofen (first generation GSMs) (Weggen, 2001) and second generation acidic and non-acidic GSMs such as GSM1 and E2012, respectively (Wolfe, 2012; Golde, 2013). First generation GSMs bind βAPP, i.e., the substrate (Kukar, 2008; Richter, 2010). These compounds lower toxic Aβ42 levels without affecting ε-site cleavage of other substrates including Notch-1 (Weggen, 2001, 2003). Second generation acidic and non-acidic GSMs bind PS1 or PS2, i.e., the catalytic subunits (Crump, 2011; Ebke, 2011; Ohki, 2011). However, the detailed mechanisms by which they selectively decrease longer Aβ species have long been unclear.Using synthetic Aβ42 peptide as a substrate and purified PS/γ-secretase from cells as an enzyme, Okochi et al. (2013) showed that PS/γ-secretase tends to bind Aβ42 longer (decreasing Kb value) and cleave it into Aβ38 with higher efficiency (increasing Kcat value) in the presence of E2012 and GSM1. The results suggest that not inhibiting but rather enhancing the activity of PS/γ-secretase to cleave Aβ42 may be a strategy for DMT.

A first generation GSM, tarenflurbil (Myriad Genetics & Laboratories), reached phase III clinical trials. However, results showed no significant effect (Imbimbo, 2009). The failure was attributed to its insufficient capacity to penetrate into the brain. Experimentally, poor central nervous system penetration of tarenflurbil was reported in studies in rodents, with a CSF/plasma ratio of only up to 1%. In addition, its Aβ42 IC_{50} is ~ 260μM.

Three second generation GSMs were developed. Compound NGP555 from Neurogenetics produced a 20–40% decrease in CSF Aβ42 and an increase in the shorter forms in rodent studies. NGP555 entered phase I clinical trials in 2015, but detailed results have not been disclosed yet. Eisai Pharmaceuticals developed E2012 and E2212 compound. The report-

ed IC50 value of E2012 for Aβ42 was 92 nM, which is much more efficient than those of first generation GSMs. E2012 entered clinical trials in 2006 and was suspended because lenticular opacity was observed in a high-dose group of a safety study using rats. E2212 entered a phase I clinical trial in 2010. E2212 has a similar pharmacological profile as E2012, reducing plasma levels of Aβ42 by about 54%, and no clinically significant ophthalmologic findings including lenticular opacity were reported (Yu, 2014). However, no further development has been reported as of now (September, 2020) for E2212.

BACE INHIBITORS

BACE1 is a type-1 membrane aspartyl protease identified in 1999 (Hussain, 1999; Sinha, 1999; Vassar, 1999; Yan, 1999). It cleaves βAPP in the luminal or extracellular surface of the plasma membrane, releases soluble βAPP (sAPPβ), and leaves the membrane stub (CTFβ), which is subsequently cleaved by PS/γ-secretase (Figure 1). A mutation close to the β-cleavage site in βAPP (A673T) reduces the ability of BACE1 to cleave it, which decreases Aβ generation by approximately 40% in vitro, reducing the risk of AD (Jonsson, 2012). Thus, BACE1 inhibitors are considered promising candidates to treat AD.

Several BACE inhibitors have been tested in clinical studies. Verubecestat entered phase II/III clinical trials for patients with mild to moderate AD in 2012 (EPOCH trial), and a phase III trial for prodromal AD in 2013 (APECS trial). The EPOCH trial and APECS trial were discontinued in 2017 and 2018, respectively. Contradictory to the expectation, patients taking verubecestat scored markedly worse than the placebo group on a cognitive test (Egan, 2018, 2019). A phase III clinical trial using atabecestat for patients with preclinical AD was discontinued for lack of efficacy. Phase II/III clinical trials using lanabecestat for patients with early and mild AD were also discontinued for lack of efficacy. Both atabecestat and lanabecestat caused cognitive decline similar to verubecestat (Henley, 2019). CNP520 (umibecestat) was used in two pivotal phase II/III studies in the Alzheimer's Prevention Initiative Generation Program. An assessment of unblinded data during a pre-planned review found worsening in some measures of cognitive function, and thus, the trial was discontinued in 2019.

BACE1 has a close homolog, BACE2. BACE2 cleaves βAPP at a theta site downstream of the alpha site, and thus, abolishes Aβ production (Sun, 2006). A trial to elevate the specificity for BACE1 was performed. E2609 (Elenbecestat) binds BACE1 with 3.53-fold higher affinity than BACE2. Elenbecestat was used in two phase III clinical trials (MISSION AD1, AD2). Both trials were discontinued in 2019 due to an unfavorable risk-benefit ratio. Detailed data of these studies will be presented in the future.

Researchers were quite disappointed and surprised that both GSIs and BACE inhibitors caused cognitive impairment. Elucidating why these BACE1 inhibitors caused such cognitive decline is critical. In addition to βAPP, BACE1 has other substrates such as the voltage-gated sodium channel (VGSC) subunit beta (Kim, 2007) and Neuregulin 1 (NRG1) type III (Willem, 2006). Through the interaction with VGSC and NRG1, BACE1 may regulate neuronal activity and myelination, respectively. Inhibition of BACE1 activity may disrupt these putative functions. Future studies are necessary to reveal whether and how disruption of neural activity and myelination by BACE inhibitors is associated with cognitive decline. Otherwise, BACE inhibitors may cause a totally unexpected phenomenon as in the case of non-TSA GSIs (Tagami, 2017).

IMMUNOTHERAPIES TARGETING Aβ

Various types of monoclonal antibodies against several forms of Aβ such as secreted forms, oligomers, protofibrils, or mature fibrils have been used in clinical studies. The approach must overcome at least two barriers. One is its low efficiency to penetrate the blood-brain barrier. Less than 0.1% of immunoglobulins injected into the bloodstream penetrate the blood-brain barrier in a mouse model of AD (Bard, 2000). The other barrier is avoiding inflammation due to immune responses against antibodies. These inflammatory reactions are classified as amyloid-related imaging abnormalities (ARIA) (Sperling, 2012). Of note, the FDA accepted the Biologics License Application for one of the monoclonal antibodies, aducanumab, for AD with priority review on August 7, 2020.

The first monoclonal antibody used in a clinical study for passive immunotherapy for AD was bapineuzumab, which entered phase III trials in 2007. Testing showed that bapineuzumab did not work better than placebo in two late-stage trials in patients with mild to moderate AD. ARIA was first reported in clinical studies using bapineuzumab, indicating a safety concerns for passive immune therapies.

Solanezumab is a humanized IgG1 monoclonal antibody that binds the central region of Aβ. Solanezumab was tested in two phase II clinical trials, EX-

PEDITION 1 and 2, which enrolled patients with mild to moderate AD. Both studies failed to show a difference in cognition and memory between the treated and the placebo group. However, less worsening of cognition in mild AD patients receiving solanezumab compared to placebo was observed. Based on the positive result, Lilly launched the EXPEDITION 3 study in 2013 which enrolled only patients with mild AD, but the trial failed to show the expected positive results. Moreover, Florbetapir PET analysis did not show a reduction in brain Aβ deposits with solanezumab treatment (Doody, 2014; Honig, 2018).

Aducanumab has a higher specificity for Aβ oligomers and aggregated Aβ forms than bapineuzumab. It has more than10,000-fold increased selectivity for aggregated Aβ compared to Aβ monomers and binds to the N-terminus of Aβ (Sevigny, 2016, 2017). In 2017 and 2018, the long-term open-label extension phase of the Multiple Dose Study of Aducanumab in Participants with Prodromal or Mild Alzheimer's Disease (PRIME study) showed slowing of cognitive decline and dose-dependent amyloid removal. The most common side effect was ARIA. However, Biogen and Eisai announced the termination of the phase III ENGAGE (221 AD301 Phase 3 Study of Aducanumab (BIIB037) in Early Alzheimer's Disease) and EMERGE (221 AD302 Phase 3 Study of Aducanumab (BIIB037) in Early Alzheimer's Disease) trials of aducanumab in March 2019 due to the result of a futility analysis (BIOGEN and EISAI, 2019). The futility analysis was conducted using data from 1748 patients. Results suggested that these trials would not reach their primary endpoint, that is, inhibiting disease progression as measured by the Clinical Dementia Rating-Sum of Boxes (CDR-SB). However, additional data from these studies became available later with a larger dataset of 3285 patients. The updated analysis led to revise results for EMERGE. It was statistically significant, especially for patients treated with a high dose of aducanumab. Patients treated with high-dose aducanumab showed a significant reduction in clinical decline from baseline in CDR-SB scores at 78 weeks (23% versus placebo, $p = 0.01$). Imaging of amyloid plaque deposition in EMERGE demonstrated that amyloid plaque burden was reduced with low- and high-dose aducanumab compared to placebo at 26 and 78 weeks ($p < 0.001$) (EISAI global, 2019). Based on the updated result, Biogen submitted a Biologics License Application for aducanumab to the FDA for the treatment of AD.

Gantenerumab is a completely human recombinant monoclonal IgG1 antibody that binds to the N-terminus and central regions of Aβ. Gantenerumab shows higher affinities for Aβ oligomers and fibrils than for Aβ monomers (Bohrmann, 2012). A phase III trial called The Marguerite RoAD study began in 2014 and evaluated monthly subcutaneous injections of gantenerumab in patients with mild AD. A portion of the result from the study was presented at the 14th International Conference on Alzheimer's and Parkinson's Disease and at the American Academy of Neurology annual meeting in 2019. PET imaging showed reduction in amyloid plaques in gantenerumab-treated patients compared to those given placebo. The safety and effectiveness of higher doses of gantenerumab have being under investigation in two phase III trials called GRADUATE 1 and GRADUATE 2 since 2019 in patients with early-stage (prodromal to mild) AD.

Crenezumab is a humanized monoclonal IgG4 that binds to the N-terminus of Aβ. Crenezumab has a particular affinity for Aβ oligomers and aggregated Aβ forms. Two phase III trials (CREAD 1 and CREAD 2) evaluating crenezumab as therapy for early AD were discontinued in 2019 because an interim analysis by an independent data monitoring committee showed that crenezumab was unlikely to meet its primary goal of halting cognitive decline as measured by the CDR-SB Score.

BAN2401 is a humanized monoclonal IgG1 antibody that binds to the N-terminus of Aβ. The antibody has a more than 1,000-fold preference for binding protofibrils over Aβ monomers and a 10- to 15-fold preference for binding protofibrils over mature fibrils (Lannfelt, 2014; Sehlin, 2012). A phase III trial called Clarity AD started in 2019 and plans to enroll 1566 patients with early AD.

PERSPECTIVES

Aducanumab will become the first DMT for AD if its use is accepted by the FDA. However, many issues remain such as financial problems due to the long-term use of costly monoclonal antibodies, how to screen preclinical AD subjects without an established blood biomarker to detect amyloid positivity in the brain, and how to prevent recurrence of Aβ accumulation after its removal or reduction by the DMT. DMT for AD has clearly progressed in an innovative manner because removal or reduction of pathological Aβ accumulation from the brain is now becoming feasible. In the next stage, deciding the optimal timing for intervention with DMT will be critical. Patients with the very early stage of preclinical AD in which amyloid accumulation has started or has progressed for a year or so may be good candidates for DMT. In such

a case, a screening method to detect amyloid positivity is needed prior to performing amyloid PET.

Three apolipoprotein E (*APOE*) alleles have been identified; *APOE*-ε2, ε3, and ε4. The *APOE*-ε4 risk allele is associated with a relatively greater risk for amyloid positivity and younger age at onset compared with the *APOE*-ε3 allele. The *APOE*-ε2 allele has opposite associations to the *APOE*-ε4 allele. At the age of 65 years, 32.0-43.9% of *APOE*-ε4+ people with normal cognition are estimated to have amyloid positivity, whereas 10.4-16.6% of *APOE*-ε4- people with normal cognition are estimated to have amyloid positivity (Jansen, 2015). At the age of 70 years, estimated amyloid positivity reaches 42.2-53.7% and 14.1-20.6% for the *APOE*-ε4+ and *APOE*-ε4- populations, respectively (Jansen, 2015). Thus, the *APOE*-ε4 risk allele will be a promising biomarker for screening for Aβ accumulation, although ethics consideration is needed. In fact, some recent clinical studies have utilized the *APOE*-ε4 risk allele for primary screening to detect Aβ accumulation.

At least one or multiple reasons may cause Aβ accumulation in the brain of each individual. Clearance of Aβ from the central nervous system may be retarded. The activities of Aβ degrading enzymes such as neprilysin or insulin degrading enzyme may be low. Abnormal elevation of total Aβ production or the Aβ42 ratio may occur to some extent, but may not be as severe as in familial AD. Currently, little data are available on whether total Aβ production or the Aβ42 ratio is elevated in sporadic AD patients. It is because the level of CSF Aβ does not reflect the level of Aβ production in the AD brain partly due to the highly aggregatable feature of Aβ. In particular, CSF Aβ42 is rather low in AD patients. New methods to deduce the Aβ production level and the Aβ42 ratio are desperately needed. In the future, BACE inhibitors and GSMs that were previously suspended may be reintroduced after Aβ accumulation is removed or reduced by anti-Aβ antibodies to prevent Aβ re-accumulation. In such a case, evaluating to what extent the Aβ42 ratio or total Aβ production is elevated in each individual prior to drug application will be ideal. Normalization of an abnormally elevated level or ratio with a minimum dose of drugs may be sufficient to prevent Aβ re-accumulation.

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