

REVIEW

Targeting the proper amyloid-beta neuronal toxins: a path forward for Alzheimer's disease immunotherapeutics

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Abstract

Levels of amyloid-beta monomer and deposited amyloid-beta in the Alzheimer's disease brain are orders of magnitude greater than soluble amyloid-beta oligomer levels. Monomeric amyloid-beta has no known direct toxicity. Insoluble fibrillar amyloid-beta has been proposed to be an *in vivo* mechanism for removal of soluble amyloid-beta and exhibits relatively low toxicity. In contrast, soluble amyloid-beta oligomers are widely reported to be the most toxic amyloid-beta form, both causing acute synaptotoxicity and inducing neurodegenerative processes. None of the amyloid-beta immunotherapies currently in clinical development selectively target soluble amyloid-beta oligomers, and their lack of efficacy is not unexpected considering their selectivity for monomeric or fibrillar amyloid-beta (or both) rather than soluble amyloid-beta oligomers. Because they exhibit acute, memory-compromising synaptic toxicity and induce chronic neurodegenerative toxicity and because they exist at very low *in vivo* levels in the Alzheimer's disease brain, soluble amyloid-beta oligomers constitute an optimal immunotherapeutic target that should be pursued more aggressively.

Introduction

Alzheimer's disease (AD) is the most common form of dementia, accounting for 60% to 80% of all dementias [1,2]. Worldwide, the prevalence of dementia was more than 35 million in 2010, and projections exceed 65 million by 2030 and 115 million by 2050 [1]. Significantly, no drugs that prevent or slow the progression of AD are currently approved. The development of effective AD therapeutics is clearly a tremendous medical challenge and should be one of society's top medical priorities.

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Despite the great need and significant societal and financial incentives, many pharmaceutical companies and investors have reduced investments in the search for new AD drugs, citing recent clinical failures of several high-profile experimental AD therapeutics and the high risks and costs of such development endeavors. The recent clinical failures also have intensified scrutiny of the 'amyloid cascade hypothesis', which spawned many of the recent experimental AD drugs targeting the amyloid-beta (A β) peptide. Nevertheless, the causal linkage between A β and AD remains strong and is supported by hundreds of studies over the past two decades [3-10]. (This is a representative sample of published reviews, and apologies are given to the authors of many excellent reviews that are not cited.)

Essentially all A β therapeutic approaches so far have targeted reducing the levels of A β monomer or A β deposits (or both) in the brain. However, today, the causal role of A β in AD is widely considered to involve soluble A β oligomers, and therapeutic strategies that selectively target soluble A β oligomers offer the potential to deliver rapid symptomatic benefit and long-term disease modification. This review describes the role of soluble A β oligomers within the amyloid hypothesis and discusses implications for current A β immunotherapies and new immunotherapies directed selectively toward soluble A β oligomers.

The amyloid cascade hypothesis

The first suggestion of an 'amyloid hypothesis' to explain the pathology of AD was that of Wong and colleagues [11], who postulated that A β -derived cerebrovascular amyloid caused seepage of A β and other substances from plasma into the brain, leading to the formation of A β plaques and possibly neurodegeneration. This was revised into the more well-known amyloid cascade hypothesis that proposed that deposition of A β as neuritic plaques caused AD and led to neurofibrillary tangles, cell

loss, vascular damage, and dementia [12]. The amyloid hypothesis linking A β to AD catalyzed much of AD and A β research over the past two decades, and key studies during that period led to important revisions of the hypothesis that highlighted the central role of soluble A β oligomers in synaptic dysfunction and loss [4,13-19].

The current understanding of the A β cascade is derived primarily from *in vitro* studies, the vast majority of which were conducted by using A β concentrations orders of magnitude greater than those found *in vivo*. The assembly of A β peptides to form soluble oligomers, protofibrils, and fibrils is well documented to be affected by the isoform of the starting peptide, how the peptide is treated prior to assembly, the nature of the buffer, pH, peptide concentration, assembly temperature and time, agitation, and presence of other peptides or biological materials [20-24]. Moreover, preparations of soluble A β species have been shown to change with time or upon dilution in different buffers, particularly in cell culture media [19]. Thus, caution must be taken in extrapolating the results and conclusions of *in vitro* studies to *in vivo* reality. Although precise mechanistic details remain to be elucidated, a multitude of studies by numerous researchers support the conclusion that monomeric A β peptides assemble to form soluble A β oligomers, which further aggregate to form fibrillar A β [17,25].

Three distinct pools of A β species exist: A β monomers, soluble A β oligomers, and insoluble fibrillar A β . Each of these pools encompasses an array of individual species. Thus, monomeric A β peptides encompass various isoforms, including A β (1-40), A β (1-42), and A β (1-43), as well as numerous N-terminal truncated isoforms. (For example, see the introductory paragraphs of Tekirian and colleagues [26].) Insoluble fibrillar A β aggregates are also known to be heterogeneous in structure and composed of various A β isoforms, both full-length as well as N-terminal and C-terminal truncated isoforms. (For example, see the introductory paragraphs of Roher and colleagues [27] and Thal and colleagues [28].)

Soluble A β oligomers are also heterogeneous and perhaps more ambiguous because of the different terminologies used by different researchers to describe them. (For an excellent review of soluble A β oligomers, see Benilova and colleagues [9].) Thus, soluble A β oligomer species reported by various researchers have been termed sodium dodecyl sulfate (SDS)-stable A β oligomers [29,30], low-n-oligomers [31-33], dimers [33-35], trimers [33,36-38], tetramers [37], paranuclei [38,39], dodecamers and A β *56 [37,40,41], amyloid-derived diffusible ligands (ADDLs) [42-44], A β oligomers [45], pre-fibrillar oligomers [46], A β globulomers [40,47-49], spherical oligomers [50], amylospheroids [51,52], protofibrils [20,53,54], and annular protofibrils [55,56]. Most of these terminologies refer to a mixture of metastable,

soluble A β oligomer species in equilibrium rather than a discrete, stable species. In many cases, there is similarity in the species comprising the different preparations. In this review, we will use the terminology soluble A β oligomers to describe A β species composed of more than one A β peptide that remain in solution following centrifugation. A β fibrils or fibrillar A β will be used as a general description of insoluble A β plaques and vascular amyloid. (The term A β plaques rather than amyloid plaques will be used to more precisely describe amyloid plaques comprising primarily A β peptides versus those comprising primarily non-A β peptides.)

It has been established that the levels of A β monomer and deposited fibrillar A β in the AD brain are orders of magnitude greater than soluble A β oligomer levels [57-65]. However, more than three decades of intense investigation has not provided a precise understanding of the extent of interconversion among the various A β species.

A β dimers have been demonstrated to form at physiologically relevant concentrations of A β monomer *in vivo* [66,67]. Numerous *in vitro* studies have shown that soluble A β oligomers and protofibrils form under similar conditions and that both species can proceed to form larger fibrillar species [20,43,53,68-74]. More recent studies provide further support for the addition of soluble A β oligomers to protofibrillar and fibrillar assemblies and also provide data indicating that oligomerization can occur via a secondary nucleation mechanism caused by fibrillar A β [73,74], possibly suggesting a mechanistic linkage between fibrillar A β and soluble A β oligomers. However, the precise mechanism of *in vivo* fibril formation has not been fully established.

A β plaques are now generally considered to be a relatively benign species [19,72,75]; however, whether A β plaques are a sink or a source for toxic soluble A β oligomers is a subject of debate. Several studies with γ -secretase inhibitors [76-79] and two studies in transgenic mice that over-express mutant amyloid precursor protein (APP) via a doxycycline-regulated promoter [80,81] show that sub-chronic or chronic suppression of A β production arrested A β plaque formation but did not result in observable clearance of existing plaques. In a related study [82], A β (1-42) immunization of Tg2576 mice prior to significant A β deposition, at an age with modest deposition or at an age with significant deposition, showed that immunization prevented additional A β deposition but did not result in significant clearance of pre-existing A β . These studies indicate that mature, dense-core A β plaques are not in significant equilibrium with soluble A β pools. Studies in transgenic mouse models of AD reporting decreased soluble A β oligomer levels and reduced cognitive deficits with increasing A β plaque levels also provide support for the concept that A β plaques may be a sink for soluble A β species [83,84].

However, in an elegant study in APP transgenic mice using a novel microdialysis technique, the half-life of low-molecular-weight A β species in hippocampal interstitial fluid following inhibition of A β production by a potent secretase inhibitor was doubled in 12- to 15-month-old mice with A β deposits compared with 3-month-old mice without A β deposits [85]. On the basis of these results, it was suggested that insoluble A β was in equilibrium with soluble A β in the interstitial fluid. In a related study using similar techniques, the temporal changes of low-molecular-weight A β species in interstitial fluid and A β levels in Tris-buffered saline (TBS), SDS, and formic acid extracts of brain tissues of 3-, 12-, and 24-month-old APP transgenic mice were reported [86]. A significant age-dependent decrease in low-molecular-weight A β in interstitial fluid and significant increases in A β species in SDS and formic acid extracts of brain tissues were found. Although A β increased approximately seven-fold from baseline in the TBS extract of brain tissues, the level of A β in TBS extracts was less than 2% of the total A β in SDS and formic acid extracts. These results indicated an age-dependent sequestration of A β as non-diffusible cell matrix and membrane-bound A β and deposited A β plaques. Acute γ -secretase inhibition of A β production in plaque-free and plaque-rich mice suggested that A β (1-42) in the interstitial fluid of plaque-rich mice was derived primarily from A β (1-42) sequestered in brain parenchyma rather than from new biosynthesis. However, it was not possible to determine whether cell matrix and membrane-bound A β or A β plaques or both forms of A β were the source of A β (1-42) in the interstitial fluid of aged mice.

In a more recent study of the temporal changes of A β species in the interstitial fluid and brain tissues of APP transgenic mice, Takeda and colleagues [87], using a 1,000-kDa-molecular-weight cutoff microdialysis probe, reported the temporal changes in soluble A β oligomer levels. In that study, consistent with previous studies [85,86], a significant, age-dependent increase in A β levels in TBS and formic acid extracts of brain tissues was found, and TBS extractable A β was less than 1% of formic acid extractable A β . However, unlike previous studies using a 35-kDa-molecular-weight cutoff microdialysis probe [85,86], a significant, age-dependent increase in interstitial fluid A β levels was found by using the larger-pore-sized microdialysis probe. The majority of the A β in interstitial fluid was determined to be higher-molecular-weight soluble A β oligomers, which showed an age-dependent increase relative to lower-molecular-weight A β species. Temporal changes in soluble A β oligomer levels in interstitial fluid and TBS brain extracts showed a significant positive correlation with formic acid A β extract levels. Comparison of high- and low-molecular-weight interstitial fluid A β levels at baseline and following acute treatment with a γ -secretase inhibitor showed slower

clearance of higher-molecular-weight A β oligomers compared with low-molecular-weight A β species.

Narayan and colleagues [88] have recently used single-molecule imaging techniques to investigate interactions between A β peptides and hippocampal cell membranes and reported results indicating that A β oligomers preferentially interact with membranes compared with A β monomer, thereby providing support for the results observed in the microdialysis studies. Thus, this study, coupled with those of the temporal changes of A β species in APP transgenic mice [85-87], does not resolve the controversy regarding the sink/source relationship between fibrillar and soluble A β species.

The sink/source relationship between soluble and insoluble A β pools is complex, not fully understood, and subject to ongoing debate. Two different forms of A β plaques are present in the AD brain: vascular amyloid plaques that are primarily composed of A β (1-40) and A β plaques that are primarily composed of A β (1-42) [89]. *In vitro* studies have shown that fibrillar A β (1-40) and A β (1-42) are in equilibrium with soluble A β [90,91] but that recycling of A β (1-40) fibrils is significantly faster than A β (1-42) fibrils [91]. Moreover, it has been reported that plaque deposition proceeds in two distinct kinetic phases: an initial, reversible deposition phase followed by a time-dependent irreversible deposition phase [92]. Furthermore, a recent study of the rates of formation of A β oligomers and fibrils provided evidence that the formation of soluble A β oligomers from monomeric A β is catalyzed by fibrillar A β [73]. This study not only indicated a mechanistic linkage between soluble A β oligomers and fibrillar A β but also provided evidence showing that fibrillar A β can be a 'source' of soluble A β via catalyzed oligomerization of A β monomer rather than via a disaggregation process. This study also provided an alternative explanation for the study by Koffie and colleagues [93], who reported a halo of soluble A β oligomers surrounding A β plaques and proposed that A β plaques were a possible source of soluble A β oligomers.

Although precise details remain to be fully elucidated, collectively, over two decades of studies on the mechanisms of formation of oligomeric and fibrillar A β , the temporal distribution of A β species *in vitro* and *in vivo*, the age-dependent effect of A β immunization, and the effects of subchronic and chronic suppression of A β production upon *in vivo* A β plaque levels show a significant, age-dependent increase in brain levels of soluble A β oligomers and deposited fibrillar A β . Collectively, the studies suggest that mature, dense-core A β plaques are not in equilibrium to any significant extent with soluble pools of A β , but that A β sequestered in the cell matrix and membranes and immature plaques is in equilibrium with soluble A β pools, and that fibrillar A β catalyzes A β monomer oligomerization, giving rise to soluble A β oligomers and growth of A β plaques.

Neuronal toxicity of amyloid-beta species

Monomeric A β , primarily the A β (1-40) and A β (1-42) peptides, is produced in various cell types throughout the body and reported to have trophic properties *in vitro* [94,95]. There are no reports suggesting that monomeric A β possesses any direct cellular toxicity at physiologically relevant concentrations. Insoluble fibrillar aggregates of A β , vascular amyloid and A β plaques, exhibit relatively low *in vitro* toxicity and have been proposed to be an *in vivo* mechanism for removal of the more toxic soluble A β species [83,96,97]. It was first suggested in 1995 that soluble A β species rather than fibrillar plaques could trigger neurotoxicity leading to AD [98], and in the subsequent decades, many studies have shown soluble A β oligomers to be the most toxic A β form, causing both acute synaptotoxicity and inducing neurodegenerative processes [5-10,99-102].

Low-picomolar levels of soluble A β oligomers have been reported to have trophic properties *in vitro* [103-105], suggesting that therapeutic targeting of soluble A β oligomers may need to modulate oligomer levels versus completely sequestering or preventing formation of soluble A β oligomers. However, the concentration of A β 42 at which enhancement of long-term potentiation (LTP) was observed in these studies was one to two orders of magnitude greater than levels of soluble A β oligomers reported in the cerebrospinal fluid (CSF) of human patients with AD, and the concentration above which inhibition of LTP was observed was an order of magnitude greater than the total levels of soluble A β species reported in the CSF of human patients with AD [65]. Thus, the relevance of the reported *in vitro* trophic properties of soluble A β oligomers to *in vivo* conditions remains to be established.

Soluble A β oligomers bind with high affinity to synapses on a subset of hippocampal and cortical neurons [19,40,106-108], indicative of specific binding to discrete cell surface receptors. In rodent hippocampal slice preparations, synaptic binding leads to rapid inhibition of LTP [19,40,109], and injection of various soluble A β oligomer preparations directly into the rodent brain leads to reversible impairment of cognitive function [31,33,110]. This aberrant signaling also causes accumulated biochemical damage within neurons [100,111,112], such as hyperphosphorylation of tau [100,111-113], suggesting a linkage between A β and tau pathologies [114-116]. Soluble A β oligomers have been isolated from extracts of postmortem AD brain tissue and from transgenic AD animal models [37,52,117-119] and have been reported to be elevated in human AD brain relative to non-demented older patients [59,61,62,65,119-124]. Importantly, a recent study suggests a correlation between CSF levels of soluble A β oligomers and cognitive deficits in human patients with AD [61,65]. These findings support the view that soluble A β oligomers interfere acutely

with normal synaptic functions and contribute significantly to the memory loss and cognitive dysfunction characteristic of AD.

Structure and activity of soluble amyloid-beta oligomer species

There is substantial ongoing debate and research concerning the structure and activity of soluble A β species [5,7-9,33,41,52,72,99,125-127]. Various soluble A β oligomer species have been reported to display synaptic toxicity or induce cognitive deficits, including dimers [33,35,128,129], trimers [32], dodecamers [33,37,40], and larger soluble A β oligomers with molecular weights of 90 to 650 kDa (20 to 150 mers) [19,130,131]. Unfortunately, the different methodologies for the preparation, characterization, and evaluation of soluble A β oligomer species by various research groups impede a direct comparison of the results reported, and few studies have directly compared the toxicities of different soluble A β species while using the same techniques.

Two studies have reported the comparative toxicities of different soluble A β oligomer species by using LDH (lactate dehydrogenase release) and MTT (oxidoreductase activity) cell viability assays. Deshpande and colleagues [45] examined the relative toxicities of purified spherical A β (1-42) oligomers [50], ADDLs [132], and fibrils [50]. However, because solutions of soluble A β oligomers in the neurobasal medium used in this study have been shown to change with time [19], it is not possible to draw definitive structure-activity conclusions from the results of this study. In the second study, Ono and colleagues [133] reported relative toxicities of purified, cross-linked A β (1-40) dimers, trimers, and tetramers, which were shown to be relatively stable under the assay conditions. In an MTT assay, half maximal effective concentration values were 67.3, 41.6, 24.5, 20.5, and 57.6 μ M, respectively, for monomer, dimer, trimer, tetramer, and fibrils. Comparable toxicity was obtained in an LDH assay. The micromolar concentrations of A β species used in this study were approximately six orders of magnitude greater than *in vivo* A β concentrations, and the relevance of cell culture MTT-type assays to the *in vivo* synaptotoxicity of A β species has been questioned [134]. Therefore, the results of the study by Ono and colleagues provide little understanding of the relative *in vivo* toxicities of soluble A β species.

More recently, cognitive effects *in vivo* were assessed in rats by using the alternating lever cyclic ratio assay following intracerebroventricular (ICV) injections of cell- and synthetically derived soluble A β oligomers [33]. Monomer and low-n-mer soluble A β oligomers derived from 7PA2 cells [29,135-137], trimer and a dodecameric species (A β *56) extracted from Tg2576 mouse brain [37], and synthetic soluble A β oligomers (ADDLs) [43]

were compared in this study. Injection of conditioned media from 7PA2 cells caused significant cognitive deficits. Evaluation of size exclusion chromatography (SEC)-enriched dimer or trimer fractions of 7PA2 cell-conditioned media showed significant cognitive deficits following injection of dimer-enriched fractions but a non-significant effect upon injection of trimer-enriched fractions. SEC-purified monomer had no effect. A β monomer was the predominant A β species in unfractionated 7PA2 conditioned media, and the amounts of dimer and trimer injected in the dimer- and trimer-enriched fractions were considerably greater than the amounts injected in unfractionated conditioned media. However, cognitive effects following injection of unfractionated conditioned media were comparable to or exceeded the observed effects following treatment with dimer- and trimer-enriched fractions. Thus, there is an inherent ambiguity regarding the results reported for 7PA2-derived A β species that is difficult to explain. One possible explanation is that a higher-order soluble A β oligomer species contributes to the cognitive deficits observed upon injection of unfractionated 7PA2 conditioned media and is a more potent inhibitor of cognitive function than A β dimer or trimer species or both. Another possible explanation is that combinations of different soluble A β oligomer species, perhaps interacting differently with different neuronal receptors, have an additive or synergistic toxicological effect. Trimers extracted from aged Tg2576 mouse brain also failed to elicit significant cognitive deficits. However, consistent with other *in vivo* efficacy studies [37], a dodecameric soluble A β oligomer extracted from aged Tg2576 mouse brain (A β *56) caused significant cognitive deficits. Synthetic soluble A β oligomers (ADDLs) also caused cognitive deficits following ICV injection. The results of this study show that ICV injection of soluble A β oligomers from different sources causes cognitive deficits in wild-type rats and that these deficits are reversible. The results of the study show that soluble A β oligomer containing conditioned media of 7PA2 cells is more potent than solutions of A β *56 obtained from aged Tg2576 mouse brains or solutions of synthetically prepared soluble A β oligomers (ADDLs). However, because it is not possible to quantitatively characterize the exact nature or distribution of soluble A β species in these different preparations, it is not possible to draw definitive conclusions from this study regarding the structure-activity relationships between individual soluble A β oligomer species.

In a more recent study, Moreth and colleagues [19] prepared, characterized, and evaluated the hippocampal binding and effects on neurotransmission of spheroidal, protofibrillar, and fibrillar A β aggregates. Under the conditions used to test for hippocampal binding and neurotransmission, the different species were shown to be relatively unchanged. Monomeric and fibrillar A β did not bind to mature hippocampal neurons (DIV21) or effect

neurotransmission at concentrations as high as 1 μ M. In contrast, spheroidal and protofibrillar A β aggregates displayed punctate binding to mature hippocampal neurons and impaired neurotransmission with nanomolar potency. Significantly, the mode of impairment of neurotransmission was different for spheroidal A β aggregates, which impaired LTP at 30 nM, compared with protofibrillar aggregates, which impaired basal neurotransmission at 100 nM. Spheroidal A β aggregates had no effect on basal neurotransmission at concentrations as high as 100 nM. Although this study was unable to address the comparative neurotoxicity of discrete soluble A β oligomers, it did show that different forms of soluble A β oligomers can trigger distinct neuronal activities.

At this point, the exact structures of the toxicologically relevant soluble A β oligomer species have not been determined to the complete exclusion of other possible structures, and analytical tools do not exist to characterize the A β oligomers that form at concentrations in the AD brain [44,138]. The numerous studies reporting neuronal toxicity for different soluble A β oligomers support the conclusion that multiple soluble A β oligomer species exhibit neuronal toxicity, rather than a single, discrete toxic species. This suggestion of multiple toxic soluble A β oligomer species may also explain the plethora of reported neuronal receptors that mediate the effects of soluble A β oligomers (Table 1) [139-165], a discussion of which is well beyond the scope of this review. (See the perspective of Benilova and De Strooper [166] for a good introduction to this complex and controversial field of study.)

Despite recognition that soluble A β oligomers are key structures causing AD memory malfunction and cognitive deficits, drug discovery efforts targeting these species have been hampered by perceived technical difficulties of generating physiologically relevant preparations of synthetic soluble A β oligomers and by differing terminologies and methodologies used by various researchers [167]. However, well-characterized and documented preparations of synthetic soluble A β oligomers have been reported by numerous researchers [19,40,43,95,100,107,108,111,168-170] that generate soluble A β oligomers with little or no detectable fibrillar A β species. With the availability of a number of well-documented preparations of different soluble A β oligomer species and tools for comparative characterization, it is hoped that additional side-by-side testing of various soluble A β oligomer preparations in different toxicity paradigms such as reversal of basal neurotransmission, LTP inhibition, changes in AMPA receptor trafficking, tau phosphorylation, and loss of dendritic spines [19,108,109,170] will be conducted and reported.

Amyloid-beta immunotherapies in development

Successful immunotherapies developed for most diseases rely upon antibodies that possess high selectivity for a

Table 1 Reported receptors that bind or mediate the toxicity of soluble amyloid-beta oligomers

Reported receptor		
Amylin receptor [139]	NMDA receptor [140-144]	Sortilin [145]
P53-Bax cell death pathway [146]	cAMP/PKA/CREB-signaling pathway [147,148]	Tau protein kinase 1/glycogen synthase kinase-3 β [51]
c-Jun N-terminal kinase [149]	α -synuclein [150]	P/Q-type calcium currents [49]
Cyclin-dependent kinase 5 [149]	EphB2 [151]	Dynamin and RhoA [152]
mGluR, mGluR5, mGluR2 [108,142,149,153,154]	TNF α [155]	Cofilin [140]
p38 mitogen-activated protein kinase [149]	PrP ^C [154,156-159]	Calcineurin [160-162]
α 7-nicotinic receptors [105]	AMPA [160]	Brain-derived neurotrophic factor [131]
Human LirB2 [163]	Insulin receptor [164]	Sigma 2 receptor [165]
ST11 [156]		

NMDA, N-methyl-D-aspartate; TNF α , tumor necrosis factor alpha.

particular target antigen connected to the disease. However, all A β -directed immunotherapies currently in clinical development are based on non-selective antibodies that bind multiple A β species. Treatment with non-selective anti-A β antibodies that bind monomeric or oligomeric A β (or both) potentially could succeed in a prodromal treatment paradigm by reducing total brain A β to levels below the toxicological threshold. However, given the high concentrations of monomeric and fibrillar A β compared with soluble A β oligomers in the AD brain [57-65] and the low levels of antibodies that penetrate the brain from the periphery [171], it will be very challenging for non-selective anti-A β antibodies that bind monomeric or fibrillar A β (or both) to show efficacy when administered to patients with mild cognitive impairment or mild-to-moderate AD. In contrast, the acute synaptic toxicity and very low brain levels of soluble A β oligomers suggest that anti-A β antibodies with selective affinity for soluble A β oligomers could provide therapeutic benefit for patients with mild cognitive impairment or mild-to-moderate AD.

It might seem difficult to generate a monoclonal antibody that does not bind monomeric or fibrillar A β yet possesses high affinity for structurally heterogeneous soluble A β oligomers. However, monomeric, oligomeric, and fibrillar A β species have been reported to have distinct conformational characteristics [172-175]. Moreover, antibodies with selective affinity for soluble A β oligomers versus monomeric and fibrillar A β have been reported [9,176,177]. These several examples of antibodies suggest that therapeutically relevant soluble A β oligomer-directed antibodies are indeed feasible.

A number of recent publications have reviewed A β immunotherapies currently in clinical trials, with an emphasis on the efficacy of the drugs for treating AD [178-187], and the clinical results will not be presented in this review, with the exception of an analysis of the brain levels of solanezumab and bapineuzumab in recently reported clinical trials. Instead, this review will

focus on a discussion of the comparative affinities of the A β antibodies in clinical development for monomeric, soluble oligomeric, and fibrillar A β species. Notably, none of the A β immunotherapies currently in clinical development selectively targets soluble A β oligomers, as discussed below and summarized in Table 2.

Solanezumab

Solanezumab is unique among A β antibodies currently in clinical development because it does not bind fibrillar A β . Solanezumab is a humanized, IgG1 monoclonal antibody derived from the murine monoclonal antibody, 266. 266 was selected for its high-affinity binding to soluble A β , although the exact nature of the soluble A β it binds was not reported. It has been suggested that 266 is a conformation-specific antibody that solely recognizes soluble A β and readily binds monomeric A β [188-190], without binding APP or the C-terminal APP cleavage product of α -secretase [189]. It has been reported that 266 selectively sequesters A β monomer and dimer in the periphery of 3-month-old APP transgenic mice [190], and definitive evidence for 266 binding of monomeric A β has been reported [191]. A study using crossed-linked soluble A β oligomers showed 266 could immunoprecipitate A β monomer and low-molecular-weight dimer, trimer, and possibly tetramer [192]. A study involving a competitive ELISA showed that 266 had a seven-fold higher affinity for monomeric A β than synthetic soluble A β oligomers [193]. Several studies have shown that 266 does not bind A β plaques or vascular amyloid [188,190,194]. Thus, according to the available data, solanezumab and 266 bind monomeric and lower-molecular-weight soluble A β oligomer species, with a preference for monomeric A β , but do not bind fibrillar A β species.

Clinical trial pharmacokinetic data for brain levels of solanezumab have not been reported. However, target engagement data reported for the phase 2, multiple ascending-dose clinical trials (Table 3) [183] provide a possible understanding of the lack of efficacy of solanezumab in clinical

Table 2 Comparison of binding affinities of amyloid-beta (Aβ) immunotherapies in development for Aβ monomer, soluble Aβ oligomers, and fibrillar Aβ

Aβ antibody	Binding affinity ^a		
	Aβ monomer	Aβ oligomers	Aβ plaque
Solanezumab/266	+++	++	–
Bapineuzumab/3D6	++	+++	+++
Crenezumab	++	+++	++
Ponezumab/D-2H6	++	++	+++
BiiB037/Ni-101.11	+	+++	+++
BAN2401/mAb158	–	+++	+++
Gantenerumab	+	++	+++
SAR228810/13C3	–	+++	+++

^aBased on published patent applications or peer-reviewed scientific publications. +, low affinity binding; ++, moderate affinity binding; +++, high affinity binding; –, very low affinity or no binding.

trials [186]. The data for the 400 mg/month and 400 mg/week dose levels show approximately a 2.7-fold increase in solanezumab bound Aβ at the higher dose level (5,858 versus 15,825 pg/mL total Aβ40 and Aβ42) [183], showing that there was incomplete target engagement at the 400 mg/month dose level used in the pivotal phase 3 trials. Solanezumab binds Aβ monomer with higher affinity than soluble Aβ oligomers and does not bind or disaggregate fibrillar Aβ. Because incomplete target engagement occurred at the dose level used in the phase 3 trials, it is unlikely any solanezumab was available to bind soluble Aβ oligomers; thus, the lack of efficacy of solanezumab at the dose levels used in the phase 3 clinical trials is not unexpected.

Bapineuzumab

Bapineuzumab is a humanized, IgG1 monoclonal antibody derived from the murine antibody, 3D6. Bapineuzumab and 3D6 are non-selective Aβ antibodies that bind with high affinity to monomeric Aβ [192], soluble Aβ oligomers [170], and fibrillar Aβ [195-197] but do not recognize APP or the product of β-secretase

Table 3 Mean cerebrospinal fluid levels of bound and unbound amyloid-beta (Aβ)40 and Aβ42 in Alzheimer's disease patients treated with solanezumab

Solanezumab dose ^a	Mean CSF Aβ40, pg/mL ^b	Mean CSF Aβ42, pg/mL ^b
100 mg Q4W	625	0.0
100 mg QW	3,750	275
400 mg Q4W	5,625	233
400 mg QW	15,000	825

^aSolanezumab phase 2 MAD data [183]. ^bMean pg/mL levels of total bound and unbound Aβ40 and Aβ42 in cerebrospinal fluid (CSF) of treated patients, derived from Figures 3A and 3B reported by Farlow and colleagues [183]. Q4W, monthly dosing; QW, weekly dosing.

cleavage of APP [198]. In a competitive ELISA assay study, 3D6 was found to bind monomeric Aβ and synthetic soluble Aβ oligomers with similar affinity [193]. Bapineuzumab/3D6 bind vascular amyloid and Aβ plaques in hAPP transgenic mice brains and human AD brains and clear vascular amyloid and Aβ plaques in hAPP transgenic mice brains [188,197,199-201]. Thus, bapineuzumab and 3D6 do not distinguish between the various Aβ species in the AD brain.

Mean CSF levels of bapineuzumab reported in the phase 2, multiple ascending-dose pharmacokinetic clinical studies were 4.9, 18.1, 27.2, and 44.7 ng/mL, respectively, at dose levels of 0.15, 0.5, 1.0, and 2 mg/kg (six infusions, 13 weeks apart) [202], which upon conversion to molar concentrations are 0.03, 0.12, 0.18, and 0.3 pmol/mL, respectively, based on a bapineuzumab molecular weight of 150 kDa. In the same studies, placebo-treated mean CSF levels of Aβ(1-42), Aβ(x-42), and Aβ(1-40) were 376.7, 537.9, and 6,705.4 pg/mL, respectively, which upon conversion to molar concentrations are 0.08, 0.12, and 1.55 pmol/mL, respectively (assuming the molecular weight of Aβ(x-42) is the same as that of Aβ(1-40)). The highest bapineuzumab dose level in phase 3 pivotal trials was 1 mg/kg (six infusions, once every 13 weeks) [187]. Thus, the total mean Aβ CSF levels were approximately an order of magnitude greater than mean levels of bapineuzumab. Because bapineuzumab binds monomeric Aβ with similar affinity to soluble Aβ oligomers and fibrillar Aβ and because Aβ soluble oligomer levels are orders of magnitude less than Aβ monomer and fibrils levels in the AD brain [58-65], it is clear that bapineuzumab was not dosed high enough in the pivotal phase 3 trials to effectively sequester soluble Aβ oligomers. Thus, the absence of efficacy of bapineuzumab in clinical trials is not unexpected.

Crenezumab

Crenezumab (MABT5102A or MABT) is a humanized, IgG4 monoclonal anti-Aβ antibody that was engineered to reduce Fcγ receptor-mediated microglia activation and minimize adverse effects due to vasogenic edema and cerebral microhemorrhage [203,204]. Crenezumab binds Aβ monomer, soluble Aβ oligomers, and fibrillar Aβ with similar affinities and binds plaques in hAPP transgenic mice and human AD brain tissues [203,204]. Similar binding of crenezumab to human Aβ(1-40) and Aβ(1-42) peptides and mouse/rat Aβ(1-42) has also been reported [204]. These results indicate that crenezumab does not possess selectivity for different Aβ species [204]. Consistent with a lack of selectivity, crenezumab prevents Aβ aggregation and disaggregates pre-aggregated Aβ species. Thus, crenezumab is similar to bapineuzumab with respect to non-selective binding of monomeric, oligomeric, and fibrillar Aβ species.

Ponezumab

Ponezumab is a humanized, modified IgG2 monoclonal antibody derived from the murine monoclonal antibody, 2H6. This progenitor murine antibody binds to monomeric A β (1-40) with high affinity but does not bind to A β (1-16), A β (1-28), A β (1-38), A β (1-42), or A β (1-43) [205]. Intracranial injection of 2H6 and its deglycosylated form into plaque-rich, 20-month-old Tg2576 mice followed by histological analysis of total A β load in the hippocampus and frontal cortex showed significant plaque reduction in both regions compared with control tissues [206], suggesting that these antibodies bind and disaggregate fibrillar amyloid. More recent studies show that ponezumab labels amyloid plaques in 14-month-old Tg2576 mouse and human AD brains [207], demonstrating binding to fibrillar A β . Binding of ponezumab to Tg2576 mouse brain plaque was comparable to the known high-affinity binding of the N-terminal A β antibody 6E10. Ponezumab was also reported to bind monomeric, oligomeric, and fibrillar forms of A β (1-40) but did not show appreciable binding to monomeric, oligomeric, or fibrillar forms of A β (1-42) [207]. Thus, although ponezumab displays selectivity for A β (1-40) versus other A β peptide isoforms, it is relatively non-selective for monomeric, soluble oligomeric, or fibrillar A β species.

BiiB037

BiiB037 is a human IgG1 monoclonal antibody derived from a patient with AD by using reverse translational medicine methodology [208]. *In vitro* BiiB037 binds fibrillar A β (1-42) with high affinity but does not bind soluble A β (1-40) [209]. Although explicit details of the binding characteristics of BiiB037 have not been publicly disclosed, results reported by Dunstan and colleagues [209] and data in the patent covering BiiB037 [208] suggest that BiiB037 is very similar, or identical, to antibody NI-101.11, which binds with high affinity to A β plaques in human AD brain tissue samples [208]. Such binding is not blocked by monomeric A β (1-16) or A β (1-42), showing selective affinity for fibrillar A β versus A β monomers [208]. Comparison of binding affinity in an A β plate-based ELISA showed greater than 100-fold-higher affinity for fibrillar A β (1-42) than for monomeric A β (1-42) [208]. SEC studies showed that NI-101.11 bound to fluorescein isothiocyanate-labeled A β (1-42) in the early-eluting peak (non-retained, large A β species) but that little binding was associated with the retained, low-molecular-weight A β peak [208]. Many researchers have deployed SEC to separate higher-molecular-weight soluble A β oligomer species from low-molecular-weight A β species [20,44,130,155,210-212], and in all cases the higher-molecular-weight, early-eluting peak contains soluble A β oligomers or A β protofibrils or both. Together

these results show that NI-101.11 binds soluble A β oligomers and protofibrils in addition to fibrillar A β . Consistent with this conclusion is the finding that NI-101.11 inhibits the formation of A β fibrils *in vitro* [208]. Thus, according to the available data, BiiB037/NI-101.11 binds soluble A β oligomers and fibrillar A β with high affinity and binds monomeric A β with low affinity.

BAN2401

BAN2401 is a humanized, IgG2a monoclonal antibody derived from the murine precursor mAb158, which was raised against A β protofibrils generated from A β (1-42)-E22G, the arctic mutant of the A β peptide [210]. Although mAb158 is claimed to be a 'highly protofibril-selective monoclonal antibody' [213], published data show that it binds fibrillar A β with a high affinity similar to that of protofibrils in a dot-blot assay [210,214] and binds fibrillar deposits in transgenic APP-ArcSwe mice brain (see Figure S2 in [213]). In a more recent study, mAb158 was shown to bind soluble A β oligomer fractions in the molecular weight range of 80 to 2,000 kDa under conditions in which protofibrillar species were not detected [119]. In this study, mAb158 showed essentially no binding to low-molecular-weight A β oligomeric species in the molecular weight range of 5 to 80 kDa. A lack of binding to low-molecular-weight A β species - monomeric to tetrameric A β (1-40), A β (1-16), and A β (17-40) - was found in an earlier study [213]. Thus, mAb158 binds higher-molecular-weight A β aggregates, including soluble A β oligomers, protofibrils, and fibrillar A β . Consistent with the binding of mAb158 to fibrillar A β , intracranial injection of mAb158 in a transgenic APP-Swe mouse model was reported to cause a significant reduction in plaque burden by 72 hours after injection [214]. In a subsequent study in a transgenic APP-ArcSwe mouse model [213], 4 months of weekly intraperitoneal injections of mAb158 to 9- to 10-month-old mice with modest plaque burden resulted in a significant reduction of A β protofibrils (74%) but no significant reduction in total soluble or insoluble A β levels in the cerebral cortex or hippocampus as determined by immunohistochemical analysis. The differences in effects of mAb158 on existing plaque burden in these two studies may be due to different administration routes (direct intracranial versus peripheral injections). As noted by Lord and colleagues [213], it may also be attributable to the APP-ArcSwe mouse model used in the more recent study, a model that exhibits more highly insoluble dense-core plaques, preventable only by early and continuous A β immunotherapy. Thus, mAb158/BAN2401 binds higher-molecular-weight soluble A β oligomers (referred to as protofibrils in the mAb158 literature) and fibrillar A β with high affinity and has low affinity for binding monomeric A β and lower-molecular-weight soluble A β oligomers.

Gantenerumab

Gantenerumab is a humanized monoclonal antibody that was optimized to have high-affinity binding of fibrillar A β and shows high-affinity binding to diffuse and dense-core plaques in human AD brain and APP transgenic mouse brain tissues [215]. Gantenerumab also binds soluble A β oligomers with high affinity and, to a lesser extent, A β monomer. The reported binding constants (Kds) for gantenerumab binding to fibrillar A β , soluble A β oligomers, and A β monomer are 0.6, 1.2, and 17 nM, respectively [215]. The dissociation constants (kds) for gantenerumab-A β complexes were reported to be 2.8×10^{-4} , 4.9×10^{-4} , and 1.2×10^{-2} , respectively, for fibrillar A β , A β oligomers, and A β monomer [215], suggesting a more rapid exchange of antibody-monomer complex compared with antibody-fibril or oligomer complexes. The selective affinity of gantenerumab for fibrillar A β and soluble A β oligomers suggests that it may be similar to BiiB013/N1-101.11 and BAN2401/mAb158. Consistent with its high-affinity binding to fibrillar A β , gantenerumab caused a significant, dose-dependent reduction in amyloid plaques in human AD brain tissue [215]. Immunohistochemical analysis of PS2APP transgenic mice brain tissue 3 days after intravenous injection of gantenerumab showed dose-dependent binding to amyloid plaques. Significantly, pharmacokinetic analysis in PS2APP transgenic mice following a single intravenous bolus dose showed sustained binding of gantenerumab to plaques in the brain for up to 9 weeks post-dosing, even though there was no detectable antibody in the plasma beyond 3 weeks post-dosing [215]. In a chronic treatment study in 5- to 6-month-old PS2APP transgenic mice, gantenerumab caused a significant reduction in pre-existing small plaques; however, there was an increase in larger plaques [215]. These results suggest that gantenerumab causes a redistribution of A β from small plaques to large plaques, possibly via disaggregation of smaller, less mature plaques. Gantenerumab-mediated disaggregation of existing plaques is consistent with the observations of vasogenic edema and microhemorrhage in human patients with AD treated with gantenerumab in clinical trials [216]. Thus, gantenerumab non-selectively binds soluble A β oligomers and fibrillar A β with similar high affinity in comparison with its lower-affinity binding of monomeric A β .

SAR228810

SAR228810 is a humanized, monoclonal antibody derived from the murine monoclonal antibody 13C3, which was raised to A β protofibrils derived from A β (1-42) [217,218]. The humanized antibody was engineered into an IgG4 framework to reduce binding to Fc γ and C1q receptors and decrease risks of amyloid-related imaging abnormalities [219]. Although specific binding characteristics of SAR228810 have not been publicly disclosed, results reported by Pradier and colleagues [219] and data in the

patent application claiming humanized variants of 13C3 [220] suggest that SAR228810 is very similar, or identical, to antibody LP09027 [220]. Antibody 13C3 and its humanized variants are reported to bind with higher affinity to SEC-separated protofibrillar A β (1-42) than to low-molecular-weight A β (1-42) [217,218,220]. As noted previously, many researchers have used SEC to separate higher-molecular-weight soluble A β oligomer species from low-molecular-weight A β species [20,44,130,155,210-212], and in all cases the higher-molecular-weight, early-eluting peak contains soluble A β oligomers or A β protofibrils or both. Moreover, as reported by Hepler and colleagues [44], the lower-molecular-weight, late-eluting peak contains predominately monomeric A β . Thus, these data show that 13C3 and its humanized variants selectively bind A β oligomers or protofibrils (or both) compared with monomeric A β . In competitive ELISA experiments, monomeric A β (1-28), A β (1-16), or A β (25-35) does not prevent binding of humanized 13C3 to plates coated with A β (1-42) [220]. Ravetch and Fukuyama [218] reported that 13C3 binds only to the higher-molecular-weight A β species contained in the supernatants from 7PA2 cells, in contrast to the non-selective A β antibody 4G8 that binds low-, medium-, and higher-molecular-weight A β species. Collectively, these data show the selectivity of 13C3 for higher-molecular-weight A β oligomers versus lower-molecular-weight A β oligomers and monomer. Antibody 13C3 was shown to bind fibrillar A β (1-42) and label A β deposits in human AD brain tissue samples [218]. Antibody LP09027 was shown to bind fibrillar A β *in vitro* and to label AB deposits in brain sections from APP-PS1 mice [220]. Thus, the reported data suggest that 13C3/SAR228810 is very similar to mAb158/BAN2401 and binds higher-molecular-weight soluble A β oligomers (referred to as protofibrils in the 13C3/SAR228810 literature) and fibrillar A β with high affinity and has low affinity for binding monomeric A β and lower-molecular-weight soluble A β oligomers.

Conclusions

The lack of efficacy observed for A β immunotherapies in clinical development is not unexpected given their lack of selectivity for soluble A β oligomers compared with monomeric or fibrillar A β and given the tremendous quantities of monomeric or fibrillar A β in the AD brain relative to soluble A β oligomers. A β antibodies optimized to bind soluble A β oligomers selectively are much more likely to succeed in AD clinical trials and should be aggressively pursued.

The prediction that immunotherapies targeting soluble A β oligomers will elicit clinical benefit is supported by studies of human A β autoantibodies, of which only a subset appears to be disease-protective (in particular, the

subset that preferentially recognizes A β oligomers) [221-223]. Thus, immunotherapeutics with high selectivity for soluble A β oligomers, which resemble these protective auto-antibodies, are expected to deliver a clinical advantage compared with the non-selective immunotherapies in clinical development.

Studies have demonstrated that antibodies with selective affinity for soluble A β oligomers can block soluble A β oligomer-mediated synaptotoxicity in cell cultures [108,224] and rapidly normalize memory deficits in transgenic AD mouse models [176]. These findings support the concept that antibodies with selective affinity for soluble A β oligomers may be a more effective therapeutic strategy than antibodies with high affinity for monomeric or fibrillar A β or both.

Abbreviations

AD: Alzheimer's disease; ADDL: Amyloid-derived diffusible ligand; APP: Amyloid precursor protein; A β : Amyloid-beta; CSF: Cerebrospinal fluid; ELISA: Enzyme-linked immunosorbent assay; ICV: Intracerebroventricular; LDH: Lactate dehydrogenase release; LTP: Long-term potentiation; SDS: Sodium dodecyl sulfate; SEC: Size exclusion chromatography; TBS: Tris-buffered saline.

Competing interests

All authors are employees or paid consultants of Acumen Pharmaceuticals, Inc. and own stock or stock options of this company.

Authors' contributions

All authors read and approved the final manuscript.

Published: 09 Jul 2014

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10.1186/alzrt22

Cite this article as: Goure et al.: Targeting the proper amyloid-beta neuronal toxins: a path forward for Alzheimer's disease immunotherapeutics. *Alzheimer's Research & Therapy* 2014, **6**:42