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Advances in the therapy of Alzheimer's disease: targeting amyloid beta and tau and perspectives for the future

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Worldwide multidisciplinary translational research has led to a growing knowledge of the genetics and molecular pathogenesis of Alzheimer's disease (AD) indicating that pathophysiological brain alterations occur decades before clinical signs and symptoms of cognitive decline can be diagnosed. Consequently, therapeutic concepts and targets have been increasingly focused on early-stage illness before the onset of dementia; and distinct classes of compounds are now being tested in clinical trials. At present, there is a growing consensus that therapeutic progress in AD delaying disease progression would significantly decrease the expanding global burden. The evolving hypothesis- and evidence-based generation of new diagnostic research criteria for early-stage AD has positively impacted the development of clinical trial designs and the characterization of earlier and more specific target populations for trials in prodromal as well as in pre- and asymptomatic at-risk stages of AD.

Keywords: Alzheimer's disease • amyloid beta immunotherapy • bapineuzumab • biological markers • clinical trials • prevention • randomized controlled trials • systems biology • solanezumab • tau immunotherapy

The pathogenesis of Alzheimer's disease (AD) is extremely intricate, given that it covers both genetic and environmental factors. Emerging evidence indicates that a spectrum of genetic factors plays a central role in the expression of AD in its late-onset (LOAD) or sporadic (sAD) forms as well as in the expression of the early-onset (EOAD) or familial (fAD) type of AD [1]. All these various genetic defects, including either mutational or susceptibility (risk) factors, have been associated with AD development and advance. Dominant mutations in the genes related to AD predict the further occurrence of AD in subjects carrying them because of their full penetrance. In contrast, susceptibility genes do not reliably cause the pathology but contribute to grow the subject's predisposition to developing AD. The genetics of AD supports a dichotomous model primarily based on the existence of early-onset and late-onset variants (EOAD and LOAD, respectively) and focused on the age of the affected individuals at symptoms first appearance (cutoff age is 65) [2] in line with the inaugural description of the disease. However, it should be emphasized that this commonly accepted dichotomy scheme between EOAD and LOAD is oversimplistic, as there are cases of EOAD without any evidence for Mendelian transmission or familial clustering; conversely, these clustering and transmission patterns are frequently reported in LOAD [1]. Notably, besides these genetic bases, non-genetic factors – for instance, environmental or epigenetic influences – are likely to significantly affect an individual's risk to develop AD [3]. The distinct endophenotypes of LOAD are the reflection of pathophysiological alterations in cascades of molecular signaling pathways that are due to the convergence of complex gene–gene interactions along with modulating influences of lifestyle, education and the environment.

The *APOE* gene has been unquestionably recognized as the major genetic risk factor available for LOAD [4]. Individuals carrying the *ε4* allele are at increased risk of AD versus those carrying the *ε3* allele. In contrast, the *ε2* allele decreases the risk [5,6]. Although the mechanisms

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accounting for the pathogenic nature of apoE in AD have not been fully elucidated, emerging data highlight that apoE contributes to AD pathogenesis not only via Aβ-dependent, but also via Aβ-independent pathways (see Yu *et al.* [4] for a detailed review of Aβ-independent roles for apoE in AD pathogenesis).

In general, EOAD patients have a more aggressive course and shorter relative survival time. They are characterized by a faster cognitive deterioration where the memory complaints could be secondary to language impairment or other neocortical function. The pathology in EOAD may be more severe with prominent synaptic fallout and neuronal loss, more severe perfusion and metabolic defects. Despite evidence suggesting differences between EOAD and LOAD, current data are inadequate as they examined isolated clinical features or cognitive functions. As the disease burden of AD is increasing with aging society, in the future it will be important to understand the etiology and characteristics of EOAD and LOAD with sufficient sample size and strong epidemiological design.

Since transgenic animal, genetic and biomarker data stress the key role of early amyloid beta (Aβ) overproduction and/or reduced clearance mechanisms, hypothesis-based Aβ-targeted pharmacological approaches are anticipated to demonstrate non-negligible clinical benefit in both fAD and sAD, more so if initiated as early as possible in the cellular and molecular disease phase (i.e., secondary prevention). Intriguingly, structures of human brain-derived Aβ fibrils, obtained by fibril seeds grown from brain extracts, have recently shown that fibrils in the brain may spread from a single nucleation site and that structural variations may correlate with variations in AD [7]. This could have a huge impact on the development of structure-specific anti-Aβ pharmacological agents. Furthermore, it would be important to know whether similar fibril structures can be generated from brain tissues and cerebrospinal fluid (CSF).

Since clinical trials with several classes of drugs have been initiated during the latest and most advanced stage in the complex disease process, no drugs for the treatment of prodromal (mild cognitive impairment [MCI]) and for the prevention of AD during preclinical and presymptomatic stages have been approved so far. With reference to the treatment of AD, no other drugs have been approved for marketing authorization since memantine in the EU and in the USA, in 2002 and 2003, respectively [8]. This has been termed as 'the lost decade' of AD therapy research. Moreover, the limited 'symptomatic' efficacy, as well as the existence of adverse effects characterizing the available drugs has led to controversy about the practicality and utility of current treatment options [8].

The aim of the present perspective is to recapitulate and critically discuss the most recent development in the area of AD drug development and the results of the clinical therapy and prevention trials and provide future directions for this important field.

Recent insights into the dementia epidemic

It should be noted that, even though demographics predict to show a catastrophic growth in the number of worldwide dementia cases, recent reports, based on population community studies or survey data, indicate an age-specific reduction of the prevalence and/or a decrease of incidence rates among individuals born later in the first half of the 20th century [9]. In particular, Manton *et al.* documented that a decay in dementia prevalence occurred during the period 1982–1999 in the US elderly population [10]. After that, Langa *et al.* reported one of the first studies suggesting a decline in the US dementia rates, using information from the US Health and Retirement Study. They found that the decline in the incidence rate tracked with education, income and improvements in healthcare, lifestyle and social factors [11]. Intriguingly, since then, this trend has been confirmed by a number of studies in Europe. In particular, one analysis has examined whether dementia incidence has changed over the last two decades by comparing dementia incidence in two independent sub-cohorts of individuals from the Rotterdam Study [12]; a Swedish study has investigated whether prevalence, survival and incidence of dementia have changed from the late 1980s to the early 2000s in central Stockholm [13]; most recently, an English study has explored whether the prevalence of dementia has been subject to alterations in the past two decades by comparing two surveys of people aged 65 years or older, named the Cognitive Function and Ageing Study I and II [14].

The inspection of these five recent studies has significantly highlighted a decline in the prevalence of dementia, relating such an encouraging and promising trend to overall enhancements in education levels, healthcare – including better prevention and treatment of key cardiovascular risk factors – lifestyle and maintaining social activities. Growing evidence indicates that early identification of AD vascular risk factors and patient follow-up to assess modifiable risk factors (e.g., obesity, insulin resistance, diabetes, deficiency of ω-3 polyunsaturated fatty acids in the diet, sedentary lifestyle and social isolation) may be a pillar of prevention. Indeed, a healthy diet coupled with physical and mental activity is increasingly applied as part of interventions in AD prevention trials [15]. As a result, research focused on preventing late-life dementias should develop ways of decreasing risk factors at both the societal and the personal levels [9]. It should be noted that a decrease in the prevalence of dementia does not necessarily mean a decrease in the prevalence of AD.

Overview on the pharmacological treatments targeting Aβ & tau protein

At present, accessible pharmacological interventions for AD consist mainly of cholinesterase inhibitors – namely, donepezil, galantamine, rivastigmine – and an *N*-methyl-D-aspartate receptor uncompetitive antagonist, that is, memantine. These drugs are able to alleviate some of the psychological and behavioral symptoms in AD patients; however, effective pharmacological therapies for AD prevention and treatment, that is, disease-modifying therapies, are still missing. In the last decade, progress in understanding the genetics and molecular pathogenesis of AD have been translated into several

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experimental approaches aimed at slowing down disease progression [16]. This led to the intensification of clinical trials evaluating various potential AD treatments [17]. Among these, compounds targeting Aβ or tau protein might represent promising therapeutic strategies. However, unfortunately, the current preclinical model systems do not adequately reflect the heterogeneity within human AD nor adequately predict efficacy of novel agents. In particular, therapeutic strategies based on lowering Aβ that have shown promise in animal models have so far failed in clinical trials. This failure of Aβ-lowering agents has caused several researchers to question the amyloid hypothesis itself.

Currently, the amyloid cascade hypothesis is the theory of AD that thus far has received the highest empirical attention postulating that accumulation of Aβ into plaques is the causative pathological event [18]. Based on this assumption, interventions that reduce Aβ load in the brain would be expected to attenuate both the neuropathological changes and functional deficits characterizing AD. Indeed, several different Aβ-lowering strategies have been developed over the past years. The therapeutic potential of clearing Aβ deposition by either active or passive Aβ immunotherapy has been the most extensively validated anti-Aβ approach in preclinical studies. Evidence for the neurotoxic activity of Aβ oligomers [19] provides the basis for the identification of compounds that block Aβ aggregation or decrease Aβ formation. Among these, small-molecule inhibitors of synthetic Aβ fibrillogenesis [20] were able to prevent synaptic plasticity impairment induced by Aβ [21]. However, this effect was obtained only under conditions in which they prevented new oligomer formation [22]. In fact, inhibitors of fibrillogenesis lead to paradoxical enhanced neurotoxicity which may derive from the release of low-n oligomers following disruption of fibril formation. Among the compounds that reached clinical trials, tramiprosate received the greater attention. This molecule has been shown to bind preferentially to soluble Aβ, thus resulting in a dose-dependent reduction in both the soluble and fibrillar amyloid burden in AD mouse model. However, despite encouraging preclinical studies [23], tramiprosate failed to show clinical efficacy in a Phase III trial (Alphase study) in patients with mild-to-moderate AD [24]. Although the negative result was ascribed by the sponsors to unexplained high inter-site variation, weak potency and poor CNS bioavailability [25], subsequent trials were not performed at higher doses. Another Aβ-lowering strategy consists in preventing the formation of Aβ oligomers by enhancing α-secretase activity or inhibiting either β-secretase or γ-secretase activity. Among these, several γ-secretase inhibitors and γ-secretase modulators advanced into clinical studies. However, recent trials of γ-secretase inhibitor and γ-secretase modulator, including semagacestat, begacestat, avagacestat and R-flurbiprofen, have been discontinued for lack of efficacy and/or side effects. The latter might depend on the non-specific cleavage of several proteins, including AβPP, Notch receptor and other substrates by γ-secretase activity [26]. Similarly, also development of β-secretase inhibitors has been hampered by the fact that this enzyme has many substrates. Among the new β-secretase inhibitors under investigation, the small-molecule MK-8931 – a β-amyloid precursor protein (APP) site-cleaving enzyme-1 inhibitor [27] – holds great potential. Merck began a recent Phase II/III trial in patients with mild-to-moderate AD and results are expected in 2018.

The *tau hypothesis* of AD has been the main competitor of the *amyloid hypothesis* [28]. Therefore, targeting abnormal tau hyperphosphorylation or tau aggregation represents the main alternative to the Aβ-lowering approach. Primary kinases involved in the phosphorylation of tau include glycogen synthase kinase (GSK-3) and cyclin-dependent protein kinase 5. Several preclinical studies in different models of AD have demonstrated the efficacy of the two structurally distinct GSK-3 inhibitors, lithium and kenpaullone [29]. Besides GSK-3, the pharmacological blockade of cyclin-dependent protein kinase 5 with either butyrolactone or roscovitine also prevented Aβ-mediated neurotoxicity [30,31]. However, only few tau-directed compounds progressed so far into clinical trials. Among these, valproate has reached Phase III trials with unsatisfactory results because there were no significant effects on cognition and functional measures [32,33]. Also, a small randomized controlled trial (RCT) with lithium in patients with mild AD failed to show any improvement in cognition, or any change in CSF biomarkers [34]. Another compound is the methylthioninium chloride or methylene blue, a widely used histology dye acting also as a tau anti-aggregant [35], although its mechanism of action still remains unclear [36]. This compound has antioxidant properties through mitochondrial modulation; it reduces Aβ oligomerization and binds to the domain responding for tau aggregation [37,38]. A new formulation (leucomethylthioninium), with a higher bioavailability, is currently undergoing two Phase III trials and results are expected in 2015 [39].

The disappointing clinical results obtained so far coupled with the evidence that AD is in nature a complex disorder involving dysregulation of multiple pathways, suggest that conventional drugs directed to a single molecular target are likely inadequate. Thus, the concept of a poly-pharmacological approach using either combinations of multiple drugs or single small molecules modulating multiple targets is nowadays emerging [40]. In addition, AD being a heterogeneous disease, much thought should be put into the design of a trial and, only then, should an enrolment strategy be developed to ensure that the target population is successfully achieved. Specifically, the first duty when considering the methodology to be used in a clinical trial is a careful definition of the characteristics of patient populations (such as age, clinical status, genetics and comorbidities).

Although therapeutic approaches leading to the decrease of Aβ levels have been reported to be encouraging in animal models, they have been disappointing in clinical trials. Consequently, the failure of Aβ-lowering agents has raised doubts on the validity of the amyloid hypothesis. This implicates the

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question of whether AD might best be viewed as the sum of various different mechanisms – some known and probably some that have yet to be disclosed. For instance, AD can be viewed as a proteinopathy; the documentation of the two types of unusual protein deposits, which are now recognized as senile plaques and neurofibrillary tangles, has offered clear disease mechanisms leading from defects in protein processing to the accumulation of abnormal intra- and extracellular deposits, to the disruption of the physiological neuronal activity and, finally, to the atrophy and death of neurons [41]. AD has also been suggested to be associated with a failure of the central cholinergic transmission [42] and it has been subsequently conceptualized as a cholinergic disorder [43]. In this regard, it has been revealed that the loss of cholinergic neurons in the basal forebrain is a consistent finding in AD patients [44] and is associated with the depletion of cholinergic markers in the cerebral cortex [42]. Interestingly, cerebral amyloid angiopathy has been demonstrated to be a major pathological feature of AD, where amyloid spreads and deposits throughout the blood vessel walls in the CNS. These pathogenic events cause a specific clinical presentation profile including cerebral hemorrhage, stroke, ischemic infarctions, cognitive impairment and dementia [45]. While the Aβ peptide is a significant molecule in AD, epidemiological studies have revealed that many well-recognized risk factors for AD – such as smoking, hypertension, atherosclerosis, stroke, microvessel pathology and diabetes mellitus – have a vascular component that decreases cerebral perfusion [46]. Accordingly, the identification of regional-cerebral hypoperfusion can preclinically detect subjects at risk for AD [46]. As a result, perturbations of cerebrovascular system are assumed to be one of the most important contributors to AD pathogenesis. Notably, *APOE ε4* also increases the risk for cerebral amyloid angiopathy and vascular dementia [6]. Since apoE4 is recognized to disrupt the blood–brain barrier integrity and to reduce small cerebral vessels [47], apoE is probably implicated in the maintenance of cognitive function by modulating the function of cerebrovascular systems. Notably, reactive astrocytes and microglia are associated with amyloid plaques in AD. In response to accumulating Aβ peptide species, microglia, the innate immune cells of the brain, generate a toxic inflammatory response that accelerates synaptic and neuronal injury. Inhibiting inflammation by deactivating glial cells might potentially reduce glial-associated inflammation that contributes to neurotoxicity, and, therefore, delay the onset of full-blown dementia [48,49].

Overall, it seems that the most fruitful way to view AD might be as a true amalgamation of all of these mechanisms.

The potential for pharmacological treatment with monoclonal antibodies targeting Aβ

Therapeutic approaches targeting Aβ have been developed with the hope of interfering with the pathogenic steps in the Aβ cascade and modifying the clinical course of the illness [50]. Genetic evidence has significantly highlighted that Aβ can lead the pathogenic process. Of note, although Aβ accumulation in the brain is probably a multifactorial process in sporadic LOAD – contrary to early-onset familial form of AD (EOFAD) – it is conceivable that the application of Aβ-targeted strategies may induce clinical benefit in all forms of AD. However, it is not at all known when during the course of illness an Aβ intervention may be effective, if at all. Based largely on observations that treatments directed toward Aβ targets have not been effective for patients with mild-to-moderate AD, and hence with a substantial amount of Aβ pathology, some expert panels have speculated that such treatment should be started earlier at a *pre-symptomatic stage*, that is, prior to the manifestation of distinct clinical symptoms or clear cognitive impairment. It is assumed that such a clinical stage would correspond to a pathological state with less Aβ burden and prior to manifest synaptic dysfunction, the appearance of large, irreparable cell depletion and the broad spread neurodegeneration [51–53]. Even though at the pre-symptomatic stages, patients are assessed as cognitively normal or with slight cognitive impairment (i.e., stage 3 pre-symptomatic, [52],) they can be identified on the basis of risk and selected biomarkers of AD pathology. It is possible as well that any progression in pathology can be monitored using dynamic molecular and imaging markers as well as tests of cognitive performance [54]. As indicated by regulatory authorities and others, the clinical validity of the preclinical AD stages and prodromal AD as diagnoses remain to be determined [55–58]. In part, this means that for drug development we require diagnoses of not just very high positive predictive values, but very high negative predictive values as well.

Since the *amyloid cascade hypothesis* postulates that Aβ deposition in the brain triggers a cascade of pathogenic mechanisms, the majority of novel strategies have been established to impede Aβ peptide deposition or to eradicate already existing amyloid deposits [59]. To this aim, the most studied targeting strategy is represented by passive *immunotherapy* with antibodies to Aβ [60].

Agents that can prevent Aβ formation and accumulation or stimulate its clearance might ultimately be of therapeutic benefit. Approaches to immunization against Aβ require: *active immunization* that employs full-length Aβ or Aβ analogues together with or without an adjuvant, or *passive immunization*, based on the use of humanized anti-Aβ antibodies or intravenous immunoglobulins [50]. The final outcome is believed to be promoted by anti-Aβ antibodies that either bind to Aβ plaques or other types of brain Aβ aggregates, thus stimulating Aβ clearance by microglia or by binding soluble Aβ in the periphery, inducing, as a result, an outflow of Aβ from the brain, or a 'sink effect' [50]. Growing evidence indicates that Aβ processing involves many enzymes and signaling pathways. Although the clinical failure of Aβ-lowering agents does not mean that the hypothesis itself is entirely incorrect, it nonetheless suggests that direct manipulation of Aβ is not sufficient *per se* as a therapeutic intervention.

Preclinical studies in transgenic mice that generate a surplus of Aβ demonstrated that antibodies directed against Aβ

Table 1. Recent amyloid beta antibody passive immunotherapy approaches in Phase II and III clinical trials; the antibodies target different epitopes of amyloid beta and differ in the ability to bind different amyloid beta conformations.

RCTs completed (examples)	RCTs ongoing (examples)
Bapineuzumab: mainly targets fibrillar forms of Aβ. Two Phase III RCTs in mild-to-moderate AD did not report clinical benefits after 18 months. Two other Phase III RCTs interrupted based on the latter results but showed null results	
Solanezumab: mainly targets soluble, monomeric Aβ. Two Phase III RCTs in mild-to-moderate AD, 2052 subjects, did not show benefits in the primary outcomes after 18 months (EXPEDITION 1 and 2 trials). Questionable benefit reported in *post hoc* pooled sample of mild AD. Negative effect in pooled moderate AD	*Solanezumab* is in an RCT in older adults with positive Aβ PET scans and without cognitive impairment (the A4 trial); and a Phase III trial of mild AD, with approximately 2100 participants (EXPEDITION 3) *Solanezumab* (and *gantenerumab*): Phase II and III RCT in carriers of mutations for autosomal dominant AD with normal cognition, or MCI, or mild AD, 2 + 3 years, approximately 240 participants (DIAN)
Gantenerumab: mainly targets Aβ plaques. 4-week Phase I RCT in 18 patients with mild-to-moderate AD, reduction in brain Aβ; high doses showed adverse effects (ARIA-E)	*Gantenerumab*: in a Phase II/III RCT in prodromal AD, 2 years, approximately 800 participants. Also in DIAN, see above
Crenezumab: mainly targets monomeric or oligomeric forms of Aβ A Phase II in mild-to-moderate AD, 2 years, approximately375 participants showed overall no significant results, but some effects in an older or younger, more or less severe, or APOE subgroup will be reported significant	An ongoing Phase II or III RCT in *PS 1* mutation carriers with normal cognition, 5 years, approximately 300 participants conducted in Columbia
BAN2401: monoclonal antibody against Aβ oligomers, Phase IIa, 60 AD participants (completed)	*BAN2401*: an 18-month Phase II RCT in 800 participants with MCI due to AD or mild AD [174]
Ponezumab PF-04360365: Phase II RCT, 175 participants, completed in 2011 (not published; development discontinued)	
	B11B 037: Phase II RCT in prodromal and early AD, 160 participants [175]
Immunoglobulin G polyclonal antibodies including anti-Aβ antibodies: 6 months, Phase II RCT in 24 participants with mild-to-moderate AD reported cognitive improvement; 6-month, Phase II RCT in 58 with mild-to-moderate AD showed no effect on cognition or AD biomarkers; 18-month, Phase III RCT in mild-to-moderate AD (approximately 390 subjects) showed no significant effect. Also in Phase II RCT in aMCI, 2 years, approximately 50 participants (completed, not published)	

Aβ: Amyloid beta; AD: Alzheimer's disease; IVIG: Intravenous immunoglobulin; aMCI: Amnestic mild cognitive impairment; MCI: Mild cognitive impairment; RCTs: Randomized controlled trials.
Data sources [176–179].

N-terminus are able to enter the brain and decrease amyloid deposits in brain tissue and cerebral vasculature [61,62]. Moreover, such antibodies inhibit the synaptotoxic actions of Aβ oligomers and enhance cognitive skill in APP transgenic mice [63,64]. Past Aβ immunotherapy trials in humans based on active immunization with $A\beta_{1-42}$ provided indications for clinical benefits [65].

Interestingly, RNA research has seen intense growth in recent years in the field of AD. In particular, post-transcriptional gene silencing via RNA interference has attracted great interest as a gene regulatory mechanism. However, RNA-induced gene silencing has yet to be fully explored even at an experimental level, and there are still many areas to this field in which understanding is lacking [66].

In the following sections, an overview is presented focused on the Aβ antibodies (Table 1), including the recently terminated *bapineuzumab* program and the ongoing *solanezumab* and *crenezumab* trials.

Bapineuzumab

Bapineuzumab (AAB-001) is a humanized monoclonal antibody to Aβ1-5 that binds to both soluble and fibrillar forms of Aβ

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and reduces amyloid burden in transgenic mice [62]. It was co-developed by Élan and Wyeth pharmaceutical companies (the Alzheimer Immunotherapy Program). Subsequently, Janssen Pharmaceutical, a subsidiary of Johnson & Johnson, acquired a substantial portion of Élan's assets and rights related to the venture, including bapineuzumab, and constituted a dedicated unit, Janssen Alzheimer Immunotherapy R&D, LLC, to manage its role in the Alzheimer Immunotherapy Program. Not long thereafter, Pfizer acquired Wyeth in 2009 and its stake in the Alzheimer Immunotherapy Program. Operationally, the development of bapineuzumab and related antibodies and vaccines continued as a joint venture between Pfizer and Janssen.

Despite evidence of vasogenic cerebral edema in Phase I trials, bapineuzumab was advanced to a multiple ascending dose Phase II trial in mild-to-moderate AD [67], where it failed to meet its primary end points, the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) and Disability Assessment for Dementia (DAD) scale for any dose. *Post hoc* exploratory analyses combined the several active dose groups into one overall treatment group and a pooled placebo group, and then analyzed outcomes on the basis of *APOE ε4* carrier status. This analysis suggested a nominal effect for the non-carriers [67]. Because of the APOE-related finding, bapineuzumab was advanced to Phase III in separate trials for *APOE ε4* carriers and non-carriers with mild-to-moderate AD. Two trials for each carrier stratum were initiated, totaling over 4000 patients altogether. Study 301 in *APOE ε4* non-carriers [68] and study 302 in *APOE ε4* carriers [69] – both conducted by Janssen Alzheimer Immunotherapy at sites primarily in North America; study 3000 in *APOE ε4* non-carriers [70] and study 3001 in *APOE ε4* carriers [71] – both conducted by Pfizer at sites primarily outside North America. However, bapineuzumab failed in AD patients with or without the *APOE ε4* allele [72].

Notably, detailed results on primary efficacy, safety and key biomarker from clinical trials 301 and 302 have been published recently [73]. These were multicenter, randomized, double-blind, placebo-controlled, parallel group studies conducted in 1121 *APOE ε4* carriers and 1331 non-carriers. The carrier study was conducted at 170 sites in the USA between December 2007 and April 2012, while the non-carrier study was conducted at 218 sites in the USA, Canada, Germany and Austria between December 2007 and June 2012. Patients aged 50–88 years were eligible for the trials; they received placebo or bapineuzumab by intravenous infusion every 13 weeks over 78 weeks (0.5 mg/kg or placebo for carrier study; 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg or placebo for non-carrier study). It should be noted that the 2.0 mg/kg dose level was discontinued early in the course of the study, and approximately 150 subjects assigned to that treatment were continued on bapineuzumab 1.0 mg/kg. Data from these subjects were not part of the primary statistical analysis for the study. Primary outcomes were the ADAS-Cog subscale/11 item-version (ADAS-Cog11, range 0–70; higher scores indicate greater impairment) and DAD (range 0–100; higher scores indicate less impairment). Biomarkers used as secondary outcomes included Pittsburgh compound B-PET amyloid burden imaging and CSF total tau and hyperphosphorylated-tau (p-tau) [73].

The primary outcomes were not significant: ADAS-Cog11 and DAD treatment differences versus placebo at week 78 were, respectively: –0.2 ($p = 0.798$) and –1.2 ($p = 0.343$) (0.5 mg/kg in carriers); –0.3 ($p = 0.642$) and 2.8 ($p = 0.067$) (0.5 mg/kg in non-carriers) and 0.4 ($p = 0.620$) and 0.9 ($p = 0.550$) (1.0 mg/kg in non-carriers). In both the carrier and non-carrier studies, amyloid-related imaging abnormalities with edema were the most notable adverse events. This increased with bapineuzumab dose and *APOE ε4* allele number and was the basis for the discontinuation of the 2.0 mg/kg dose in protocol 301 during the course of the studies. CSF p-tau, a marker of neurodegeneration, was observed to be lower with bapineuzumab treatment in both *APOE ε4* carriers and non-carriers. Furthermore, a decreased rate of brain amyloid accumulation on Pittsburgh compound B-PET was observed in *APOE ε4* carriers treated with bapineuzumab, but not in non-carriers [73].

In summary, the four Phase III clinical trials did not show clinical benefit for intravenous bapineuzumab on primary or secondary outcome. The meaning of its effect on lowering CSF p-tau is uncertain but could represent an effect on further neurodegeneration. The bapineuzumab development program has been discontinued. A Phase II trial of bapineuzumab given subcutaneously also apparently showed no significant outcomes. For this reason, on 16 July 2013, Johnson & Johnson announced the discontinuation of the Phase II trial testing the subcutaneous formulation of bapineuzumab [74].

Solanezumab

Solanezumab (LY2062430, Eli Lilly and Company, Indianapolis, IN, USA) is a humanized IgG1 derivative of an anti-Aβ mouse mAb named m266 that recognizes a distinct epitope located in the central portion of the human Aβ peptide (against residues 13–28 of Aβ). It reacts with various N-terminally truncated species of Aβ that are known to exist alongside full-length $Aβ_{1-42}$ in AD senile plaques [75]. The biochemical properties of solanezumab differ from other monoclonal antibodies, in that, first, the antibody targets the central domain of Aβ, and, second, because of this property, it has been suggested to be more effective than other antibodies at clearing N-terminal truncated or modified forms of the Aβ peptide [76]. By comparison, bapineuzumab acts upon the N-terminal portion (amino acid residues 1–5) of the Aβ peptide [73]. Also by comparison with bapineuzumab, solanezumab preferentially binds to the soluble form of Aβ, with little or no affinity for senile plaques. Soluble oligomeric Aβ are believed to be more neurotoxic than Aβ fibrils or deposits [77].

Treatment with solanezumab apparently reversed memory deficits without influencing brain Aβ content in a PDAPP AD mouse model [78]. In Phase II trials, solanezumab was reported

to be safe while increasing CSF and plasma concentrations of $A\beta_{1-40}$ and $A\beta_{1-42}$ [76]. Solanezumab did not show any effect on behavioral outcomes as evaluated by ADAS-Cog [79]. In spite of the absence of efficacy in Phase II, two Phase III randomized, double-blind, placebo-controlled trials were undertaken in patients with mild-to-moderate AD: EXPEDITION 1 [80] and EXPEDITION 2 [81]. The two trials together randomized 2052 patients with mild-to-moderate AD; Mini-Mental State Examinations from 16 to 26, patients aged 55 and older. Placebo or solanezumab (400 mg) were infused intravenously every 4 weeks for 18 months. Initially, the co-primary end points were the ADAS-Cog11 and the Alzheimer's Disease Cooperative Study-Instrumental Activities of Daily Living inventory (ADCS-ADL) at week 80 [82]. After analyzing the results of EXPEDITION 1, the statistical analysis plan of EXPEDITION 2 was modified to designate the ADAS-Cog14, which includes three additional items relevant for mild AD patients [83], as the co-primary outcome in the subgroup with mild AD [82].

In both EXPEDITION clinical trials, the two primary end points, both cognitive (ADAS-Cog subscale) and functional (ADCS-ADL), were not met in the overall mild-to-moderate AD study samples [82]. On the safety side, some minor adverse effects occurred more often in the solanezumab compared with placebo groups; these included lethargy, rash, malaise (in EXPEDITION 1) and angina (in EXPEDITION 2) [84]. Solanezumab therapy was associated with a low incidence of amyloid-related imaging abnormalities with edema and hemorrhages [82] consistent with preclinical reports and the lack of targeting fibrillar amyloid [85].

According to Eli Lilly and Company's press release, the pre-specified secondary analysis of pooled data in patients with mild AD apparently showed a significant slowing in cognitive decline by 34% ($p = 0.001$) compared with placebo, as measured by the ADAS-Cog14 subscale, and a non-significant reduction by 17% in functional decline ($p = 0.057$) compared with placebo, as measured by the ADCS-IADL subscale, in mild AD patients [86,87]. However, the slope reductions depend largely on the extent of change in the placebo groups. An ongoing open-label, uncontrolled extension study, EXPEDITION-EXT [88], in patients who completed either the EXPEDITION 1 or the EXPEDITION 2 study will likely offer further safety data. In summary, while the primary end points were not met in EXPEDITION 1 and EXPEDITION 2 trials, data reported in the pooled mild AD patient subsets of the trials suggested a cognitive effect.

Based on the analysis above in the pooled mild AD patients, the development of this molecule for the management of AD-associated dementia continues with a third Phase III clinical trial in mild AD, called EXPEDITION 3 [89,90]. Some researchers believe, however, that studies in the prodromal phase of AD or in asymptomatic individuals will yield a greater effect [91]. The Anti-Amyloid Treatment in Asymptomatic AD trial (A4) is an ongoing trial of solanezumab for people over 65 who have borderline episodic memory impairment, and a positive amyloid PET scan, funded by Eli Lilly and NIH, and will serve as a secondary prevention trial [92,93].

Therefore, a better understanding of the role of immune responses in AD and their impact on immunotherapy is essential in the design of alternative or combinatorial immunotherapy approaches in AD [94].

The potential role of tau-targeted therapies for AD

In AD patients, tau pathology correlates better with the degree of dementia than amyloid plaque burden [95–97] and reduction of Aβ with immunotherapy does not translate into cognitive improvement, making tau an attractive target for therapy [98]. According to the classic scheme of Braak and Braak [99], neurons belonging to the superficial layer of the entorhinal cortex are the first to be affected by an accumulation of tau followed by a pattern involving progressively limbic and association cortices. Thus, in theory, targeting the first tau deposition with selective antibodies could block the further propagation of tau pathology. Transgenic mice overexpressing APP are the most commonly used animal models of AD; however, unlike AD patients, these mice do not develop neurofibrillary tangles despite extensive Aβ accumulation. Multigenic APP-Tau transgenic models develop neurofibrillary tangles similar to those seen in AD brain and are particularly suited to the study of the relation between Aβ accumulation and tau pathology [100].

In these transgenic models, removal of intraneuronal Aβ via active immunization, reduces neural accumulation of tau, if tau is not yet aggregated [101]. On the other hand, in AD, the reduction of cortical Aβ produced by immunization which is accompanied by a significant reduction of CSF p-tau produces no cognitive improvement [98]. This suggests that reducing tau via the Aβ pathway may not be a valid strategy.

A strategic target could be to prevent initial formation of tau seeds (tau oligomers) and subsequent spreading of tau aggregates. In particular, targeting soluble extracellular tau with anti-tau antibodies may block the spread of tau pathology from one neuron to another.

Several animal studies of tau, targeting immunotherapy, have been reported [102] in some cases, the tau protein vaccine caused a severe encephalomyelitis, triggered by the unphosphorylated tau protein, suggesting a need for extreme caution in designing immunization protocols in humans. On the other hand, several studies on animal models have demonstrated that a reduction of tau pathology may produce an improvement in cognitive performance in the absence of obvious adverse effects [103–107]. Immunizing transgenic rats with recombinant misfolded truncated tau, before the occurrence of behavioral symptoms, reduces the level of both tau and p-tau and delays the onset of sensory-motor deficits without causing adverse effects [108]. Thus, preclinical immunization in animal studies performed with either tau peptides or tau antibodies suggest a novel strategy for immunotherapy of AD [98,109]. Animal data show that, as for Aβ, an optimal tau-vaccination should target preferably pre-filament oligomeric species at specific phosphorylation sites.

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Table 2. Anti-tau therapy drugs in development.

Drug name, category and clinical trials	Mechanism of action	Company
Tau aggregation or deposition inhibitors		
Paclitaxel	Tau deposition and aggregation inhibitor; microtubule stabilizing agent	Generic
Davunetide, AL-108, NAP Phase II clinical trial in aMCI patients: negative	Tau deposition and aggregation inhibitor; microtubule stabilizing agent	Allon Therapeutics–Biogen Idec
BMS-241027	Tau deposition and aggregation inhibitor; microtubule stabilizing agent	Bristol-Myers Squibb
TRx0237, (methylthioninium chloride, methylene blue) LMTX: two Phase III clinical trials	Inhibitor of tau protein aggregation	University of Aberdeen, UK, and TauRx Therapeutics
Pyridazines	Tau aggregation inhibitors	University of Pennsylvania
BLV-0703	Tau aggregation inhibitor	Bioalvo
ReMIND NV	Tau deposition inhibitor	Roche
Nicotinamide Phase II clinical trial	Inhibitor of microtubule polymerization	Generic
Kinase inhibitors		
GSK-3 inhibitors (various)	Kinase inhibition (GSK-3)	AstraZeneka, Takeda, CrystalGenomics
Valproate, divalproex sodium Phase III clinical trial: negative and worsening	Inhibitor of the kinase GSK-3	Generic
Tideglusib, NPO3112, NP-12 Two Phase II clinical trials	Inhibitor of the kinase GSK-3	Noscira
SAN-161	Inhibitor of the kinase GSK-3	Sanoimmune
Lithium chloride: two negative and one positive Phase II clinical trials. Not being developed.	Kinase inhibitor	Generic
Tau protein modulators	Enzyme modulator	Biogen Idec
Immunotherapies		
Monoclonal antibodies	Passive tau immunization	AC Immune, Prothena Elan, Neotope Biosciences
Supra-antigen vaccines	Active tau immunization	AC Immune
AADvac-1 clinical Phase I/II	Vaccine : synthetic peptide targeting pathological tau protein	Axon Neuroscience

GSK-3: Glycogen synthase kinase-3.

Since both oligomerization of Aβ and phosphorylation of tau appear at early stages of the disease, even before the appearance of clinical symptoms, an early intervention with anti-tau oligomer antibodies seems necessary. Studies in transgenic mouse strains carrying different expressions of pathological events suggest that tau aggregation correlates with synaptotoxicity but not necessarily with the presence of neurofibrillary tangles and that recovery of functional deficits may occur after reduction of aggregation-prone tau species [110]. Therapeutic efforts should specifically target certain soluble tau species, to reduce p-tau levels and aggregation, or to promote degradation. Microtubule-stabilizing drugs such as paclitaxel have shown significant improvement of fast axonal flow and microtubule density in mice [111]. Tau phosphorylation represents another critical target for tau-oriented therapy. Kinases and phosphatases, involved in tau hyperphosphorylation and dephosphorylation respectively, represent two excellent enzymatic targets (Table 2) [112]. A small number of kinase inhibitors have been tested in animal models,

however, all of these kinases have other substrates beside tau and inhibition of phosphorylation of these other substrates could be dangerous (Table 2).

The current state of anti-tau therapy in humans parallels the early phases of the anti-Aβ treatment approach, while it is not clear which type of Aβ or tau to target, for how long to treat or immunize and at which stage of the disease to intervene. It is also not clear which conformational state (oligomers, or truncated tau, or aggregated tau) is responsible for neuronal dysfunction and degeneration and how tau exerts its toxicity and, like for Aβ, it is not known at which disease stage tau aggregation blockers would be most effective [98].

Several approaches targeting tau pathology are being considered in AD therapy (Table 2). Small molecular weight compounds developed to inhibit formation of tau oligomers and fibrils by blocking tau/tau aggregation have already been tested in humans. The dye methylene blue, a tau anti-aggregant, has been reported to have a positive therapeutic effect in a Phase II clinical trial [113], but results have not been replicated. New Phase II and III trials are in progress with a new formulation of the compound to treat mild and moderate AD patients.

So far, no kinase inhibitor has advanced to a late clinical phase. Mainly GSK3 inhibitors, including lithium chloride have been tested but failed to show efficacy despite the fact that in one of the trials, a decrease in CSF p-tau was seen [114]. A 2-year Phase III trial of sodium valproate also did not show an effect on cognition (Table 2) [32].

Animal studies suggest that anti-tau immunotherapy would be more effective in the early stages of functional impairment. Therefore, an effective vaccine should target early stages and pre-filament tau species rather than mature stable tangles. Also, animal studies indicate that active tau immunotherapy using tau-epitopes, similarly to Aβ immunotherapy in humans, may involve serious potential risks of inflammatory reactions and encephalitis suggesting that passive immunization may represent a safer approach.

Three different types of immunotherapies in humans have been recently proposed but, so far, only one of them has reached clinical Phase I with a synthetic vaccine targeting misfolded truncated tau (Table 2). Up to now, a serious limitation for clinical trials targeting tau has been the lack of selective tau biomarkers and PET tracers binding specifically to tau and p-tau to evaluate treatment effect. New classes of selective PET tau-binding ligands are been tested, both in transgenic mice and human subjects with normal cognition, AD and cortico-basal syndrome with promising results [115].

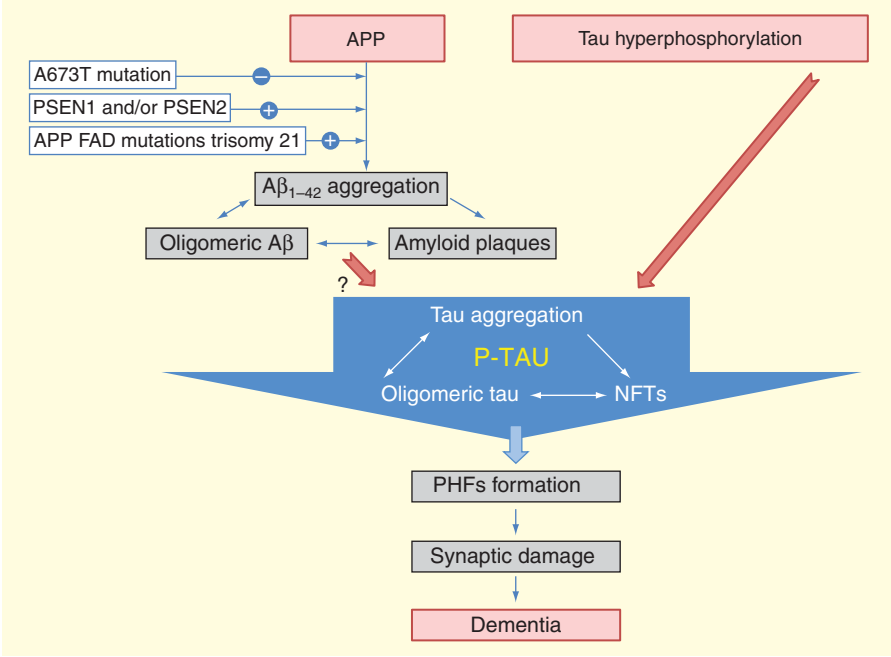

Figure 1. Update of the amyloid cascade hypothesis suggested by Hardy and Higgins [18] integrating both the original theory and the essential part played by tau in Alzheimer's disease pathogenesis.
Aβ: Amyloid beta; APP: Amyloid precursor protein; FAD: Familial Alzheimer's disease; NFTs: Neurofibrillary tangles; PHFs: Paired helical filaments; PSEN1: Presenilin 1; PSEN2: Presenilin 2; p-tau: Hyperphosphorylated-tau.

An update of the amyloid cascade hypothesis proposed by Hardy and Higgins [18] that integrates both the original theory and the essential part played by tau in AD pathogenesis is presented in Figure 1. This modified cascade hypothesis underlines the fact that the treatment target must be related to the underlying molecular mechanism; the timing of the clinical intervention should take into account the specific phase of the disease and biomarkers should quantitatively reflect the expected effect of treatment on the target. This conceptual revision might promote the discovery of new biomarkers and facilitate the translation of preclinical findings into clinical interventions.

The prevention initiatives: DIAN, API & A4 – the role of early clinical trials

Large efficacy trials of anti-amyloid therapies, including anti-aggregation agents, γ-secretase modulators and inhibitors and immunotherapies, have been conducted. To date, all have been negative. The γ-secretase inhibitor semagacestat [116] and the N-terminus anti-amyloid mAb bapineuzumab [73], in particular, demonstrated target engagement in the CNS yet no beneficial effect on cognition in mild-to-moderate AD. To some, this represents substantial evidence refuting the amyloid therapeutic hypothesis. But there are explanations for these failures that do not preclude the development of successful interventions targeting amyloid.

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The spectrum of AD spans many years. It is now clear that an early, asymptomatic stage precedes the syndromes of MCI and dementia by a decade or longer. By the time significant symptoms occur, substantial neurodegeneration is evident. Even if, as most believe, amyloid is the principal driver of the neurodegenerative process, it is plausible, perhaps likely, that minimal or no cognitive or clinical benefit can be expected from anti-amyloid interventions at symptomatic stages [52]. Is it feasible to conduct therapeutic trials at the asymptomatic stage of disease? Indeed, several such trials have now been launched, taking various approaches.

Genetically determined AD presents an opportunity for early intervention. Every individual carrying an AD-causing mutation of presenilin 1, presenilin 2 or the APP will develop AD, with a generally predictable age of onset. Mutation carriers can be identified by screening members of affected families; with or without disclosure of genetic testing results, such individuals can be invited to participate in trials of potential disease modifiers. Two trials, each a collaboration between academic groups and industry partners, have been launched. One, led by the Alzheimer's Prevention Initiative (API), involves primarily a large family in Colombia affected by a presenilin 1 mutation [117]. The second, led by the Dominantly Inherited Alzheimer's Network (DIAN) [118], includes multiple families with various autosomal dominant mutations. Each of these programs involves a RCT of one or more anti-amyloid interventions over a period of years, aiming to alter the natural course of AD as indicated by biomarker and cognitive outcomes.

Down syndrome is another genetically determined form of AD, with a very high incidence of the disease by the fourth, fifth and sixth decades. Again, this presents an opportunity to identify and treat individual at very high risk before the onset of symptoms. Biomarker studies to facilitate trial design are under way in the Down syndrome population, and prevention trials are expected to begin quite soon [119].

Can prevention trials be conducted in the population at risk for sAD? The asymptomatic stage of sAD can be identified on the basis of biomarkers of brain amyloid accumulation, either CSF $A\beta_{1\text{-}42}$ or amyloid PET scanning. Evidence for brain amyloid in the absence of AD symptoms is consistent with the recently formulated research criteria for the diagnosis of preclinical AD [120]. The first large trial of a potential disease-modifying agent in preclinical AD, the A4 trial [121], is now enrolling participants. The A4 trial – a collaboration between the ADCS (an academic consortium funded by the National Institute on Aging) and Eli Lilly – is testing the mAb solanezumab. Participants, clinically and cognitively normal individuals between the ages of 65 and 85 who have elevated brain amyloid by PET scan, are randomly assigned to receive infusions of the antibody or placebo every 4 weeks for 3 years. The primary outcome measure is represented by a cognitive composite, the preclinical Alzheimer's cognitive composite [122]. The ADCS-preclinical Alzheimer's cognitive composite consists of the Free and Cued Selective Reminding Test total and list learning, Logical Memory II delayed recall, Mini-Mental State Examination and Digit Symbol Substitution Test [122]. Additional cognitive, clinical and biomarker measures are collected.

Prevention trials as described here need not be restricted to anti-amyloid interventions. Indeed, many have proposed that even at the earliest, asymptomatic stage of disease, even in amyloid-related genetic subgroups, therapy targeting other aspects of the neurobiology, alone or in combination with anti-amyloid measures, should be studied. The recent exciting development of tau PET imaging [123] presents an opportunity for characterizing the earliest stages of AD, for identifying individuals who may be particularly appropriate for anti-tau interventions, and for determining the impact of therapies on disease progression.

Prevention trials are now under way in genetically determined AD as well as sAD; more are in the planning stages. Though these trials are costly and time-consuming, they may present the best hope for effective disease-modifying therapy for AD.

Clinical trials for AD: regulatory viewpoints & requirements

In spite of the remarkable progress in understanding the molecular underpinnings of AD, there are still no efficacious treatment options for modification of the natural course of AD or its prevention. Approved therapies as cholinesterase inhibitors or the *N*-methyl-D-aspartate receptor antagonist memantine showed statistical significant improvements in cognition and global or functional outcomes, however, effect sizes are small and are questioned to be clinically meaningful in many patients. This is in contrast to positive results in preclinical models aimed at rescuing synaptic dysfunction or ameliorating behavioral impairment in animal models [16], which have yet to be translated into disease-modifying medicinal products for patients with AD. Based on the recent clinical trial failure of bapineuzumab and the very modest results from two major Phase III studies for solanezumab, all stakeholders raise questions from their different angles regarding: following the right models and theories on the pathogenesis of AD; including the right patients at the right stage of disease (e.g., preclinical AD vs mild-to-moderate AD) with the right compound (e.g., mechanism of action); appropriateness of the therapeutic targets and selection of end points and fostering new study designs (adaptive designs and combination therapy).

The EU and US regulators are reacting to these issues, so recently the EMA has published a concept paper clarifying the need for updating our regulatory guidance for clinical trials regarding AD. The EMA update will focus on:

- The impact of new diagnostic criteria for AD including early and even asymptomatic disease stages on clinical trial design;
- The choice of outcome parameters and need for distinct assessment tools with regard to the different disease stages in AD and other dementias (different signs and symptoms, differences in change over time, severity);

- Assessment of efficacy and safety in different age groups (e.g., old vs very old);
- Potential use of biomarkers and their temporal relationship with the phases of AD in different stages of drug development (mechanism of action, use as diagnostic test, enrichment of study populations, stratification for subgroups, safety and efficacy markers, etc.);
- Design of long-term efficacy and safety studies;
- Usefulness of combination therapy and corresponding study designs;

The FDA has distributed a draft guidance for industries [124] allowing alternative targeting of interventions at the early stages of AD (preclinical-mild). According to this new guidance, the FDA suggests potential approaches to clinical trial design and execution that allow for regulatory flexibility and innovation [55]. There they cover the selection of patients for trials at early stages of AD and for this there is consensus between the AD research community and regulatory bodies that clinical diagnosis of early cognitive impairment might be coupled with specific appropriate biomarkers reflecting *in vivo* evidence of AD pathology. New diagnostic criteria addressing these issues have been established and are under validation by various working groups [54,56,125–128]. Most biomarkers include brain Aβ load, as measured by PET and CSF levels of Aβ and tau proteins [129,130], however, there is a clear move to update the amyloid hypothesis and to look for new biomarkers for the different disease stages [98,131].

However, adequate validation of these biomarkers is still lacking despite many cross-sectional and more than 150 longitudinal studies related to the biomarkers of interest, which included subjects who had objective cognitive impairment but no dementia at baseline. The authors conclude in their review that the body of evidence for these imaging and CSF biomarkers is still limited and highly variable across the different types of biomarkers [132]. As far as the CSF biomarkers are concerned, it was recently reported that the overall variability of data coming from a total of 84 laboratories remains too high to allow the validation of universal biomarker cutoff values for the specific intended use [133], which underpins the urgent need for better harmonization and standardization of these methods. Moreover, the use of biomarkers as end points in earlier stages of drug development is well established for regulators and there are examples to approve medicinal products on the basis of their effects on validated surrogate markers, such as antihypertensives, or cholesterol-lowering products. However, these examples have been considered as validated surrogate markers as they allow substitution for a clinically relevant end point. In their validation, a link between a treatment-induced change in the biomarker and long-term outcome of the relevant clinical measure was undoubtedly established. Therefore, the regulatory requirements on biomarkers used as end points in clinical trials are high as outlined earlier [134]. In consequence, the EU regulators help applicants in their research and development by issuing opinions on the acceptability of using such biomarkers or a distinct methodology in clinical trials. Since 2011, The EMA's Committee for Medicinal Products for Human Use (CHMP) has adopted and published several qualification opinions for use in the development of medicines for AD. In these qualification, opinions biomarkers are accepted for identification and selection of patients at the pre-dementia stage of the disease as well as for selection of patients for clinical trials in mild and moderate AD. In September 2013, a qualification opinion for a novel model of disease progression and trial evaluation in mild and moderate AD was adopted by CHMP. The simulation tool is intended to provide a quantitative rationale for the selection of study design and inclusion criteria for the recruitment of patients.

In the diagnostic area, the first approvals of radiopharmaceuticals (Florbetapir [^{18}F]; Florbetaben [^{18}F]) for PET imaging of Aβ neuritic plaques in the brain by the European Commission on the recommendation of the CHMP have been another step forward. These diagnostic agents can be used in patients who are being evaluated for AD and other causes of cognitive decline. Flutemetamol (^{18}F), another diagnostic radiopharmaceutical for amyloid load is currently under evaluation by the CHMP; the FDA has already approved all three of them. However, interpretation of these PET amyloid scans is not without hurdles: amyloid positivity does not reliably distinguish between clinical diagnoses, so that neuropsychiatric normal people as well as those with MCI, AD and other neurodegenerative diseases can all be 'amyloid positive'. Moreover, some patients with 'typical' signs and symptoms of AD show negative scans. So for the time being a positive amyloid scan must be considered in the full clinical picture of a patient, on the other hand, a negative amyloid scan indicates that the likelihood of cognitive impairment due to AD might be low, nevertheless, further data on sensitivity and specificity are needed [135–138].

Another issue in future clinical trials is the appropriate choice of clinical end points. In established AD, the CHMP guidance requires co-primary end points in cognition (mandatory) together with functional or global outcome measures; moving now to earlier asymptomatic or prodromal stages of AD might change this requirement. So the FDA suggests for clinical trials focusing on patients in whom overt dementia seems imminent, the use of a single scale that combines assessment of both cognition and function such as the score on the Clinical Dementia Rating Sum of Boxes [55]. For patients whose disease is at an even earlier clinical stage, it might be possible to approve a drug through an accelerated procedure pathway on the basis of assessment of only cognitive symptoms in the USA. There the accelerated approval mechanisms will allow drugs that address an unmet medical need to be approved on the basis of a surrogate end point or an intermediate clinical end point (i.e., a sensitive cognitive measure). In the EU, a similar approach is possible via a 'conditional' approval, which implies that the applicant accepts after such a preliminary approval the obligation to carry out further long-term clinical studies to confirm clinical efficacy and safety. Only after the approval and long-term treatment, it would be

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Table 3. Proposed risk and protective factors for late-onset dementia and Alzheimer's disease.

Risk factors	Protective factors
Age Genetic Familial aggregation *APOE* ε4 Different genes (e.g., *CR1, PICALM, CLU, TREM2, TOMM40*) have been proposed [180]	**Genetic** Different genes (e.g., *APP, APOE* ε2) have been proposed [180]
Vascular and Metabolic Cerebrovascular lesions Cardiovascular diseases Diabetes mellitus and pre-diabetes *Midlife positive association but late-life negative association* Hypertension High BMI (overweight and obesity) High serum cholesterol	**Psychosocial factors** High education and SES High work complexity Rich social network and social engagement Mentally stimulating activity
Lifestyle Smoking High alcohol intake	**Lifestyle** Physical activity Moderate alcohol intake
Diet Saturated fats Homocysteine	**Diet** Mediterranean diet Polyunsaturated (PUFAs) and fish-related fats Vitamin B_6, B_{12}, folate Antioxidant vitamins (A, C, E) Vitamin D
Others Depression Traumatic brain injury Occupational exposure (heavy metals, ELF-EMFs) Infective agents (herpes simplex virus type I, *Clamydophila pneumoniae*, Spirochetes)	**Drugs** Antihypertensive drugs Statins HRT NSAIDs

ELF-EMFs: Extremely low frequency electromagnetic fields; HRT: Hormone replacement therapy; PUFAs: Polyunsaturated fatty acids; SES: Socio-economic status.

possible to properly follow the amelioration of cognitive and behavioral disorders as well as the slowing of the progression of neurodegenerative lesions as shown by neuroimaging techniques [129,130]. Pharmaceutical industry- as well as investigator-initiated activities from academia are encouraged to seek scientific advice on their development programs as soon as possible with the regulators, if they intend to use new methods to define the patient population or specific study designs and assessment tools. For instance, Richard *et al.* recently proposed a new memory test for improving the diagnostic accuracy in subjects with MCI [139]; another approach came from Donohue *et al.*, who presented the ADCS-preclinical Alzheimer's cognitive composite [122] (see also section 'The prevention initiatives: DIAN, API & A4 – the role of early clinical trials'). The development and validation of such new assessment tools is encouraged by regulators.

The EMA guideline on the clinical investigation of medicines for the treatment of AD will be updated on the basis of new knowledge obtained from the validation of the new diagnostic criteria, the use of biomarkers in clinical evaluation and other recent trends in research and development. It has already been acknowledged that AD is more a continuum of different disease stages and that the focus of new drug development has to shift to early, even clinical asymptomatic disease stages. It is desirable that regulators and all involved stakeholders work together to decide on the most adequate study designs for the distinct stages of disease of AD prevention and treatment.

Perspectives on prevention trials for AD

Dementia and AD are multifactorial and heterogeneous conditions, which result from interactions between genetic and environmental risk factors over the lifespan. Indeed, evidence from longitudinal observational studies shows that risk factors for dementia and AD are diverse biological and lifestyle-related factors (as summarized in Table 3 adapted from [15]). In contrast to epidemiological studies, less evidence is available from RCTs showing that interventions focusing on modifiable factors can reduce the risk of cognitive impairment and dementia/AD. It has recently been suggested that up to half of AD cases are the result of modifiable risk factors, and a 10–25% decrease in such factors may prevent 3 million AD cases worldwide [140].

Based on evidence from observational findings, prevention initiatives aim to decrease the incidence of dementia/AD through reducing risk factors and promoting healthy and active lifestyle. Some studies have focused on unimodal preventive interventions, and it was concluded that their effects are rather modest [141]. More recently, a few studies have shifted to multidomain interventional studies that simultaneously target several risk factors, which is deemed more appropriate considering the heterogeneous etiology of dementia and AD [142].

Currently, in Europe, there are three ongoing RCTs to prevent cognitive impairment and dementia/AD: The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), the Multidomain Alzheimer Prevention Trial (MAPT) and the Prevention of Dementia by Intensive Vascular Care (PreDIVA) study [143–146].

Table 4. Characteristics of selected randomized controlled trials for prevention of cognitive impairment, dementia and Alzheimer's disease based on multidomain interventions.

RCT	FINGER	MAPT	PreDIVA
Sample size	1260 community dwellers, from previous population-based observational cohorts	1680 community dwellers	3700 community dwellers
Main inclusion criteria	Dementia risk score >6 and mild degree of cognitive impairment	Frail elderly people (subjective memory complaint, slow walking speed, limitation in IADL)	All elderly within GP practices, non-demented (MMSE >23)
Age at enrolment	60–77 years	≥70 years	70–78 years
Study design	Multicenter, randomized, single-blind, parallel-group trial	Multicenter, randomized, double-blind controlled trial	Multisite, open, cluster-randomized parallel group
Intervention	Multidomain: nutritional guidance, physical activity, cognitive training, increased social activity and intensive monitoring and management of metabolic and vascular risk factors	Multidomain: vascular care, nutritional advice, exercise advice, cognitive training and/or DHA 800 mg/day	Multidomain: nurse-led vascular care including medical treatment of risk factors, nutritional advice, exercise advice
Duration	2 + 5 years extended follow-up	3 + 2 years extended follow-up	6 years
Outcomes	Primary: change in cognitive function (neuropsychological test battery, Trail Making, Stroop), Secondary: dementia, cardiovascular events, depression, disability, quality of life, health resources utilization, AD biomarkers change	Primary: change in cognitive function (Grober and Buschke memory test) Secondary: cognition (MMSE, CDR), functional status, depression, health resources utilization, AD biomarkers change	Primary: dementia, disability Secondary: cognitive decline (MMSE, VAT), depression, cardiovascular events
Status	Intervention was completed in March 2014	Ongoing, will be completed in 2014	Ongoing, will be completed in 2015

CDR: Clinical Dementia Rating Scale; DHA: Docosahexaenoic acid; FINGER: Finnish Geriatric Intervention Study to Prevent Cognitive impairment and Disability; GP: General practitioner; IADL: Instrumental activities of daily living; MAPT: Multidomain Alzheimer Prevention Study; MMSE: Mini-Mental State Examination; PreDIVA: Prevention of Dementia by Intensive Vascular Care; RCT: Randomized controlled trial; VAT: Visual Association Test.

While the trials have some methodological differences related to recruitment strategies, target population and selected multimodal interventions, they also have several similarities. All RCTs use a multidomain interventional approach to target several dementia risk factors and reduce the risk of developing dementia. The studies aim to promote lifestyle changes, closely monitor metabolic and vascular risk factors and increase adherence to treatments for such conditions. They are also similar in excluding individuals with dementia at baseline, and their primary outcome is cognitive status and incidence of dementia. Their secondary outcomes comprise quality of life, mood disorders, functional status, utilization of health resources and adherence to the intervention trials. These studies have subsidiary data on structural and functional neuroimaging and biomarkers allowing the investigation of underlying mechanisms and the prevention program effects on biomarkers (for a summary of the three trials, see Table 4, adapted from [15]).

FINGER [143] [147] is a 2-year multicenter RCT that aims to prevent cognitive impairment, dementia and disability among 1260 older adults aged 60–77 years at the study baseline. Participants were randomly selected from population-based surveys (FINRISK and FIN-D2D) that provide extensive retrospective information. Eligible participants were at risk for dementia based on the CAIDE Dementia Risk Score and the Consortium to Establish a Registry for AD neuropsychological test battery [148,149]. The multidomain intervention consists of physical activity, nutritional guidance, cognitive training, social activity and monitoring/management of metabolic and vascular risk factors (impaired glucose tolerance, obesity, hypertension and dyslipidemia). Participants in the control group are given regular health advice. The primary outcome after 2 years is cognitive performance measured by a comprehensive Neuropsychological Test Battery composite Z score [143]. The 2-year intervention was recently finalized (spring 2014) and participants' experiences with the intervention were very positive and the dropout rate was low (11%). A 7-year extended follow-up will start in 2015 to assess the long-term effects of the intervention on the risk for dementia/AD and the secondary outcomes.

MAPT [145,146,150] is a multicenter 3-year RCT investigating frail older adults in France. A total of 1680 participants have been enrolled and randomized into one of four groups: ω-3, multidomain intervention, ω-3 plus multidomain intervention

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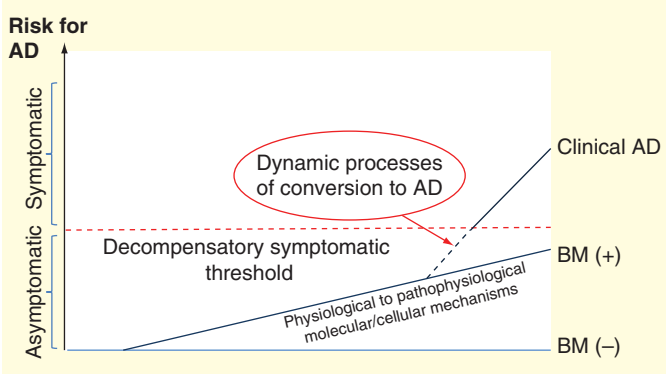

Figure 2. Hypothetical model of progression from an asymptomatic at-risk stage for sporadic AD to biologically manifest presymptomatic AD to clinically manifest prodromal and dementia stages due to AD. Dynamic factors contributing to disease conversion at a certain point are currently mostly unknown. Biomarkers serve as guideposts throughout all stages of sporadic AD. Current hypothesis-driven and exploratory biomarker research is focused on establishing dynamic profiles and disease signatures from AD asymptomatic to prodromal to dementia stages, even indicating altered biochemical or topographical changes before reaching a critical disease threshold. AD: Alzheimer's disease; BM: Biomarkers.

or placebo. The multidomain intervention involves group sessions (for physical activity, nutrition and cognitive training) and personalized consultations to manage risk factors. The goal of the study is to investigate the efficacy of the treatments on cognitive changes measured at the 3-year follow-up, using the Grobe and Buschke Test (memory recall) [145,146]. An extended follow-up will be carried out 5 years after the baseline assessments.

PreDIVA [144] (ISRCTN29711771) [151] is an open cluster RCT in the Netherlands among 3700 older adults aged 70–78 years, who were recruited from primary care settings, with a follow-up duration of 6 years. The goal of the study is to investigate the effects of nurse-led intensive vascular care (in primary care) on reductions of dementia incidence and disability (assessed using the Academic Medical Center Linear Disability Scale). The study also examines whether vascular care impacts cognitive functioning, incidence of vascular events, mood disorders and mortality. Intensive vascular care consists of personalized care based on consultations with the study nurse. The nurse tailors interventions for medical (diabetes, hypertension, hypercholesterolemia) and lifestyle-related (body weight, smoking habits, diet, physical activity) risk factors. The control group receives standard care according to Dutch general practice [144].

Investigators of the three trials – FINGER, MAPT and PreDIVA – have recently established the European Dementia Prevention Initiative network [152] to facilitate international collaborations while harmonizing and sharing data analyses. The initiative allows investigators to share experiences regarding methodological challenges and optimal strategies to further improve future prevention trials. Consistently, European Dementia Prevention Initiative has started a joint project titled the Healthy Aging Through Internet Counseling in the Elderly, which has been funded by the EU 7th framework grant [153]. The goal of Healthy Aging Through Internet Counseling in the Elderly is to prevent dementia and cardiovascular diseases among elderly populations. Through a user-friendly Internet platform, participants consult with a nurse who provides comprehensive advice on management of vascular and lifestyle risk factors.

Currently, most of the prevention RCTs fall into one of two categories. This review section has focused on the trials targeting broad population-based samples with multifactorial risk profiles and using multidomain lifestyle-related interventions. The second category of trials targets highly specific clinic-based samples with a well-defined biological risk factor (e.g., genetic or Aβ), and uses anti-amyloid agents (e.g., ADCS-A4, API, DIAN trials; see also section 'The prevention initiatives: DIAN, API & A4 – the role of early clinical trials') [15]. In the future, it will be important to fill the gap between these two categories and combine characteristics of both vascular/lifestyle interventions and disease-modifying drugs in order to tailor prevention programs for different at-risk groups as well as the prodromal stages of AD.

Future collaborative initiatives will require large-scale multinational prevention trials where data sharing and joint analyses are carried out. Such innovative projects will allow investigators to maximize the usage of existing infrastructures while optimizing the development of new cohorts/databases as well as novel complex infrastructures. Findings will also identify specific windows of opportunity during which prevention initiatives can have maximal benefits for dementia prevention. Collectively, the studies will be important in demonstrating not only the effectiveness of such intervention trials, but also their feasibility and cost–effectiveness, which have marked implications for dementia/AD prevention and public health.

Moving toward earlier disease stages – preclinical issues

In the field of 'disease modifier' intervention, there is already a systematic trend to shift from late clinical AD dementia stages to the earlier prodromal stages of the disease. When such mechanistic drugs in the prodromal target population will become approved and available, the consequent next step will be to initiate treatment in asymptomatic subjects at risk for AD in order to delay the progression to and the onset of clinical signs and symptoms. Today, we know very little about the natural pathophysiological history in these asymptomatic at-risk subjects. A much improved knowledge of the evolution of AD-related processes, however, will be the invaluable basis for the successful design of adequate clinical trials. In this respect, it will be crucial to clearly define the underlying dynamic processes that push risk profiles in patients over a disease threshold with escalating pathophysiology, preceding the conversion to a clinical disease (Figure 2).

Subjects at a preclinical state of AD can now be recognized by the presence of *in vivo* evidence of pathology associated with AD. This is the only way by which they can be identified since they should have no detectable clinical changes. If the presence of *in vivo* evidence of a biological signature is a fundamental feature that favors a further conversion to a clinical

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disease, it is not definitely established that all cognitively normal subjects who are biomarker-positive will develop the disease during their lifetime. Until now, the risk of conversion to a clinical AD has been established around 25% after 3 years [154] or after 5 years of follow-up for subjects who are at stage 2, that is, subjects with evidence of both amyloid and neuronal injury markers [155]. The conversion rate definitely increases with the duration of follow-up [156] but longer follow-ups are needed to ascertain the fact that those subjects will all convert to AD. Several factors may influence positively or negatively the risk and the date of conversion to a clinical disease as it has been demonstrated in other neurodegenerative diseases. Therefore, an *in vivo* evidence of AD-associated pathology does not automatically imply a progression to clinical AD. We need to provide answers to some of the following questions: will these cognitively normal biomarker-positive individuals all convert to AD? In case that they will not, is it possible to identify, before the onset, those subjects who will definitely progress and convert to a clinical disease? In other words, what is the natural history of the preclinical states of the disease? And when in fact does the 'disease' along the physiological and pathophysiological continuum start? How should we define the disease threshold triggered by dynamic factors from the asymptomatic at-risk stage (perhaps differentiating a true primary from a secondary prevention stage for therapy)? With the accompanying ethical considerations concerning these questions, what should we disclose about the status of these asymptomatic individuals at risk for AD and their risk? Can we treat someone for a disease that he/she will never develop?

If the presence of a pathophysiological marker increases the risk and is a *necessary* condition for a further conversion to clinical AD, this might not be sufficient. A dynamic process is postulated that activates the clinical onset. The process is the result of a complex and specific algorithm, which takes into account the risk/preventive factors previously discussed. This algorithm is important to identify *in vivo* if we keep in mind the possibility of preventive intervention with disease modifier treatments in asymptomatic at-risk subjects. When such drugs become available, an important step will be to identify the dynamic process of conversion in order to screen those patients who can be treated in order to avoid treating any biomarker-positive subjects including those who will never become symptomatic. This dynamic process, which drives or precedes the appearance of clinical disease, can be hopefully identified by early and preclinical brain changes.

Expert commentary

From the advent of the first transgenic mouse model of brain amyloidosis [157], and the significant finding of the vibrant impact of active immunization on aggregated amyloid peptides on this model [158], anti-amyloid immunotherapy has been considered the primary approach to develop and improve disease-modifying drugs. However, the development of an active vaccine was rapidly stopped as a consequence of meningoencephalitis in a small fraction of treated patients [159]. While energies turned to the production of innocuous active vaccines in order to diminish toxicity due to cellular immunity, many companies focused the attention on passive immunotherapy. This strategy is characterized by a substantially higher control since it allows the use of a mAb with manageable dose and frequency and it is free of risk of cellular immune response [160].

Phase III trials for two passive immunotherapies, that is, the Aβ-targeting monoclonal antibodies bapineuzumab (Janssen/Pfizer/Elan) and solanezumab (Eli Lilly and Company), failed to meet their primary cognitive and functional end points. However, pooled data from the mild AD subsets of the two solanezumab trials displayed a subtle decrease in cognitive decay at the end point of the 18-month study.

The Phase III bapineuzumab and solanezumab clinical trials were initiated in 2008 and 2009 based on a less clear knowledge of the molecular etiology of AD and were directed to patients with mild-to-moderate AD. (As discussed above, some experts believe this is too late to intervene by targeting Aβ, although there is an ongoing large trial of solanezumab for mild AD sponsored by Lilly.) However, it should be noted that these null results do not necessarily invalidate Aβ-targeting mechanisms. In this regard, one example might be represented by *Gammagard*^{TM} (Baxter International Inc., Deerfield, IL, USA), an intravenous immunoglobulin, attained from pooled plasma of healthy human blood donors, which contains natural anti-Aβ antibodies [161]. In an 18-month Phase II study executed on 24 mild-to-moderate AD patients [162], the drug has been observed to slow clinical decline and brain shrinkage. After that, the Phase III study, called the Gammaglobulin Alzheimer's Partnership, has been carried out in collaboration with the ADCS at 45 centers in the USA and Canada. After 18 months of treatment, mild-to-moderate AD patients receiving either the 400 or 200 mg/kg dose have not shown statistically significant differences in the rate of cognitive decline versus the placebo group [163,164]. However, a subgroup analyses revealed that those with moderate AD and *APOE ε4* carriers, all taking the 400 mg/kg dose, exhibit a positive difference in change from baseline in cognition as compared with placebo, as measured by ADAS-Cog and the Modified Mini-Mental State Examination. Nevertheless, full results have not been published [163,164].

Genetic evidence has substantially emphasized that Aβ can guide the mechanisms of the pathology; thus, decreasing its production or inducing its clearance are attractive goals [165]. Although Aβ accumulation is probably a multifactorial process in sporadic LOAD – in opposition to the autosomal dominant form of AD – amyloid-targeted interventions are assumed to provide clinical benefit in all forms of AD, if they are commenced very early, that is, before marked synaptic dysfunction, permanent extensive cell depletion and neurodegeneration occurred [52]. Although there is yet no clinical evidence for this, the recent finding that a genetically confirmed decrease in Aβ production by 40% provides evident protection against AD [166] further supports the idea that the timing of an Aβ-targeted intervention may be important for clinical response. In addition, general consensus concerning the pathophysiological processes causing AD progression is mirrored by the novel revised criteria for the diagnosis of AD-related dementia, MCI

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and preclinical disease [120]. Therefore, the concept of a biomarker-defined pre-symptomatic stage of AD may provide a foundation for early-stage clinical trials [127,167].

Such an exciting debate on the new AD diagnostic criteria has encouraged the development of novel study designs for prevention trials in at-risk and preclinical stages of AD. The prevention initiatives – namely, DIAN, API and A4 – are separate, but interconnected, long-lasting projects. Their leaders have formed an umbrella group referred to as Collaboration for Alzheimer's Prevention in order to maintain a regular dialogue as they plan and implement their preclinical treatment trials. One goal is to avoid duplicating effort on their part and on the part of the many outside partners necessary to pull off public–private trials. Another is to ensure that the initiatives find regulatory solutions jointly and construct their trials in such a way that as many data as possible can be compared and shared with the field at large. While each of the three initiatives is unique – for instance, in which population it enrolls and in how it negotiates with pharmaceutical companies – Collaboration for Alzheimer's Prevention exists to exploit their synergies. For example, DIAN will assist in the selection and training of the US sites, most likely including some DIAN sites, for the API's first trial of crenezumab in preclinical mutation carriers. This is done in order to distribute the burden, risk and potential benefit of this trial between Colombia and the USA [168].

The key source of hope is the current shift in industry to concept of preclinical AD diagnoses by employing biomarkers and diagnostic imaging-based strategies in conjunction with earlier clinical intervention [161]. Undoubtedly, the goal should be that of working in the direction of an adequate understanding of the neurobiology of AD to support the design of primary prevention trials [160].

Five-year view

We recognize that Aβ- and tau-based approaches have not led to clinical effective therapeutic strategies. This emphasizes the requirement for more pathophysiological studies on Aβ and tau as well as for identifying novel therapeutic targets. In this regard, the negative results of several previous Phase II/III trials highlight the need to critically assess the following points before embarking on further long and expensive trials:

- Is the mechanism of action plausible?
- Does the drug distribute to its site of action/target?
- Does it engage the target?
- Is there evidence of biological effect on plausible disease-relevant biomarkers?

Given the devastating consequences that AD will have on economy and society, research and development for AD is presently witnessing a 'call to action' in order to address the yet unmet needs of patients and their caregivers. In particular, it seems necessary to continually ameliorate definitions and criteria as well as conceptual models of AD and focus on the need for developing better infrastructures and research methods [131,169].

The very high extent of heterogeneity characterizing the genotypes of AD is reflected in the extensive variations in the age of onset, the neuropathological lesions, the pattern and the types of behavioral-clinical manifestations. These remarks highlight the complexity of the multigenic nature of this pathology. As a result, the extreme complexity of AD involves the necessity for integrating knowledge-information from several distinct but parallel sources (i.e., systems or networks) using both clinical and laboratory-based approaches including: high-throughput molecular profiling methodologies, multimodal brain imaging, neurophysiological measures and psychometric cognitive evaluation. As reported by Misgeld *et al.* [170], the understanding of neurodegenerative disorders such as AD needs a systems biology-based approach that spreads beyond the traditional boundaries by classical definitions of research areas. In the forthcoming years, future therapeutics development of AD pathology will be informed by advances in studies of whole genomes derived from individual patients and the introduction of more advanced systemic analyses resulting from combined transcriptomics, proteomics and metabolomics/lipidomics approaches that span from single molecules to pathway signatures in patients [171,172].

From the point of view of pure basic research, the development of the 'omics' disciplines has provided the approaches for detecting novel molecular biomarkers from biofluids, cells and tissues. High-throughput technologies show the capacity to collect large different amounts of data with reference to a definite phenotype or disease status in an unbiased way. In order to amalgamate these heterogeneous data, the development of tools is required not only for data storing and mining, but also for modeling the data in the context of disease pathophysiology [171,173]. Of note, since data attained at different levels may carry complementary information on AD pathophysiology, their integration is thought to improve the diagnosis and the interpretation of the disease [171,173] and the elucidation of the commonalities and dissimilarities between the inherited and sporadic forms of AD. Intriguingly, the integration process across different research disciplines in neurology is expected to result in a new field properly designated as *systems neurology*, a new integrated and interdisciplinary research field [170].

From a more clinical perspective, the establishment of consortia together with the implementation of unprecedented collaborative research networks are expected to provide large, representative EOAD and LOAD study samples that can be utilized to longitudinally characterize the natural history of the disease and to understand how novel neurochemical, genetic and imaging biomarkers can predict the disease course, help monitor the response to treatment and aid in clinical decision-making. The combination of advances in the genetics of AD, along with the applications of systems biology is expected to yield new insights into the etiology of the disease and its downstream pathophysiological processes.

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ANR-10-IAIHU-06. H Hampel declares no competing financial interests related to the present article. During the last 36 months, he has received lecture honoraria and/or research grants and/or travel funding and/or participated in scientific advisory boards and/or as a consultant to diagnostic, biotechnology and pharmaceutical companies involved in the manufacture and marketing of biomarkers and/or diagnostics and/or drugs or medicinal products for cognitive impairment and Alzheimer's disease including Boehringer-Ingelheim, Bristol-Myers Squibb, Elan Corporation, Novartis, Eisai Inc., Pfizer Inc., Sanofi-Aventis, Roche Pharmaceuticals and Diagnostics, GE Healthcare, Avid, Eli Lilly and Company, GlaxoSmithKline-Biologicals, Jung-Diagnostics, Cytox, Takeda, Isis Pharmaceutical Inc. He is co-inventor in pending patent submissions relating to biological markers and/or diagnostics and has not received any royalties. S Lista declares no conflict of interest.

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PS Aisen serves on a scientific advisory board for NeuroPhage; he has served as a consultant to Elan, Wyeth, Eisai, Bristol-Myers Squibb, Eli Lilly and Company, NeuroPhage, Merck, Roche, Amgen, Genentech, Abbott, Pfizer, Novartis, Bayer, Astellas, Otsuka, Daiichi, AstraZeneca, Janssen, Medivation, Ichor, Toyama, Lundbeck, Biogen Idec, iPerian, Probiodrug, Somaxon, Biotie, Cardeus, Anavex, Kyowa Hakko Kirin Pharma, Medtronic, Abbvie, Cohbar; and he receives research support from Eli Lilly, and the NIH (NIA U01-AG10483 [PI], NIA U01-AG024904 [Coordinating Center Director], NIA R01-AG030048 [PI] and R01-AG16381 [Co-I]).

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Key issues

- The pathogenesis of Alzheimer's disease (AD) is extremely complex because it covers both genetic and environmental factors. A spectrum of genetic factors plays a central role in the expression of AD in late-onset or sporadic forms.
- Currently, accessible AD pharmacological interventions consist of cholinesterase inhibitors and an *N*-methyl-D-aspartate receptor uncompetitive antagonist, memantine. These drugs can alleviate some of the psychological/behavioral symptoms in AD patients. Effective pharmacological therapies for AD prevention and treatment – that is, disease-modifying therapies – are still missing.
- Progress in understanding the genetics and molecular pathogenesis of AD have been translated into experimental approaches aimed at slowing down the development of the disease. As a result, clinical trials assessing various potential AD treatments have been intensified. Among these, compounds targeting amyloid beta (Aβ) or tau protein likely represent the most promising therapeutic strategies.
- Approaches to immunization against Aβ require: *active immunization* utilizing full-length Aβ or Aβ analogues together with or without an adjuvant; *passive immunization*, based on the use of humanized anti-Aβ antibodies or intravenous immunoglobulins.
- Phase III clinical trials for intravenous *bapineuzumab* did not show clinical benefit on primary or secondary outcome. The meaning of its effect on lowering cerebrospinal fluid p-tau levels is uncertain but could represent an effect on further neurodegeneration. The bapineuzumab development program has been discontinued.
- Regarding *solanezumab*, while the primary end points were not met in EXPEDITION 1 and EXPEDITION 2 trials, data reported in the pooled mild AD patient subsets of the trials suggested a cognitive effect. Based on the analysis in the pooled mild AD patients, the development of solanezumab for managing AD-associated dementia continues with a third Phase III clinical trial in mild AD, called EXPEDITION 3.
- The current state of *anti-tau therapy* in humans parallels the early phases of the anti-Aβ treatment approach while it is not clear which type of Aβ or tau to target, for how long to treat or immunize and at which stage of the disease to intervene. It is also not clear which conformational state (oligomers or truncated tau or aggregated tau) is responsible for neuronal dysfunction and degeneration and how tau exerts its toxicity and, like for Aβ, it is not known at which disease stage tau aggregation blockers would be most effective.
- The debate on the new AD diagnostic criteria has encouraged the development of prevention trials in at-risk and preclinical stages of AD. Such prevention initiatives – namely, *DIAN*, *API* and *A4* – are separate, but interconnected, long-lasting projects.
- Currently, in Europe, there are three ongoing *randomized controlled trials* to prevent cognitive impairment and dementia/AD: The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability, the Multidomain Alzheimer Prevention Trial and the Prevention of Dementia by Intensive Vascular Care study. All RCTs use a multidomain interventional approach to target several dementia risk factors and reduce the risk of developing dementia.
- In the forthcoming years, future therapeutics development of AD pathology will be informed by advances in studies of whole genomes and by the introduction of more advanced systemic analyses resulting from combined transcriptomics, proteomics and metabolomics/lipidomics approaches that span from single molecules to pathway signatures in patients. Thus, a *systems biology*-based approach is expected to elucidate the pathogenesis of AD.

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