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The amyloid cascade and Alzheimer's disease therapeutics: theory versus observation

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Abstract

The identification of amyloid-β precursor protein (APP) pathogenic mutations in familial early onset Alzheimer's disease (AD), along with knowledge that amyloid-β (Aβ) was the principle protein component of senile plaques, led to the establishment of the amyloid cascade hypothesis. Down syndrome substantiated the hypothesis, given an extra copy of the APP gene and invariable AD pathology hallmarks that occur by middle age. An abundance of support for the amyloid cascade hypothesis followed. Prion-like protein misfolding and non-Mendelian transmission of neurotoxicity are among recent areas of investigation. Aβ-targeted clinical trials have been disappointing, with negative results attributed to inadequacies in patient selection, challenges in pharmacology, and incomplete knowledge of the most appropriate target. There is evidence, however, that proof of concept has been achieved, i.e., clearance of Aβ during life, but with no significant changes in cognitive trajectory in AD. Whether the time, effort, and expense of Aβ-targeted therapy will prove valuable will be determined over time, as Aβ-centered clinical trials continue to dominate therapeutic strategies. It seems reasonable to hypothesize that the amyloid cascade is intimately involved in AD, in parallel with disease pathogenesis, but that removal of toxic Aβ is insufficient for an effective disease modification.

The Amyloid-β cascade hypothesis

Genetic evidence

The linkage of pathological lesions to amyloid-β (Aβ) along with discoveries in genotype–phenotype relationships led to the amyloid cascade hypothesis [1] (Fig. 1). The hypothesis held then, as it does now, that Aβ is causative of the neurodegenerative processes in Alzheimer's disease (AD). Genetic evidence was apparent in Down syndrome patients, who constitutively overexpress Amyloid-β precursor

protein (APP) gene and consequently develop hallmark lesions of AD early in life [2]. Mutations in the carboxy-terminal portion of APP causing hereditary AD or cerebral hemorrhage were also cited and continue to be compelling evidence in favor of the amyloid cascade [3, 4].

Since the original hypothesis, a large body of supporting literature has accumulated. A number of additional APP mutations causing familial AD (FAD) have been described, the majority occurring within or near the transmembrane region of AβPP. Pathogenic mutations in presenilin 1 and 2 genes (PSEN1, PSEN2) also cause familial AD, with PSEN1 mutations being the most common among dominantly inherited FAD kindreds. Presenilin proteins are the catalytic subunits of the gamma-secretase complex required along with beta secretase 1 (BACE1) for Aβ peptide processing and secretion [5]. Sequential C-terminal Aβ cleavage by gamma secretase generates Aβ fragments of differing lengths, with 40 amino acid (Aβ40) and 42 amino acid (Aβ42) being the most common. An increased Aβ42:Aβ40 ratio is described in plasma and cultured fibroblasts from familial AD patients [6, 7], suggesting Aβ42 as the pathogenic Aβ species [8]. The predominance of Aβ42 in affected areas of the AD brain tissue lacking cerebral amyloid angiopathy (CAA) supports this claim [9],

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Fig. 1 Amyloid-β cascade hypothesis. Amyloid-β precursor protein (*APP*) gene expression is followed by consecutive protease processing by β-secretase 1 (BACE1) and γ-secretase complex (PSEN1 (presenilin 1), PSEN2 (presenilin 2), nicastrin and APH1 (anterior pharynx-defective 1)) releasing the amyloid-β peptide, including pathogenic species Aβ42. Aβ42 undergoes conformational change, assembles into oligomers (2–100 units) and protofibrils (>100 kDa), which are neurotoxic, and ultimately deposits as senile plaques
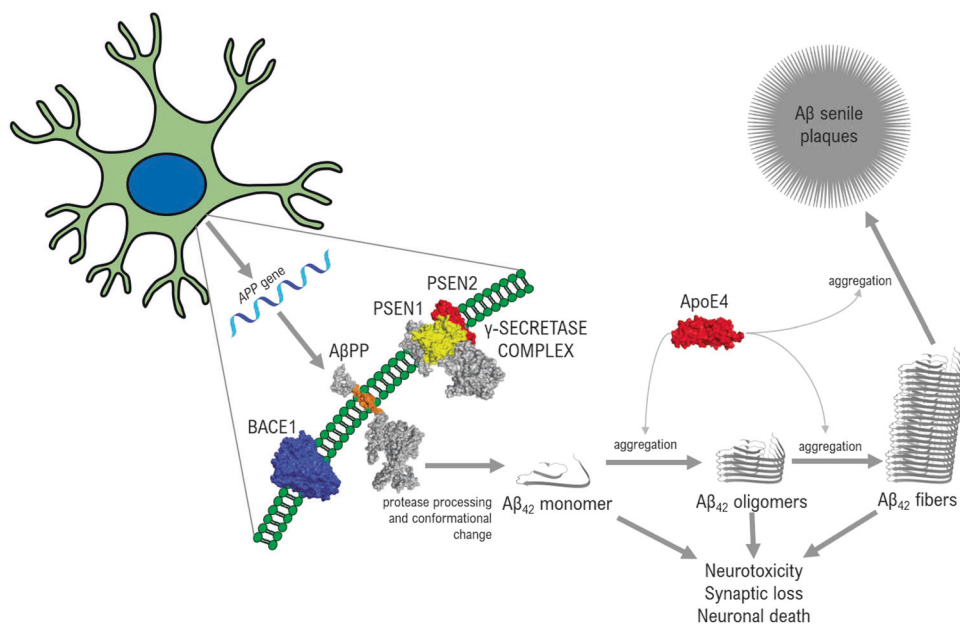
although data in sporadic AD are sparse and somewhat contradictory [6]. The tendency for Aβ42 to spontaneously self-assemble into fibrils is thought to underlie its pathogenicity [10]. It is generally accepted that Aβ40 is relatively more abundant in cerebral amyloid angiopathy [8], although Aβ42 may "seed" blood vessels initially and may be present to a variable extent [9, 11, 12]. Interestingly, Aβ40 may inhibit fibrillar aggregation [13, 14], which is more in line with a bystander role for Aβ40 in CAA.

Apolipoprotein E

Apolipoprotein E (ApoE) is the glycoprotein product of the polymorphic APOE gene on chromosome 19 [15], first described in 1973 as a component of very low density lipoprotein (VLDL) [16]. Initial interest in ApoE was centered on its role in cholesterol-induced hyperlipidemia, as its primary role is the transport and delivery of cholesterol and other lipids in the plasma and central nervous system. The potential role of ApoE in AD and amyloidogenesis was discovered serendipitously in the early 1990's with Affected Pedigree Analysis linking familial clusters of late-onset AD to the long arm of chromosome 19 [17]. Independent experiments by Strittmatter et al. showed variable binding of ApoE, one of a number of candidate proteins transcribed in the region of 19q identified by linkage analysis, to Aβ as a function of APOE polymorphism [18]. The three major allelotypes (e2, e3, and e4) differ at positions 112 and 158, which affects ApoE conformation, receptor binding affinity, and stability [19–22].

Importantly, ApoE e4 allele is the most significant genetic risk factor for AD identified to date, and has a gene dosage effect in terms of disease risk, age at onset, and Aβ

neuropathology [23–26]. In line with the amyloid cascade hypothesis, ApoE binds Aβ in an isoform-specific manner, with e4 showing the lowest binding affinity [27], consistent with other data indicating its role in Aβ clearance [28]. ApoE has also been shown to stabilize Aβ oligomers [29]. The association between ApoE genotype and AD appears to be stronger in women [30, 31], less robust for African-Americans and Hispanics [32], and appears to diminish somewhat with advanced age. ApoE4 is nevertheless heavily enriched in AD patients, with 65–80% of AD patients carrying at least one Apo e4 allele [33], compared to worldwide frequency of about 14%. E2 is said to be "protective" as noted above, reducing the AD risk by about half [34, 35], possibly related to greater protein stability [36].

Aβ oligomers

The lack of eloquence with respect to fibrillary Aβ deposits and neurological signs indicates a level of complexity to AD pathogenesis, unaccounted for by hallmark lesions [37]. Accordingly, the revised amyloid cascade hypothesis tends to minimize insoluble Aβ as the neurotoxic species, in favor of low-n assembly fibrillar intermediates [38, 39], while senile plaques deposit either en passant, or as an anatomically imprecise reservoir in dynamic equilibrium with toxic soluble Aβ oligomers [6, 40]. Interestingly, beta sheet content tends to increase as a function of oligomer size, while neurotoxicity of dimers, trimers, and tetramers is 3-fold, 8-fold, and 13-fold higher, respectively in comparison with Aβ monomers [41].

Oligomers of Aβ42 are neurotoxic to synapses and impair memory in rats, whereas senile plaque cores do not

impair long-term potentiation [42]. The concept of a toxic penumbra of Aβ42 oligomers has been raised, consistent with data in APP/PS1 transgenic mice suggesting a halo of decreasing synapse loss for a distance of 50 μm from Aβ senile plaques [43]. Aβ oligomers have been shown to correlate with disease severity [44]. Human AD brains versus non-AD controls with comparable Aβ burden examined by oligomer specific ELISA showed higher oligomer to senile plaque ratios in AD patients [45]. This suggests a qualitative difference in AD versus plaque-rich controls that might explain substantial senile plaque burden with apparently intact cognition. Remarkably, Aβ oligomer levels distinguished demented from non-demented patients in all cases studied, consistent with the concept that a physical limit is reached in AD at which point Aβ oligomers would perhaps diffuse onto synaptic membranes or other hydrophobic surfaces initiating neurodegeneration [46]. Aβ Oligomers have been implicated in an extensive array of molecular outcomes [47]. Preliminary attempts to identify oligomeric forms in vivo as potential AD biomarkers, including quantification by ELISAs, flow cytometry, misfolded protein assays, and surface fluorescence distribution analysis, have been encouraging [47], in agreement with the role of Aβ oligomers in disease.

The complexity of oligomer biology, however, is substantial (reviewed in [48]). Nosology alone is unwieldy. Aβ Oligomers may be separated by number of mononers, e.g., dimer, trimer, tetramer, 12-mer, 32-mer etc. There may be differences in three-dimensional structure and arrangement, such as "globulomers," annular protofibrils, or linear protofibrils. There may be a mixture of Aβ oligomeric species with overlapping properties in dynamic equilibrium. Sample preparation using detergents can induce oligomerization artifacts. Aβ species may vary by concentration, temperature, pH, salt, metal ions, and other chemical variables. Toxicity assays vary widely, with no standards for interlaboratory comparison. Toxicity may be measured by acute changes in morphology of dendritic spines, apoptosis, ex vivo hippocampal electrophysiology, neurotransmission in cell culture, or neurobehavioral changes in rodents, which imperfectly model AD biology. The collective literature further provides no clarity in terms of which of the bewildering array of Aβ oligomer species are more or less relevant to trigger neurotoxicity, but implies instead the existence of a soluble "Aβ soup" [48] as an amorphous explanation to complex biology.

Aβ prions

Differences in conformation of neurotoxic proteins and the possibility of protein-mediated, non-Mendelian transmission of phenotypic information raises the issue of the "Aβ prion". Both Aβ and pathological prion protein (PrPsc) share protease resistance [49]. Both Aβ and PrPsc may be capable of conformational templating, or "seeding." [50] Aβ may undergo misfolding into distinct conformers or "strains," both in vitro and in vivo [51]. Aβ seeds inoculated into brain show features of Aβ aggregation, and neuroanatomical propagation [52]. Increased Aβ deposition is described in subjects with and without iatrogenic CJD (Creutzfeldt-Jakob Disease) [53]. Both Aβ and PrPsc may cause a secondary tauopathy [54]. Perhaps most compelling is the study by Cohen et al. [10], in which a distinct, rapidly progressive AD phenotype was associated with distinctly structured Aβ42 species (30–100 monomers) [55]. This parallels sporadic CJD subtypes with distinct differences in disease progression and pathologic phenotype on the basis of PRNP codon 129 polymorphism and the size of the PrPsc conformer [56], i.e. strain. This study also confirms the pathogenic relevance of Aβ42 over Aβ40, in that Aβ40 remained largely as soluble monomers with no features suggesting strain variation with phenotype.

The analogy of Aβ to PrPsc is lessened somewhat by the fact that PrPsc and CJD pathology do not appear in asymptomatic patients. PrPsc is indicative of clinical disease and its absence is indicative of absence of clinical disease [57]. Horizontal transmission of Aβ proteinopathy, either iatrogenically in humans, or by serial passage of distinct Aβ conformers associated with distinct phenotypes in experimental animals, is not described. Establishing standardized procedures for laboratory determination of prion-like transmission is another challenge. It remains to be seen whether the "Aβ prion" will translate into a viable target for AD therapy.

Targeting Aβ for disease modification

It is clear from the above discussion that identifying a specific Aβ species (monomer, dimer, oligomer, fibrillar, senile plaques, etc.) as a target for therapy is a formidable challenge. The numerous oligomeric species may be more or less relevant, transient, and immunogenic [48]. The potential conformations and antigenicity of the spectrum of potential oligomers may be impossible to define by current methodology. Pathogenic relevance of a given species may vary depending on diverse factors such as ApoE genotype [10], race [58], genetic factors not fully characterized [59], or other co-factors not yet characterized. A given Aβ species may relate to selective vulnerability (classic AD, versus limbic predominant AD or hippocampal sparing AD [60]), or other endophenotypes such as rapid disease progression. To this is added the complexities of immunology [61], blood brain barrier penetration, dosing, and cerebral and systemic toxicity. In the event of successful target engagement (which is not demonstrated in most trials to date [62]),

human factors such as clinical trial attrition rates [61] and diagnostic inaccuracy [63] may degrade statistical assessment.

Proposals for disease-modifying therapy require targeting of AD biology [64]. Ancillary features of AD biology, such as neuroinflammation and oxidative stress, provide myriad additional targets to neurotoxic Aβ, but these tend to be viewed as significant only insofar as they represent effector processes from downstream of the Aβ cascade [63]. Despite the challenges and repeated failures in clinical trials, Aβ remains the primary target for disease-modifying therapies (Fig. 2). Attempts at species-specific targeting is on-going (Fig. 3). Specific linear Aβ domains (e.g. N-terminal, C-terminal, mid domain) have been used to
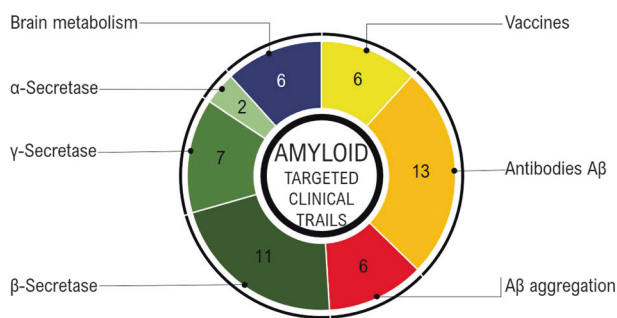
generate antibodies [65]. These may target monomers and fibrillar Aβ species, since the N-terminal domain remains exposed to the external or cellular environment in fibrillar Aβ arrangements [61] while the C-terminal is not. Antibodies may target protofibrils (>100 kDa) specifically [66], or other oligomerization states for unclear reasons [67]. The IVIg construct theoretically targets oligomers and fibrils, since the 0.5% of antibodies that bind Aβ exhibit very little monomeric Aβ binding [68]. Constructs that target protease cleavage rely on the premise of Aβ overproduction as the driver of disease, or modulation of the Aβ cleavage, rather than clearance of Aβ species (Fig. 4). Numerous other approaches and epitope specificities will likely be added as time goes on. Table 1 lists amyloid-targeted therapeutic intervention attempts completed or in progress, indicating the agent, and putative target/mechanism, although it is apparent that targets may be presumed more than actually targeted in any given construct because of inherent complexities.

Selected Aβ targeted constructs in phase 3 clinical trials

Bapineuzumab

Bapineuzumab or AAB-001 is a humanized IgG1 version of the mouse monoclonal antibody 3D6 that binds the N-terminus of Aβ, an exposed epitope in fibrillary Aβ deposits. Following an unpublished phase 1 study showing potential treatment benefit, the initial phase II study included primary outcomes that trended toward improved

Fig. 2 Distribution by molecular target of amyloid-related clinical trials. Diagram shows selected amyloid-related clinical trials of compounds divided into their molecular target. For Immunotherapies, compounds were subclassified into vaccines and therapeutic antibodies. Small drugs were classified into compounds that interfere with Aβ aggregation, modulators of β-secretase, γ-secretase or α-secretase, or compounds with overall effects on neuronal/brain metabolism
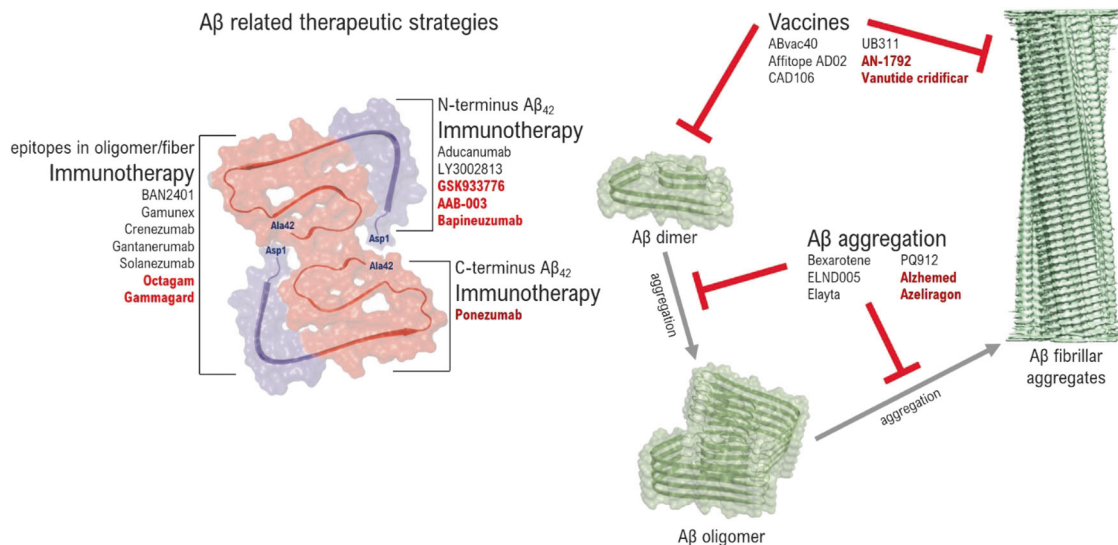

Fig. 3 Schematic of Amyloid-β therapeutic strategies. Diagram shows selected Aβ related compounds divided into immunotherapies that target N- or C-terminus or epitopes present in high-order oligomers of fibrillar assemblies. Additional compounds intended as Aβ-vaccines or to interfere with Aβ-aggregation into fibrillar aggregates. Compounds in red text are inactive/discontinued in clinical trials. Despite the diversity of Aβ species targeted, clinical trials to date have failed to demonstrate disease modification. Atomic structures from PDB 5OQV and EMB-3851, were modeled with PyMOL and UCSF Chimera
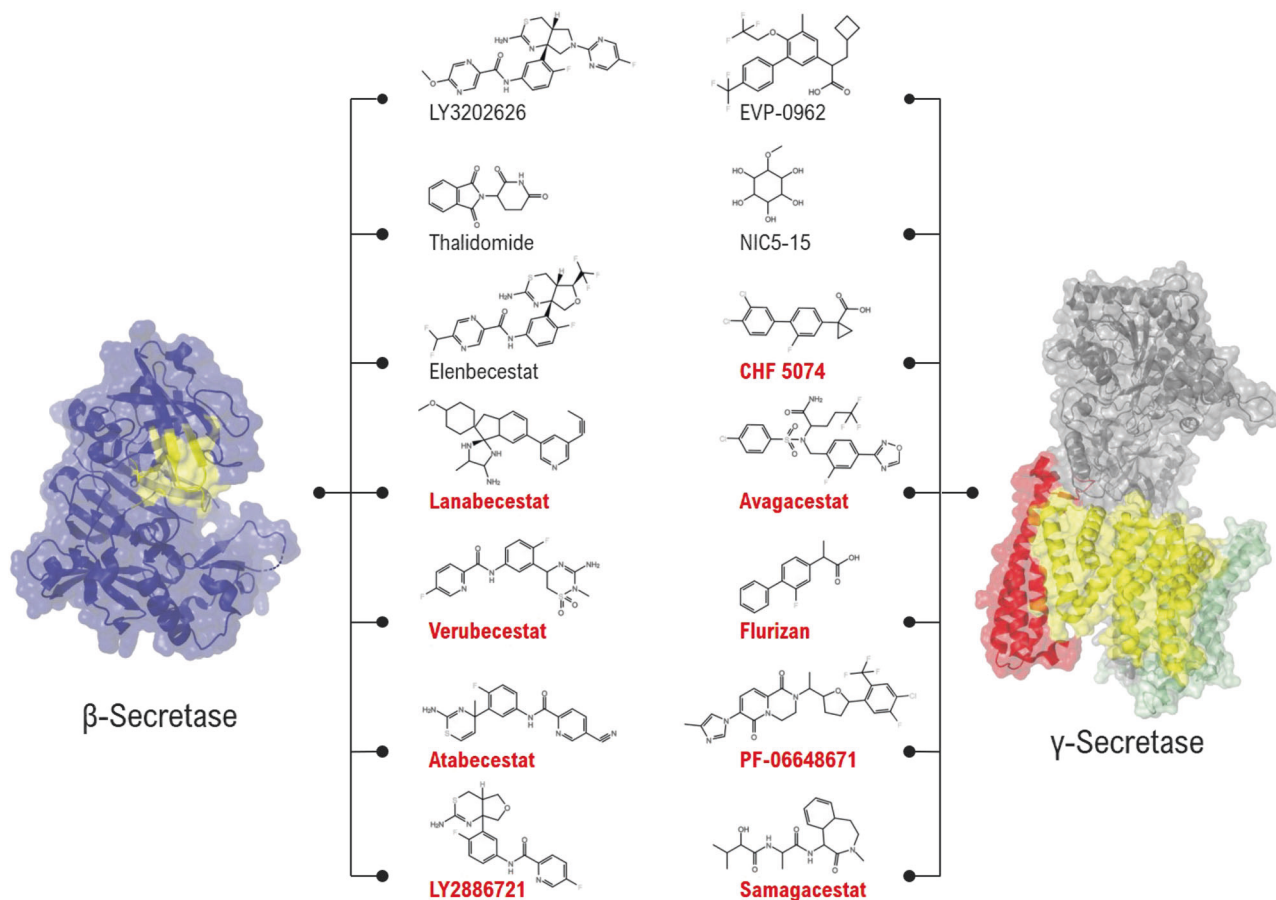
Fig. 4 Schematic of Secretase therapeutic approaches. Modulators of β-secretase (BACE1) and γ-secretase complex. BACE1 structure from PBD 6EJ2, yellow area indicates the catalytic site where most of the compounds exert effects as modulators or inhibitors. γ-secretase complex structure from PDB 5A63, yellow area indicated the PSEN1 domain and red area the PSEN2 domain. Compounds in red text indicate that they are inactive/discontinued. Secretase inhibition has been disappointing to date, in some cases performing worse than placebo, with increased adverse reactions (e.g Semagacestat). Atomic were structures modeled with PyMOL and UCSF Chimera

cognitive scores but with emerging neurovascular complications, prompting post hoc analyses [69].

A meta-analysis of six phase 3 randomized clinical trials ($n = 2380$) showed no differences from placebo among four doses (0.15, 0.5, 1, and 2 mg/kg), except for the 0.15 mg/kg dose, which showed significant worsening on the ADAS-cog11 scale [70]. A second phase 3 trial that excluded ApoE e4 carriers because of neurovascular complications also showed no clear benefits [61]. Biomarker analysis suggested exposure-dependent reductions in brain amyloid burden [71]. Studies differ on whether Bapineuzumab was associated with increased ventricular volume by magnetic resonance imaging [71, 72]. Amyloid-related imaging abnormalities with vagogenic edema (ARIA-E) increased with increasing dose in non-APOE e4 carriers, and was significantly increased in APOE e4 carriers compared to non-carriers [73]. ARIA-E occurred despite the attempts to reduce this complication by lowering the dose [74]. Treatment was also complicated by amyloid-related imaging abnormalities with microhemorrhage (ARIA-H).

Solanezumab

Solanezumab or LY2062430 is a humanized IgG1 monoclonal antibody that recognizes the mid region of Aβ. It is derived from mouse monoclonal antibody m266 that recognizes the Aβ16-24 epitope [62]. It is thought to bind monomeric, soluble Aβ rather than fibrillar or senile plaque Aβ [75], with only 0.1% blood brain barrier penetration [61]. Two phase 3, double-blind trials involving a total of 2052 patients with mild-to-moderate AD received 400 mg intravenous solanezumab or placebo every 4 weeks for 18 months [76]. Primary outcomes were Alzheimer's Disease Assessment Scale (ADAS-cog) and the Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale (AADCS-ADL). Neither clinical trial showed significant improvement in primary outcome measures, notwithstanding nonsignificant trends in subjects with mild AD [61]. In contrast to Bapineuzumab, there was no difference between treatment and placebo groups with respect ARIA-E or ARIA-H. This may be attributed to a lack of fibrillar Aβ binding.

Table 1 Some Aβ-targeting therapeutic constructs with completed, terminated, or on-going clinical trials in phase 2 or phase 3

Agent	Target	Status	Findings of trials
ABvac40	Aβ (Active vaccine)	Recruiting	Phase 2– pending
Acitretin Synonyms: Soriatane, Neotigason, RO 101670	Aβ synthesis (via increased α-secretase)	Completed	Phase 2– pending
Aducanumab Synonyms: BIIB037	aggregated Aβ	Recruiting	Phase 3–pending.
Affitope AD02	Aggregated Aβ	Completed	Phase 2–no benefit or worse than placebo; limited data available.
Alzhemed Synonyms: Vivimind, Tramiprosate, NC-531, homotaurine, 3-APS	Aβ-glycosaminoglycan binding	Completed	Phase 3–no benefit overall; possible benefit for e4 carriers
AN-1792 Synonyms: AN-1792	Aβ (Active vaccine).	Terminated	Phase 2–no benefit; Aβ plaque clearing; meningoencephalitis in 6%
Atabecestat Synonyms: JNJ-54861911	Aβ synthesis (BACE1)	Active, not recruiting	Phase 2/3–discontinued due to elevated liver enzymes
BAN2401 Synonyms: mAb158	Aβ protofibrils	Active	Phase 2–no benefit (questionable benefit at high dose); Aβ reduction on PET.
Bapineuzumab Synonyms: AAB-001	Fibrillar and soluble Aβ	Terminated	Phase 3–no benefit; neurovascular complications; reduced Aβ in e4 carriers
Bexarotene Synonyms: Targretin	Soluble and insoluble Aβ	Completed	Phase 2–no benefit
CAD106	Aβ (active vaccine)	Recruiting	Phase 3–pending. Some neurovascular complications in phase 2.
CNP520	Aβ synthesis (BACE1)	Recruiting	Phase 2/3–pending
Crenezumab Synonyms: MABT5102A, RG7412	Oligomeric and fibrillar Aβ	Active	Phase 3–pending; possible benefit at high doses in phase 2
Elayta Synonyms: CT1812	Aβ oligomer receptor (competitive inhibition)	Recruiting	Phase 2–pending
ELND005 Synonyms: AZD-103, scyllo-inositol, cyclohexane-1,2,3,4,5,6,-hexol	Aβ aggregation	Completed	Phase 2–no benefit
Elenbecestat Synonyms: E2609	Aβ synthesis (BACE1)	Recruiting	Phase 3–pending
Epigallocatechin gallate (EGCG) Synonyms: Sunphenon EGCg	Aβ synthesis (α-secretase modulation)	Completed	Phase 2/3–results not published
EVP-0962 Synonyms: EVP 0015962	Aβ synthesis (γ-secretase modulation)	Completed	Phase 2–results not published
Flurizan Synonyms: tarenflurbil, r-flurbiprofen, MPC-7869	Aβ synthesis (γ-secretase modulation)	Terminated	Phase 3–no benefit; increased adverse reactions; no clear reduction in Aβ
Gammagard Synonyms: Intravenous immunoglobin, IVIg	Unclear	Complete	Phase 3–no benefit, decreased plasma Aβ42
Gantenerumab Synonyms: RO4909832, RG1450			
Gamunex Synonyms: Intravenous Immunoglobulin, Human Albumin Combined with Flebogamma	Fibrillar Aβ	Active	Phase 3–no benefit; no change in CSF Aβ42; new phase 3 initiated in 2017.
Lanabecestat	Unclear	Active	Pending
Synonyms: AZD3293, LY3314814	Aβ synthesis (BACE1)	Terminated	Phase 3–results not published
LY2886721	Aβ synthesis (BACE1)	Terminated	Phase 2–results not published; terminated due to liver toxicity
LY3002813 Synonyms: N3pG-Aβ monoclonal antibody	Aggregated Aβ3-42.	Recruiting	Phase 2 combined with BACE inhibitor pending
LY3202626	Aβ synthesis (BACE1)	Recruiting	Phase 2–results pending
NIC5-15 Synonyms: Pinitol, D-pinitol	Aβ synthesis (γ-secretase modulation)	Completed	Phase 2–results not published
Octagam 10% Synonyms: Intravenous immunoglobin, NewGam	Unclear.	Enrolling	Phase 2–no benefit, no change in plasma Aβ
Ponezumab Synonyms: PF-04360365	Soluble and fibrillar Aβ (1–40)	Completed	Phase 2–no benefit, no change in brain Aβ
PQ912	Pyroglutamate Aβ	Completed	Phase 2–no benefit; possible decreased pyroglutamate Aβ oligomers in CSF
Semagacestat Synonyms: LY4501139 dihydrate, hydroxylvaleryl monobenzocaprolactam	Aβ synthesis (γ-secretase inhibition)	Completed	Phase 3–worse than placebo; increased adverse reactions
Solanezumab Synonyms: LY2062430	Soluble, monomeric Aβ	Terminated	Phase 3–no benefit
Thalidomide Synonyms: Thalomid	Aβ synthesis (BACE1)	Unknown	Phase 2–no benefit; poor tolerability
UB-311	Aβ1-14 (active vaccine)	Active	Phase 2–pending.
Vanutide cridificar Synonyms: ACC-001, PF-05236806	Aβ1-7 (active vaccine)	Completed	Phase 2–no benefit, one serious adverse event
Verubecestat Synonyms: MK-8931, MK-8931-009	Aβ synthesis (BACE1)	Terminated	Phase 3–no benefit; increased adverse reactions

Clinical trial data was retrieved from clinicaltrials.gov (accessed April 2018–Aug 2018), alzforum.org/therapeutics (accessed May/Aug 2018), and journal articles. The list provided is a brief representation of the outcome data to date. Please see clinicaltrials.gov, Alzforum, and the reference list for more comprehensive clinical trial information.

Noteworthy with respect to targeted soluble versus insoluble Aβ was a study examining experimental administration of 3D6 (mouse version of Bapineuzumab) [77]. In this study, an increased frequency of microhemorrhages in experimental mice was noted in the absence of plaque removal. These data were at odds with the notion that clearing of cerebral amyloid angiopathy co-segregates with clearing of plaque. The investigators concluded that antibodies binding to soluble and insoluble Aβ lack therapeutic plaque lowering, and that soluble Aβ blocked target engagement in existing plaque. Whether plaque lowering is truly therapeutic remains an open question [78].

Intravenous Immunoglobulin G

Intravenous Immunoglobulin G (IVIg) is polyclonal IgGs pooled from healthy donors. The rationale for a potential Aβ-targeted therapy is based on the findings that anti-Aβ antibodies in the blood and CSF are reduced in AD compared to controls [79] and that commercial preparations of IVIg contain natural anti-Aβ antibodies [80]. About 0.5% of all antibodies in IVIg bind with Aβ. Similarly, a retrospective national database analysis demonstrated a risk reduction in the diagnosis of AD following IVIg administration [81]. The precise target of anti-Aβ antibodies in IVIg preparations is unclear, however. IVIg has been shown to inhibit fibril formation [82], Aβ-mediated toxicity [83], and Aβ aggregation [84], and enhance Aβ clearance [85]. IVIg administered to APP/PS1 transgenic mice suggested binding within the brain but not of senile plaques [84].

In a phase 3, double-blinded trial [86], 390 subjects with mild to moderate AD were randomly administered IVIg (0.2 or 0.4 g/kg every 2 weeks for 18 months) or placebo. Outcome measures included ADAS-cog11 and ADCS-ADL. Significant decreases in Aβ42 were observed with IVIg treatment, but with no differences in ARIA-E or ARIA-H. No beneficial effects on cognition outcomes was demonstrated.

Tramiprosate

Tramiprosate (known as Alzhemed, Vivimind or NC-531) is a glycosaminoglycan mimetic thought to prevent Aβ aggregation via soluble Aβ binding [62]. In a mass spectrometry study, a 10-fold molar excess of tramiprosate led to 50% binding of Aβ40 and Aβ42 [87], although the high concentrations of both substances required to demonstrate binding, and lack of activity in the aqueous phase raise questions about the actual in vivo anti-aggregation properties of this compound [62]. Overall, other than the general concept that tramiprosate is thought to prevent Aβ amyloidosis, the precise Aβ species targeted by tramiprosate is unclear.

In a phase 3, double-blind, placebo-controlled trial in 67 medical centers in the United States and Canada, 1052 subjects with mild to moderate AD were randomly assigned tramiprosate (100 mg and 150 mg BID) and placebo [88]. Primary outcomes included ADAS-cog and Clinical Dementia Rating—Sum of Boxes (CDR-SB). No significant differences in cognitive function were detected, although the treatment group showed less hippocampal volume loss. The negative results were disappointing in light of promising phase 2 clinical trial data suggesting up to 70% reduction of Aβ42 in the CSF [62], although interestingly, in post hoc analysis with ApoE subclassification, e4 homozygotes show statistically significant improvement in ADAS-cog score [89].

Semagacestat

Semagacestat or LY450139 is a small molecule gamma-secretase inhibitor that acts by functional inhibition rather than competitively inhibiting enzyme complex active sites [90]. The precise mechanism of action is unclear; given gamma-secretase inhibition, it is not specific to any Aβ aggregation state or conformation, but theoretically reduces proteolytic cleavage of transmembrane domain of AβPP and consequently release of Aβ42. Semagacestat has been shown to reduce Aβ in brain, CSF, and plasma of PDAPP transgenic mice with human APP V717F mutation [90]. Phase 1 studies in normal human volunteers showed a dose-dependent reduction in plasma Aβ42 [91], whereas subjects with mild to moderate AD showed a reduction in plasma but not CSF Aβ in a small phase 2 cohort study [92].

In a phase 3 clinical trial, 1537 subjects with probable AD were randomized to 100 mg or 140 mg Semagacestat, or a placebo dose, daily. Primary outcome measure was cognitive function based on ADAS-cog and ADCS-ADL. The ADAS-cog and ADCS-ADL scores worsened in all groups. ADCS-ADL scores in the high dose group were significantly worse than placebo ($p < 0.001$). Significant side effects were also noted in the treatment groups, including weight loss, skin cancers, infections, and laboratory test abnormalities. Withdrawal from the trial due to adverse events was also significantly more frequent in the treatment group ($P < 0.001$).

The Semagacestat trials and related studies have since been criticized for lack of rigorous preclinical characterization of Semagacestat as an effective or reliable Aβ lowering agent and its low therapeutic index [63]. It has since been termed a "pseudo-inhibitor" of gamma secretase [93]. A shift in direction may be the pursuit of gamma-secretase modulators that alter the cleavage site to produce an ostensibly more benign, non-amyloidogenic Aβ species while leaving proteolytic function intact [63].

Tarenflurbil

Tarenflurbil or Flurizan is a non-steroidal anti-inflammatory agent that is said to modulate (rather than inhibit) gamma secretase by apparently shifting cleavage sites in favor of shorter, more benign Aβ species [94], but does not alter diverse functions associated with Notch processing [95]. Tarenflurbil reduces Aβ42 production in cell culture and experimental models [96], although dose responses were not established in vitro [62]. Subsequent studies in Tg2576 mice showed limited modulation of Aβ42 production [97]. Efficacy in AD treatment may also be limited by its greater inhibition of COX1 than Aβ42 [97].

While conclusions of specificity of Tarenflurbil for its proposed Aβ42 modulation may have been premature, a multicenter phase 3 clinical trial of mild AD was undertaken [98]. Eligible participants were randomized to placebo, 400 and 800 mg tarenflurbil. Primary outcomes included ADAS-Cog and ADCS-ADL. CDR-sb, mini-mental status exam (MMSE), Neuropsychiatric inventory, quality of life scale, Caregiver Burden Inventory, and 70-point version of ADAS-Cog were secondary outcomes. No difference in primary outcomes was detected in tarenflurbil treatment groups versus placebo. Secondary outcome analyses showed either no difference or marginally favored the placebo group. Some adverse events were more frequent in treatment groups, such as dizziness, anemia, pneumonia, herpes zoster, gastrointestinal ulcer, and eosinophilia. Tarenflurbil treatment groups were more likely to discontinue the study because of an adverse event. The placebo group was more likely to experience a stroke or a transient ischemic attack. Due to the combined negative outcomes with tarenflurbril, the clinical trials were terminated and compound discontinued.

Verubecestat

The development of different small molecules functioning as beta-secretase (BACE1) inhibitors was slowed somewhat by the challenge of targeting a large active site in intact neurons [63, 99]. However, through application structure-based rational design and screening, researchers discovered verubecestat (also known as MK-8931 or MK-8931-009), and confirmed hydrogen bonding interactions with the BACE1 catalytic site [100]. Verubecestat shows inhibition of production of Aβ40, Aβ42, and sAPPβ in vitro, and is a potent inhibitor of mouse BACE1 as well as purified human BACE2 [101]. Verubecestat reduces plasma, CSF, and cortical Aβ40 in rats and cynomolgus monkeys. Once-daily oral administration to cynomolgus monkeys for 9 months produced reduction of CSF Aβ40, Aβ42, and sAPPβ, and cortical Aβ40 and sAPPβ. Chronic administration in animal models was well tolerated.

In a phase 3 clinical trial, 1958 subjects with mild to moderate AD were randomized to 12 or 40 mg per day Verubecestat, and placebo [101]. Primary outcomes included ADAS-cog and ADCS-ADL. The trial was terminated for futility, with no differences in primary outcome between treatment and placebo groups. A number of adverse events were more common in the verubecestat groups, including rash, falls and injuries, sleep disturbance, suicidal ideation, weight loss, and hair color change.

Lanabecestat

Lanabecestat (AZD3293 or LY3314814) is a selective human BACE1 inhibitor shown to reduce plasma, CSF, and brain Aβ40, Aβ42, and sAPPβ in mouse, guinea pig, and dog models. It was reasonably well tolerated in a phase I study demonstrating a dose-dependent reduction of CSF Aβ [102]. A phase 3 trial was recently terminated due to futility. The specific results have not been published at the time of this writing.

Aducanumab

This monoclonal IgG1 antibody designed to selectively target aggregated Aβ epitopes to slow neurodegeneration and reduce AD progression has reached phase 3 trials. Initially, the antibody showed reduction of soluble and insoluble Aβ in the brain of Tg2576 mice in a dose-dependent manner, followed by significant reduction in brain Aβ in patients with prodromal or mild AD in a time (up to 54 weeks of treatment) and dose (1, 3, 6 or 10 mg/kg) dependent manner, as measured by PET imaging and showing some signs to slow the rate of cognitive decline [103, 104]. Some adverse events caused withdrawal of a subset of patients. Overall, the study concluded that aducanumab reduces brain Aβ plaques with some clinical benefit. Ongoing phase 3 trials will continue until the end of 2022 (trials NCT02477800 and NCT02484547) at sites of North America, Australia, Europe and Asia in 1350 patients with early AD [105]. Outcomes will focus on efficacy of monthly doses of aducanumab in treatment of AD symptoms and cognitive changes on patients.

Noteworthy Phase 2 trials

AN-1792

Although it did not progress to phase 3, AN-1792 or AIP 001 an active immunization formulation deserves mention. In a seminal study, Schenk et al. immunized PDAPP transgenic mice with synthetic Aβ42 before the onset of Aβ plaque deposition and at an older age when plaques are well established [106]. Immunization of younger mice prevented

Aβ plaque deposition, neuritic dystrophy, and gliosis, while immunization of older mice markedly reduced AD pathology. These preclinical results not surprisingly met with considerable enthusiasm for disease modification in AD. A Phase 1 study in AD patients subsequently showed acceptable tolerability of Aβ42 (AN-1792) with QS-21 as adjuvant. 372 patients with mild to moderate AD were then randomized to IM injections of AN-1792 or placebo in a multicenter Phase 2 study, however, the study was terminated after early reports of meningoencephalitis. Follow-up revealed meningoencephalitis by clinical examination in 18 out of 298 (6%) study subjects [107], although it is difficult to know how many subjects were affected neuropathologically. One case examined at autopsy showed evidence of plaque clearing, but also showed extensive T cell infiltrates involving leptomeninges and parenchymal tissue, encompassing apparent immune-mediated attack on blood vessels (i.e. vasculitis) involved by CAA, as well as a subacute leukoencephalopathy [108]. Other cases showed convincing evidence of plaque clearing in the absence of meningoencephalitis, including one case of Lewy body dementia. Some follow-up studies suggested less functional decline in antibody responders, but the overall follow-up data, including neuropathological examination of eight subjects, pointed to no benefit from Aβ clearance[108]. Antibody response was also related to cerebral atrophy by MRI [109].

In retrospect, the active approaches to immunotherapy may have had the disadvantages of polyclonal antibody responses that are variable across patient populations and with age. This could lead to persistent adverse effects or blunted immune responses. Active approaches have therefore proceeded more slowly since the AN-1792 phase 2 trial. Several agents are in development, including ACC-001, CAD106, and Affitope AD02, and may be worth pursuing given the daunting cost and logistics of administering passive monoclonal antibody to a large population of AD patients [63].

BAN2401

BAN2401 is the humanized version of murine antibody mAb158, derived from immunization with Aβ-protofibrils containing the intra-Aβ Arctic mutation E22G. The Artic mutation has been shown to enhance Aβ protofibril formation in vitro, and intraneuronal Aβ accumulation in vivo. BAN2401 likewise shows selectivity for protofibrils compared to monomers, and greater binding affinity for protofibrils than fibrils [110]. Transgenic mice carrying Swedish and Artic mutations were cleared of senile plaques if BAN2401 was administered prior to the appearance of senile plaques in young mice, whereas older mice with established plaques showed no clearing of fibrillary Aβ. Lowering of protofibrillar Aβ (i.e., larger than 100 kDa)

occurred in all age groups. In addition, researchers have suggested that Artic mutation carriers lack fibrillar Aβ based on PET imaging studies using Pittsburgh Compound B, which is consistent with neuropathological investigations demonstrating morphologically atypical Aβ plaques with relatively limited Congo red staining in autopsy brain of symptomatic patients. Taken together, these data suggest that BAN2401 is relatively specific for protofibrillar Aβ.

Safety and tolerability of BAN2401 in mild to moderate AD were investigated and found to be acceptable across a range of doses tested [110]. A recent phase 2 clinical trial of 856 patients with mild cognitive impairment (MCI) due to AD, or mild AD with evidence of brain amyloid by PET imaging were randomized to one of different five dose regimens: 2.5 mg/kg biweekly, 5 mg/kg monthly, 5 mg/kg biweekly, 10 mg/kg monthly and 10 mg/kg biweekly, or placebo. Outcome measures determined at 18 months included predefined conventional statistics on ADCOMS, ADAS-Cog, and Mini-Mental State Examination (MMSE). Incidence of ARIA-E (edema) was less than 15% in patients with ApoE e4 alleles at the highest dose tested, although substantially fewer patients with e4 alleles received the highest dose because of the association between ARIA-E and e4 alleles. Reduction in amyloid on PET imaging was also demonstrated with treatment. Statistically significant slowing of disease progression was noted only with the highest treatment dose; however, the failure to normalize for ApoE genotype in the high dose group mitigates those results. The treatment with antibody BAN2401 (mAb158) rescued neuronal Aβ-induced death and substantially reduced accumulation of Aβ in astrocytes in co-cultures from embryonic cortex of C57/BL6 mice [111]. Recent data related to the BAN2401 phase II trials suggested cognitive benefit, but normalizing patients treatment groups to APOE4+revealed that treated group had similar decline rates than placebo controls [112]. The study evaluated safety, tolerability and efficacy of BAN2401 (clinitrialtrials.gov: NCT01767311) for over 18 months in 856 patients with MCI showed dose-dependent Aβ reduction and 26–30% slowing of cognitive decline. The outcomes of this trial revealed acceptable tolerability overall, although specific data were not available as a function of *APOE*.

BAN2401 results are significant in that proof of concept, or reduction in brain amyloid by PET imaging, is demonstrated, but with arguable clinical/therapeutic efficacy as noted, suggesting both the existence and the clinical insignificance of the amyloid cascade. It is also noteworthy that clinical trial strategies targeting amyloid-β increasingly steer away e4 alleles, despite e4 being the most significance genetic risk factor for developing AD, and having a close relationship with Aβ aggregation. The challenge of targeting a process that bridges normal cellular physiology and manifest as insoluble deposits is becoming increasingly apparent.

Failure of construct versus the null hypothesis

The remarkable amount of past and on-going efforts to target Aβ by a variety of mechanisms are evidence that Aβ neurotoxicity is settled science, yet outcomes from many clinical trials tend to suggest otherwise. It may be useful to recall that the foundation for all proteinopathy constructs lies in hallmark lesions, which are entirely empirical observations. SP and NFT are evidence only that some complex processes have taken place in the affected brain areas. This is as opposed to neoplasia, for example, in which morphology captures the essence of the disease, and presents an unambiguous target. SP and NFT occur in parallel with the essence of the disease, which is neurodegeneration, and present a less clear or specific target. Strictly speaking, the notion that hallmark lesions in AD represent neurotoxicity, or surrogates for targetable neurotoxic protein species, is still theoretical.

The above is the case despite the enormous superstructure assembled since identification of lesion constituents. The complex data concerning such phenomena as low-n assembly intermediates or self-propagating Aβ prions, tend more to highlight the malleability of the original hypothesis, founded in Mendelian genetics [1], for example, and revised over time to encompass purely post-translational phenomena, such as oligomers [39] and non-Mendelian, prion-like transmission of phenotypic information [10, 113]. Collective strategies that target the spectrum of Aβ species, from soluble Aβ, to insoluble Aβ, to both soluble and insoluble Aβ, to Aβ production, are de facto admissions that Aβ theory is instead unsettled. The spectrum of approaches further indicates that a broad diversity of Aβ mechanisms remain in play, for example that Aβ senile plaques sequester toxic oligomers and many other functional components of the neurons until a physical limit is reached [46], or alternatively that Aβ plaques are poorly soluble, benign aggregates, downstream from toxic soluble mediators [63]. This remains the case despite repeated demonstrations that Aβ lowering does not modify disease.

Outcome data in clinical trials are often viewed as evidence for or against the amyloid cascade hypothesis, but this may be a false dichotomy. A negative trial may, in some cases, indicate proof of concept, i.e. the existence of the amyloid cascade, and no disease modification. This would suggest that complex biological processes not fully understood mute the impact of the amyloid cascade on disease expression, or that Aβ metabolism on balance may have a partly protective role in the relentless neurodegeneration of AD. New adverse phenomena, such as ApoE e4 carrier susceptibility to ARIA-E [73] and other adverse reactions to therapy [107], in the face of target engagement [90], Aβ reduction [71, 86], plaque clearance [108], and unaltered neurological deterioration [70, 76, 86, 88, 90, 101], suggest that this may be the case. Alternatively,

failure may be partly explained by deficiencies in the clinical studies. This includes, but are not limited to, suboptimal trial design, inadequate dosing, recruitment of patients at advanced stages of AD, genetic heterogeneity of selected patients, inconsistencies in cognitive assessment, and inadequate target engagement [114, 115].

Conclusions

Aβ-targeted therapeutic constructs rely on the amyloid cascade hypothesis and the inherent neurotoxicity of Aβ, for which there is an abundance of supporting literature. Nevertheless, a significant body of cumulative data now exists from the highest evidence base—randomized, blinded, placebo-controlled clinical trials—demonstrating target engagement and Aβ clearing, with no clinical benefit. If the scientific mode of inquiry is to challenge theory with observation, the neuroscience field seems obligated to revise the original theory, or discard Aβ altogether as the primary driver of neurodegeneration in AD.

Compliance with ethical standards

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