Update on Alzheimer's Disease Therapy and Prevention Strategies

W. Vallen Graham,[1] Alessandra Bonito-Oliva,[1] and Thomas P. Sakmar[1,2]

[1]Laboratory of Chemical Biology and Signal Transduction, The Rockefeller University, New York, NY 10065; email: sakmar@rockefeller.edu

[2]Department of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Division of Neurogeriatrics, Karolinska Institutet, 141 57 Huddinge, Sweden

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Abstract

Alzheimer's disease (AD) is the primary cause of age-related dementia. Effective strategies to prevent and treat AD remain elusive despite major efforts to understand its basic biology and clinical pathophysiology. Significant investments in therapeutic drug discovery programs over the past two decades have yielded some important insights but no blockbuster drugs to alter the course of disease. Because significant memory loss and cognitive decline are associated with neuron death and loss of gray matter, especially in the frontal cortex and hippocampus, some focus in drug development has shifted to early prevention of cellular pathology. Although clinical trial design is challenging, due in part to a lack of robust biomarkers with predictive value, some optimism has come from the identification and study of inherited forms of early-onset AD and genetic risk factors that provide insights about molecular pathophysiology and potential drug targets. In addition, better understanding of the Aβ amyloid pathway and the tau pathway—leading to amyloid plaques and neurofibrillary tangles, respectively, which are histopathological hallmarks of AD—continues to drive significant drug research and development programs. The main focus of this review is to summarize the most recent basic biology, biochemistry, and pharmacology that serve as a foundation for more than 50 active advanced-phase clinical trials for AD prevention and therapy.

THE SEARCH FOR DISEASE-MODIFYING AGENTS

Alzheimer's disease (AD) is the most common cause of dementia, which is defined as a significant, persistent, and progressive memory loss combined with cognitive impairment and personality change. The primary risk factor for AD is old age, so as the demographics of many societies change, the prevalence of AD and other age-related dementias is increasing. The direct societal cost of AD is second only to cancer care, but the overall societal cost of AD is underestimated because the burden of care of AD patients, especially in the early phases, often falls on family members and informal care providers. The challenge of AD and other dementias for society, in terms of healthcare systems, research and drug-discovery infrastructures, and public policy, was reviewed recently in a major *The Lancet Neurology* Commission report (1).

Despite decades of study of the basic biology of AD and significant pharmaceutical industry efforts to develop therapies, there is no effective therapy available to cure AD or to inhibit significantly the progression of AD symptoms. Although multiple so-called disease-modifying agents have been tested, the most recently approved drug of any kind for AD, memantine, was approved by the US Food and Drug Administration (FDA) back in 2003. Consequently, the strategy to identify and test drugs for AD has recently shifted to disease prevention, with clinical testing carried out in at-risk populations.

One significant challenge for drug development in AD has been a lack of validated objective diagnostic criteria and robust biological markers of disease that might be useful as clinical endpoints and efficacy standards. This limitation, together with the extremely long, symptom-free prodromal phase that characterizes AD, results in the enrollment in clinical trials of patients with already advanced pathophysiological signs of the disease. Therefore, the timing of the treatment must be considered as a possible factor in the success rate of these drugs and highlights the need for better diagnostic tools. Recent progress in developing novel biomarkers, including imaging strategies, with high predictive value at predementia stages, and in genetic risk factor analysis, has led to some renewed optimism. Many pharmaceutical companies and public–private–corporate partnerships continue to focus on AD prevention and treatment strategies. The main aim of this review is to highlight the most recent ongoing clinical trials for AD therapeutic agents with a particular focus on the underlying basic biology and pathophysiology.

CURRENTLY APPROVED DRUGS

Effective pharmacological therapy for cognitive impairment related to prodromal AD and mild AD dementia remains a major unmet need in clinical practice. Only four drugs are currently approved and marketed for the treatment of AD-associated dementia (**Table 1**), and their utility is limited. Three of these drugs act on central nervous system (CNS) cholinergic pathways, including donepezil, galantamine, and rivastigmine. All three drugs have anticholinesterase activity, and galantamine, which is a natural-product alkaloid, is also active as an allosteric modulator at nicotinic acetylcholine receptors. Each of these drugs is now available in generic formulations and is approved for mild to severe dementia, although they are often used for patients in earlier predementia stages associated with significant progressive memory impairment based on cognitive testing results.

Memantine is the drug most recently approved for AD in the United States and, notably, it is the first approved AD drug to target the *N*-methyl-D-aspartate (NMDA) receptor and glutaminergic pathways (2). Excess glutamate at excitatory synapses with associated cytotoxicity, possibly due to decreased glutamate reuptake from microglia, has recently been implicated as a pathophysiological mechanism in AD, and glutaminergic modulation affects dendritic spine clustering in a

Table 1 Update on Alzheimer's disease therapeutic agents[a]

Target type	Name	Therapy type	Status	Company
Cholinergic	Donepezil	Small molecule	Approved	Eisai Co., Ltd., Pfizer, Inc.
Cholinergic	Galantamine	Small molecule	Approved	Janssen Pharmaceutica, Ortho-McNeil Pharmaceutical, Sanochemia Pharmazeutika, Shire PLC, Takeda Pharmaceutical Company
Cholinergic	Rivastigmine	Small molecule	Approved	Novartis Pharmaceuticals
Glutaminergic	Memantine	Small molecule	Approved	Forest Laboratories, Inc., H. Lundbeck A/S, Merz Pharma
Glutaminergic	Riluzole	Small molecule	Phase II	Sanofi S.A.
γ-Secretase inhibitor	Semagacestat	Small molecule	Discontinued	Eli Lilly & Co.
γ-Secretase inhibitor	Avagacestat	Small molecule	Discontinued	Bristol-Myers Squibb
γ-Secretase inhibitor	EVP-0962	Small molecule	Phase II	FORUM Pharmaceuticals Inc.
BACE inhibitor	BI 1181181	Small molecule	Discontinued	Boehringer Ingelheim, Vitae Pharmaceuticals, Inc.
BACE inhibitor	RG7129	Small molecule	Discontinued	Roche
BACE inhibitor	LY2811376	Small molecule	Discontinued	Eli Lilly & Co.
BACE inhibitor	LY2886721	Small molecule	Discontinued	Eli Lilly & Co.
BACE inhibitor	E2609	Small molecule	Phase II	Biogen, Inc., Eisai Co., Ltd.
BACE inhibitor	AZD3293	Small molecule	Phase III	Eli Lilly & Co., AstraZeneca
BACE inhibitor	CNP520	Small molecule	Phase II/III	Amgen, Inc., Novartis Pharmaceuticals
BACE inhibitor	JNJ-54861911	Small molecule	Phase II/III	Janssen Pharmaceutica, Shionogi
BACE inhibitor	Verubecestat	Small molecule	Phase III	Merck & Co., Inc.
Aβ clearance	AN-1792	Immunotherapy (active)	Discontinued	Janssen Pharmaceutica, Pfizer, Inc.
Aβ clearance	Bapineuzumab	Immunotherapy (passive)	Discontinued	Pfizer, Inc., Johnson & Johnson Pharmaceutical Company, Janssen Pharmaceutica, Elan Pharmaceuticals, Inc.
Aβ clearance	AAB-003	Immunotherapy (passive)	Phase I	Janssen Pharmaceutica, Pfizer, Inc.
Aβ clearance	GSK933776	Immunotherapy (passive)	Discontinued	GlaxoSmithKline PLC
Aβ clearance	Solanezumab	Immunotherapy (passive)	Phase III	Eli Lilly & Co.
Aβ clearance	Crenezumab	Immunotherapy (passive)	Phase III	Genentech, Inc.
Aβ clearance	Gantenerumab	Immunotherapy (passive)	Phase III	Chugai Pharmaceutical Co., Ltd., Hoffmann-La Roche
Aβ clearance	BAN2401	Immunotherapy (passive)	Phase II	BioArtic Neuroscience AB, Biogen, Inc., Eisai Co., Ltd.
Aβ clearance	Aducanumab	Immunotherapy (passive)	Phase III	Biogen, Inc.
Tau stabilization	Epothilone D	Small molecule	Discontinued	Bristol-Myers Squibb

(*Continued*)

Table 1 (*Continued*)

Target type	Name	Therapy type	Status	Company
Tau aggregation inhibitor	Rember TM	Small molecule	Discontinued	TauRx Therapeutics Ltd.
Tau aggregation inhibitor	TRx0237	Small molecule	Phase III	TauRx Therapeutics Ltd.
p-Tau clearance	AADvac-1	Immunotherapy (active)	Phase I	Axon Neuroscience SE
p-Tau clearance	ACI-35	Immunotherapy (active)	Phase I	AC Immune SA, Janssen Pharmaceutica
Microglial activation inhibitor	Alzhemed™	Small molecule	Discontinued	Neurochem, Inc.
Microglial activation inhibitor	Azeliragon	Small molecule	Phase III	Pfizer, Inc., TransTech Pharma, Inc., vTv Therapeutics
Microglial activation inhibitor	Ibuprofen	Small molecule	Discontinued	
Microglial activation inhibitor	Flurizan™	Small molecule	Discontinued	Myriad Genetics, Inc.

[a]Drugs or drug candidates discussed in the article are listed. For a more comprehensive list, see Reference 1.

mouse model of disease (3). Accordingly, riluzole (**Table 1**), an inhibitor of glutamate release and postsynaptic glutamate receptor signaling, is in a phase II trial in mild AD patients.

The results of early limited clinical trials for the four approved drugs for AD dementia are difficult to generalize in order to provide useful guidelines for clinical practice.The long-term (for example, greater than six months) safety and efficacy of the drugs are not entirely clear. There is no clear evidence that any of the currently available drugs modifies primary pathological processes that underlie disease. However, the drugs seem to provide some symptomatic relief and are generally administered as palliative therapy with the aim of slowing the decline in quality of life, including in patients already receiving dementia care in institutional settings.

POTENTIAL THERAPEUTIC AGENTS TARGETING MOLECULAR PATHWAYS

Amyloid β and the Amyloid Hypothesis

Amyloid β (Aβ) refers to a set of hydrophobic peptides of 39–43 amino acid residues, predominantly $A\beta_{42}$ and $A\beta_{40}$, whose pathological aggregation is implicated in neuronal degeneration and cognitive decline in AD. Aβ is derived from amyloid precursor protein (APP) in a two-step proteolysis reaction by two membrane-bound enzyme complexes, β-secretase and γ-secretase. Specifically, β-secretase cleaves near the N terminus of the Aβ domain of APP to generate secreted APP-β and a membrane bound C-terminal fragment (C99) containing the entire Aβ domain, which is further cleaved by γ-secretase to generate Aβ peptides of different lengths. Alternative processing of APP by α-secretase generates secreted APPα and α-CTF, the latter also cleaved by γ-secretase. Aβ aggregates eventually self-assemble into organized macrostructures in which the constituent Aβ monomers display characteristic secondary β-sheet and supersecondary reverse turn structures (**Figure 1*a***). These transient "protofibrils" damage neurons and go on to

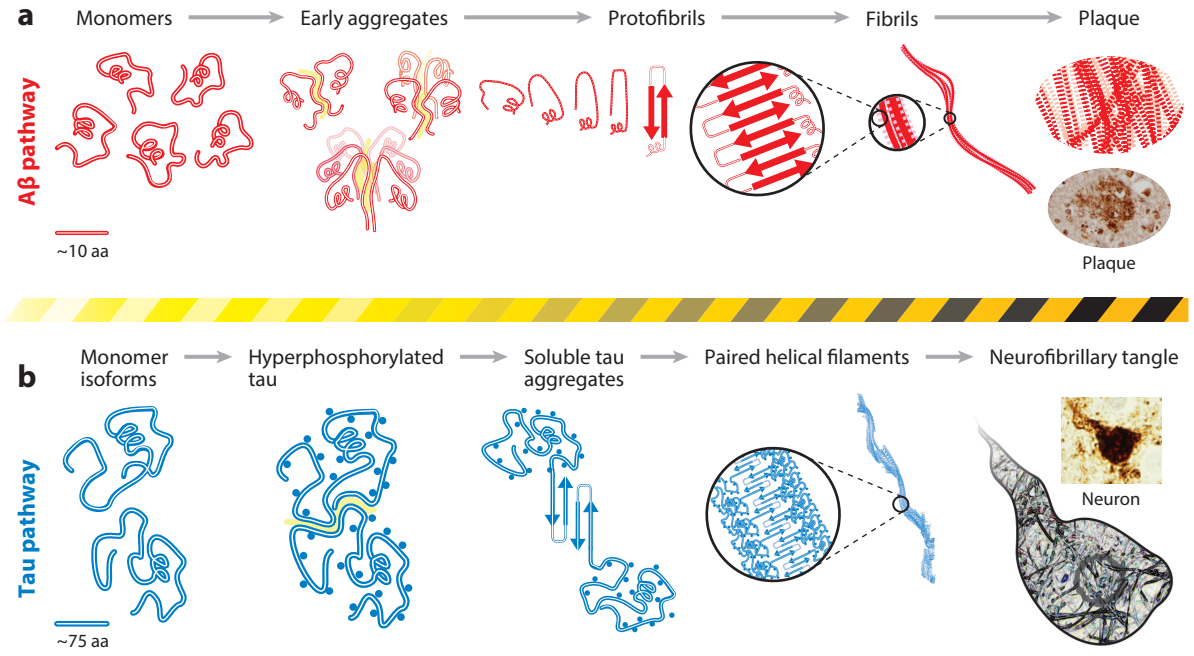

Figure 1

Alzheimer's disease (AD) is characterized by neuronal death, which is usually correlated with the appearance of amyloid plaques and neurofibrillary tangles (NFTs). (*a*) The Aβ pathway leading from monomers of Aβ peptides (predominantly 40 and 42 amino acid residues in length) to insoluble AD plaques is depicted schematically (not drawn to scale). The processing of amyloid precursor protein (APP) to create Aβ peptides is described in the text. Aβ spontaneously self-assembles to form aggregates and then protofibrils, which are a heterogeneous class of soluble prefibrillar species with a characteristic secondary and supersecondary structure. Protofibrils go on to form fibrils, which "mature" to form plaques that disrupt normal tissue architecture. Congo red–staining plaques are a histopathological hallmark of AD. The Aβ pathway is primarily an extracellular pathway. Therapeutic strategies that target the Aβ pathway are described in the text. Drugs and biologics in development, including monoclonal antibodies, are designed to influence each step. (*b*) The tau pathway leading from monomers of microtubule-associated protein tau isoforms to NFTs is depicted schematically (not drawn to scale). Native functional tau monomer isoforms are about 350–440 amino acid residues in length. Mutation, proteolysis, association with polyamines, or a combination of factors causes hyperphosphorylation (indicated by blue dots) by cellular kinases and both disulfide-dependent and -independent dimerization. Soluble tau aggregates subsequently assemble and form so-called paired helical filaments that go on to form intracellular NFTs, a process that causes cell death. The tau pathway is primarily an intracellular pathway that affects neurons. Therapeutic strategies that target the tau pathway, described in the text, focus mainly on limiting pathological tau phosphorylation that drives early aggregation. Illustration: Karina Åberg.

form linear filaments and fibrils that deposit in specific regions of the brain as plaques, which are histologically pathognomonic for AD when stained with Congo red dye.

Based in part on the identification in 1991 of mutations in the APP gene (4) and in genes encoding one subunit of the γ-secretase complex that cleaves APP, presenilin (5, 6), the amyloid cascade hypothesis (7, 8) suggested that the pathological aggregation of Aβ described above was the primary cause of disease. Therefore, β- and γ-secretase have attracted strong interest as potential targets for drugs that might reduce Aβ production. However, as discussed below, progress has been limited. The extreme complexity of plaque production also suggests multiple approaches aimed at halting pathological Aβ aggregation by targeting specific molecular intermediates in the pathway from monomers to plaques (**Figure 1*a***).

Modulation of Amyloid β Production

Modulating Aβ production has proven difficult, in part because of the intricacy of the γ-secretase complex. Each of its four subunits is regarded as a potential therapeutic target: nicastrin (NCSTN), presenilin (PEN-1), anterior pharynx-defective 1 (APH-1), and presenilin enhancer 2 (PEN-2) (9). Because γ-secretase complex is now known to cleave up to 50 different type 1 transmembrane protein substrates besides APP, the identification of a selective and specific inhibitor, ideally for only APP processing, represents a tremendous drug-development challenge.

Among its numerous downstream effects, γ-secretase plays a key role in controlling the proteolysis of the transmembrane domain of the Notch receptors, with critical consequences for many different kinds of cell differentiation events and eventually the expression of genes that control cell fate (10). The effects of γ-secretase inhibitors on Notch proteolysis can lead to toxic effects, including gastrointestinal bleeding and immunosuppression (11, 12). The interaction with Notch is among the reasons why numerous γ-secretase inhibitors have failed advanced-phase clinical trials (**Table 1**). Semagacestat (Eli Lilly & Co.) not only failed to achieve endpoints related to slowing disease progression but also appears to have worsened some patients' symptoms (13), and Avagacestat (Bristol-Myers Squibb) caused serious adverse events such as cerebral microbleeds, dose-dependent glycosuria, and nonmelanoma skin cancer (14).

A new generation of γ-secretase inhibitors and modulators, including some nonsteroidal anti-inflammatory drugs (NSAIDs), are designed to reduce $A\beta_{42}$ selectively, with little or no effect on physiological Notch cleavage (15) (**Table 1**). Currently in a phase II trial, EVP-0962 (FORUM Pharmaceuticals, Inc.) is a γ-secretase modulator that reduces the production of $A\beta_{42}$ by shifting the APP cleavage toward the production of shorter and less toxic Aβ peptides, without affecting Notch cleavage.

The regulation of γ-secretase complex activity is still far from being understood in detail. For example, the composition of the complex as well as other γ-secretase-associated proteins might affect substrate selectivity and cleavage (16). Among them, SLC2A13 is a multi-transmembrane protein highly expressed in the brain that affects Aβ production dose-dependently without affecting Notch cleavage (16). Furthermore, silencing the expression of Erlin-2 or NADH dehydrogenase ubiquinone iron-sulfur protein 7 (NDUFS7) downregulated Aβ production, and silencing expression of tubulin polymerization promoting protein (TPPP) caused upregulation of Aβ, each without affecting Notch cleavage (17, 18). Therefore, modulation of the γ-secretase complex through targeting γ-secretase-associated proteins might be a viable therapeutic approach.

After the apparent failure of early γ-secretase-based drug candidates, a β-secretase—namely β-site APP cleaving enzyme 1 (BACE1)—became the favored target in the chase for Aβ-centered therapeutics. Because BACE1 is the first enzyme to cleave APP, it plays a crucial role in the generation of Aβ, and BACE1 message levels are increased in both AD patients and animal models of disease (19). β-Secretase is a type 1 transmembrane aspartic acid protease related to the pepsin family with two aspartic acid protease motifs (Asp-Thr/Ser-Gly-Ser/Thr) in its catalytic domain that form the active site of the enzyme. Since its discovery in 1999, BACE1 has been heavily pursued as a small-molecule drug target (20). The BACE2 homologue has 64% amino acid similarity to BACE1, suggesting that it might also be a β-secretase. Although animal studies have not found any causative link between BACE2 and pathological Aβ in the brain, it seems too early to exclude any possible off-target effects of BACE1 inhibitors on BACE2 (21).

The first generation of BACE1 inhibitors comprised noncleavable peptide-based transition-state analogues (e.g., BI 1181181, Boehringer Ingelheim, Vitae Pharmaceuticals, Inc.) (**Table 1**). They failed because of low oral bioavailability and low blood–brain barrier penetration. The second-generation BACE1 inhibitors were supposed to be more lipophilic and to cross plasma

and endosomal membranes to reach the BACE1 active site (**Table 1**). However, many compounds failed advanced-phase clinical trials because of liver toxicity (e.g., RG7129, Roche; LY2811376 and LY2886721, Eli Lilly & Co.). More recently, a potent third-generation small-molecule BACE1 inhibitor showed satisfactory pharmacokinetics and provided encouraging clinical data in ongoing studies [phase II, E2609 (Biogen, Inc., Eisai Co., Ltd.); phase II/III, AZD3293 (AstraZeneca, PLC, Eli Lilly & Co.), CNP520 (Amgen, Inc., Novartis Pharmaceuticals), JNJ-54861911 (Janssen Pharmaceutica, Shionogi); phase III, Verubecestat (Merck & Co.) (**Table 1**)].

One recent novel and promising approach utilizes anti-BACE1 antibodies (Abs) characterized by high target specificity and excellent serum pharmacokinetics. Atwal et al. (22) showed that anti-BACE1 Abs delivered systemically in various animal models reduced Aβ concentrations in the periphery as well as in the brain. An alternative immunotherapy approach consists of site-directed Abs that affect the BACE-APP complex by targeting the β-secretase cleavage site of APP. Therefore, anti-APP Abs would preserve BACE activity directed toward non-APP substrates and selectively interfere with the BACE-APP complex, inhibiting both intracellular and extracellular Aβ formation (23). One challenge in the use of an immunotherapeutic approach for neurodegenerative disease therapy is limited blood–brain barrier penetration of conventional Abs and immune effector activation, but these challenges can often be addressed through protein engineering.

Increasing Amyloid β Degradation: The Defective-Clearance Hypothesis

Aβ is constantly metabolized, and its net content in the brain results from the equilibrium between overall rates of production and clearance. Numerous peptidases and proteinases, known collectively as Aβ-degrading proteases (AβDPs), affect Aβ levels. The finding that patients with sporadic AD tend to have deficient cerebrospinal fluid clearance of Aβ (24) supports the hypothesis that increased Aβ levels may be caused not only by elevated production but also by diminished degradation (25). Although AβDPs work together to degrade Aβ, their specific regional and subcellular localization results in different pools of peptide, with possible differences in pathogenicity (26). Functionally, AβDPs can be classified as endogenous or pathogenic regulators depending on their preferential activity under physiological or pathological conditions, respectively (26).

The degradation effect of AβDPs on Aβ monomers, and in some cases Aβ fibrillar forms, has paved the way for pharmacological as well as gene-therapy approaches. Examples of pharmacological treatments are drugs that enhance Aβ degradation directly by stimulating the expression of AβDPs (27) or indirectly by inhibiting the endogenous inhibitors that regulate AβDPs (28). For example, increased endogenous levels of the AβDP cysteine protease cathepsin B (29), as well as decreased levels of its inhibitor cystatin C (30), result in increased Aβ degradation and neuroprotection in animal models.

Immunotherapy to Increase Amyloid β Clearance

The first report of a clinically relevant immunotherapy treatment, published in 1999, showed that active immunization achieved by using synthetic human $A\beta_{42}$ significantly reduced Aβ plaque formation (31) and prevented memory deficit (32) in AD animal models. However, when these findings were applied to patients, an active immunization study with the full-length $A\beta_{42}$, AN-1792 (Janssen Pharmaceutica, Pfizer, Inc.) (**Table 1**), was halted despite the proven reduction of Aβ levels after 6% of the patients developed severe meningoencephalitis (33). As a consequence of this failure, passive immunization with systemic infusion of monoclonal Abs (mAbs) directed at Aβ was developed as an alternative. This approach showed the potential to prevent oligomerization and fibril formation (34, 35) and to dissolve Aβ aggregates (36, 37). Despite low blood–brain barrier penetration, mAbs provide high specificity and affinity toward their antigen, low toxicity, and good plasma pharmacokinetics.

It has been suggested that the reduction of Aβ induced by mAbs can be explained by two distinct, but not necessarily alternative, mechanisms: microglia activation through Fc receptors and the "peripheral sink effect." Whereas the first mechanism might lead to inflammation, the peripheral sink effect blocks Aβ deposition by binding to and reducing soluble Aβ circulating in the bloodstream and might draw out Aβ from the brain (38). The high number of mAb candidates that have failed clinical trials suggests that the choice of mAb epitope is crucial. The N terminus of the Aβ protein is freely accessible in both monomeric and aggregated forms, but mAbs directed against the N-terminal tail have failed owing to adverse events.

Bapineuzumab (Pfizer, Inc., Johnson & Johnson Pharmaceutical Company, Janssen Pharmaceutica and Elan Pharmaceuticals, Inc.) (**Table 1**) is a humanized mouse IgG1 mAb that binds an N-terminal epitope ($A\beta_{1-5}$). Interestingly, both the therapeutic and adverse effects of bapineuzumab treatment depend on ApoE4 genotype. ApoE4 carriers did not show any improvement but developed much more severe vasogenic edema. In non-ApoE4 carriers, the drug seemed to be safe and well tolerated; it dose-dependently cleared cerebral Aβ and moderately slowed cognitive decline. However, phase III clinical development was terminated because primary clinical endpoints were not met, and possibly because of the risk of vasogenic cerebral edema. A derivative of bapineuzumab, AAB-003 (**Table 1**), modified to reduce the mAb effector function on microglial activation, is currently in phase I trials. GSK933776 (GlaxoSmithKline, PLC) (**Table 1**), a humanized mAb directed against the N-terminal tail of Aβ, was engineered to have an inactive Fc in order to avoid the risk of vasogenic edema. Despite the apparent general safety of the mAb, a clinical trial for AD was suspended.

The safety profile of mAbs against the central epitope of Aβ is more promising. Solanezumab (Eli Lilly & Co.) (**Table 1**) binds to soluble monomers and reduces $A\beta_{42}$ levels by shifting the equilibrium toward the production of shorter and less toxic Aβ species, demonstrating the peripheral sink effect discussed above. Solanezumab recognizes a central epitope region $A\beta_{16-24}$ and, interestingly, the ApoE4 genotype seems not to affect drug action. Phase III trials are ongoing to confirm solanezumab's efficacy as a disease-modifying agent and possible disease-prevention agent.

Unlike solanezumab, the mAb crenezumab (Genentech, Inc.) (**Table 1**) shows high affinity for aggregated Aβ, including oligomers, fibrils, and plaques, and low affinity for monomeric Aβ. Crenezumab binds to the central epitope region of $A\beta_{12-23}$, which seems to be most responsible for aggregation. Importantly, crenezumab contains a human IgG4 backbone that translates into reduced effector function on microglia, promotion of microglial Aβ phagocytosis, and reduced proinflammatory response. Clinical data indicate that crenezumab reduces plaques with no vasogenic edema risk, and it is currently being tested in a phase II trial and in a five-year prevention trial. Gantenerumab (Chugai Pharmaceutical Co., Ltd., Roche) (**Table 1**) is the first fully human anti-Aβ IgG1 that appears to recognize both the N-terminal tail ($A\beta_{3-12}$) and the central region ($A\beta_{18-27}$). It has moderate binding affinity to monomers and oligomers and potently binds to and degrades fibrils (39). Its proposed mechanism of action is based on the recruiting of microglia and activation of phagocytosis. It seems to be well tolerated and is currently in a phase III trial for AD and in a phase II/III trial for dementia prevention.

Accumulating data point to the soluble intermediate protofibril aggregates in the Aβ aggregation pathway as the most toxic species (40). A new generation of mAbs has been engineered to bind to the transient and soluble protofibrils with the dual goal of reducing toxicity and preventing further aggregation. BAN2401 (BioArtic Neuroscience AB, Biogen, Inc., Eisai Co., Ltd.) (**Table 1**) was obtained by immunizing mice with protofibrils originating from the "Arctic mutant" form of Aβ42, a mutated protein associated with a high-protofibril-content form of AD (41). BAN2401 is a humanized mAb that recognizes large (>100 kDa) protofibrils and does not seem to bind

significantly to fibrils or monomers. It is currently in phase II trials and seems to be generally safe and well tolerated.

Finally, produced through a "reverse translational medicine" approach, aducanumab (Biogen, Inc.) (**Table 1**) is a fully human IgG1 mAb obtained from a screen of healthy advanced-age donors with normal cognition who are hypothesized to harbor naturally developed mAbs against Aβ. The mAb selectively targets and dose-dependently reduces amyloid deposition and slows cognitive decline. Aducanumab is currently in a phase III trial (42).

Inhibiting Protein Aggregation: The Role of Molecular Chaperones

Molecular chaperones mediate proper protein folding and help to assure physiological protein conformation during cellular stress. In some cases, chaperones mediate the transfer of misfolded proteins to the proteasome for degradation (43). In recent years, increasing evidence suggests that molecular chaperones might modulate the aggregation of amyloid proteins and therefore play a key protective role in neurodegenerative diseases characterized by protein misfolding. This hypothesis relies on the observation, supported by in vitro and animal studies, that several chaperones and chaperone-like proteins, including the extracellular chaperone clusterin and several heat shock proteins (HSP70, HSP90, and DNAJ) (40–46), share the ability to decrease significantly pathological aggregation of proteins such as Aβ (44), islet amyloid polypeptide (IAPP) (45), the yeast prion protein Ure2p (46), and the polyglutamine peptide (47).

Importantly, each chaperone seems to interfere with amyloid aggregation through a different mechanism, and the efficiency of the aggregation inhibition appears to be a function of the specific aggregation phase that the chaperone affects (44). Indeed, the aggregation of amyloid proteins results from a variety of microscopic chemical events, such as primary nucleation, fibril elongation, fibril fragmentation, and secondary nucleation (48). The nonlinearity of this process (49) generates a heterogeneous population of protein species ranging from monomers to oligomers and from protofibrils to long fibrils, and each of these species represents a possible chaperone target. According to the kinetic model recently presented by Arosio et al. (44), the effect of the chaperone changes the relative contribution of each microscopic event and changes the overall rate of aggregation. This model allows analysis of changes in kinetic rates in order to understand the microscopic events affected by the chaperone and therefore make predictions about the aggregate species that should be targeted. For example, chaperones that preferentially inhibit primary nucleation bind intermediate aggregates rather than monomers (e.g., DNAJB6-mediated inhibition of $A\beta_{42}$), whereas proteins that selectively affect secondary nucleation affect the catalytic activity of the fibril surface (e.g., the molecular chaperone domain BRICHOS-induced effect on $A\beta_{42}$ aggregation). Some chaperones and chaperone-like proteins can interfere with more than one microscopic event, achieving a more potent inhibition of aggregation (e.g., αB-crystallin and Bri2 BRICHOS affect both elongation and secondary nucleation of $A\beta_{42}$) (44).

In this scenario, it is worth noting that the chaperone-like protein nucleobindin-1 (NUCB1) appears to bind several different amyloid proteins by recognizing the common structure of soluble intermediate species and stabilizing short, nontoxic protofibrils (A. Bonito-Oliva, S. Barbash, T.P. Sakmar, W.V. Graham, unpublished observations). Interestingly, brain specimens from AD patients show reduced levels of NUCB1 (50, 51).

Together, these data indicate that chaperones and chaperone-like molecules are potential tools to prevent and/or inhibit early aggregates of Aβ. Understanding the mechanisms that regulate the interaction between given chaperones and specific amyloid proteins offers precious insights into potential new therapeutic approaches. Although still in the preclinical research phase, the use of endogenous chaperones or engineered recombinant versions represents a novel and intriguing approach to the potential treatment of AD and other neurodegenerative diseases.

Neurofibrillary Tangles as Drug Targets

Neurofibrillary tangles (NFTs) are a primary histological marker of AD and are composed of hyperphosphorylated, aggregated tau protein (**Figure 1*b***). The molecular events leading to NFT formation are unclear, but the field has made great strides over the past few years (52). As understanding of the required pathological events leading to NFT formation and neurodegeneration expands, several potential drug targets are being uncovered.

Physiologically, tau is a natively unfolded, hydrophilic, microtubule-binding protein found primarily in neurons in the brain. The six main isoforms of tau in the adult brain result in varying affinities for microtubules, so splicing events may play a role in the propensity for aggregation in tauopathies (53). Pathological tau can cause disturbances of microtubules leading to neuronal degeneration, and aggregated tau is cytotoxic. Many of the emerging targets described below have pleiotropic activities and therefore represent challenges for robust therapeutic targeting. Perhaps the best path forward is to lower the level of tau in the brain through targeting tau expression, stabilizing tau conformations, or clearing hyperphosphorylated tau aggregates.

Regulation of gene expression and splicing mechanisms are emerging targets in tau pathophysiology. Tau knockout mice lack an obvious phenotype, although they have age-dependent deficits in axonal microtubule density (54). Furthermore, tau deficiency can protect from Aβ-induced toxicity (55, 56). Antisense targeting of tau to decrease expression diminishes seizures in mice, indicating that tau may mediate a pathological signaling network in neurons (57). Recently, miR-219 was shown to bind the 3′ untranslated region of tau RNA and downregulate its expression (58). In AD, miR-219 is downregulated, possibly indicating a direct role in pathogenesis of the disease and an opportunity for therapeutic intervention.

The tau protein can undergo many forms of posttranslational modification, including acetylation, ubiquitination, glycosylation, and phosphorylation. The most extensively studied modification is phosphorylation. Hyperphosphorylated tau is the form of the protein found in the paired helical filaments that make up NFTs. These posttranslational modifications lead to disruptions in microtubule binding and destabilization of the cytoskeleton, which in turn leads to the observed neurodegeneration and neuronal cell death.

Because hyperphosphorylated tau leads to cytoskeletal disruptions along axons, a reasonable therapeutic approach would be to restabilize the microtubules to preserve neuronal health and axonal transport. Paclitaxel-derived products represent a class of compounds that stabilize microtubules (**Table 1**). Epothilone D (Bristol-Myers Squibb) is a derivative of paclitaxel that has good brain penetrance (59). Epothilone D has been shown to reduce transport deficits and protect from cognitive impairment in tau transgenic mice (59, 60). Epothilone D has been evaluated in a phase I trial, although it is not currently being pursued as a therapeutic for AD.

Targeting Tau Aggregates

Although tau is thought to be natively unfolded, there is evidence that the protein can form a "paper-clip" shape where the N-terminal and C-terminal repeat regions loop back on each other, likely preventing rapid aggregation (61). Truncations can disrupt this conformation, which leads to higher propensity for aggregation, probably because the stabilized loop structure is disrupted. Paired helical filaments (PHFs) consist of the repeat regions at the core and the N and C termini forming a "fuzzy coat" (62). Aggregated tau has been described as a two-layered polyelectrolyte brush because of the heterogeneity of the N- and C-terminal structures (63).

Aggregation occurs through a nucleation-dependent elongation mechanism (64). In vitro–generated tau seeds derived from disrupted PHFs can induce tau aggregation in cell culture and in mice (65). In fact, tau may adopt stable seed structures, displaying prion-like characteristics

(66, 67). Similar to Aβ plaques, mature tau fibrils may be protective and absorb toxic oligomers, although they may sequester other cell components causing neurotoxicity. Studying early tau aggregates such as oligomers and protofibrils has been challenging because of the heterogeneity of the protein species and the lack of tools and assays for study.

Prevention of aggregation regardless of phosphorylation or other tau modification is a therapeutic approach. Derivatives of methylene blue have been shown to disrupt the aggregation of tau, thereby reducing oxidative stress, preventing mitochondrial damage, and preserving cognitive function in mice (68, 69) (**Table 1**). The clinically applied derivative, Rember TM (TauRx Therapeutics Ltd.), showed some significant improvement of AD-related symptoms, although there were side effects. A second-generation version, TRx0237 (TauRx Therapeutics Ltd.), is currently in a phase III trial.

Active immunization against phosphorylated tau is a viable approach for eliciting the activation of the immune system and production of high-affinity Abs against the target. Two active immunization vaccines (**Table 1**) are currently in clinical trials: AADvac-1 (Axon Neuroscience SE), a tau 294–305 linked to keyhole limpet hemocyanin with an N-terminal cysteine, and ACI-35 (AC Immune SA, Janssen Pharmaceutica), a tau 393–408 (pS396, pS404) tetra-palmitoylated phospho-tau peptide (70, 71).

There are also a number of passive immunization trials in progress (recently reviewed in 72). The targets of these Abs can be generalized into four categories: hyperphosphorylated tau, conformations of tau, fragments of tau, and total tau. Targeting hyperphosphorylated or specific conformations of tau has the advantage of clearance of pathological tau and tau aggregates, but there may be inherent issues with this approach because of the heterogeneity of the disease system. Clearance of total tau or fragments of tau may have therapeutic value because there is some evidence that downregulation of tau expression has benefit (discussed above).

EMERGING THERAPEUTIC TARGETS AFFECTING CELLULAR SYSTEMS

Targeting Neuroinflammation

After Aβ plaques and tau NFTs, neuroinflammation is the third neuropathological correlate of AD. AD brain tissue presents clear evidence of astrogliosis and other inflammation-related signs surrounding amyloid plaques. Microglia are a heterogeneous group of cells constantly motile in the CNS, where they control many homeostatic functions and mediate the CNS immune response. Microglia continuously scavenge the healthy brain in search of signs of damage and are ready to provide trophic support for neurons. In the case of brain insult, they represent the main form of active immune defense; they migrate to surround the damaged area and clear cellular debris (73). Despite the vital and dynamic role of microglia in the healthy brain, they are subjected to both physiological and pathological aging. During physiological aging, the structural and functional brain changes are accompanied by a chronic mild inflammation state (74). Different hypotheses have been formulated to explain the role of microglia during physiological aging. On the one hand, microglia have been described as progressively more sensitive to oxidative stress and DNA damage, showing reduced motility and altered gene expression (75). On the other hand, it has been postulated that a lifelong exposure to insults results in microglia hypersensitivity that favors proinflammatory instead of anti-inflammatory behavior (76).

In this regard, the fact that age is the most predominant risk factor for many neurodegenerative diseases cannot be neglected. In cases of chronic insult, as during AD or other neurodegenerative diseases, microglia cells present clear pathological alterations. For a long time, these changes have

been considered signs of microglia reaction to the neuroinflammatory state, but recent advances in biotechnology and genetics paved the way for descriptive genome-wide association studies (77) and functional analysis of expression quantitative trait loci on isolated microglia cells (see 76 for review). Analysis of the correlation between single-nucleotide polymorphisms and disease or specific endophenotypes of the disease led to the identification of several susceptibility loci, many of them related to immune functions (e.g., APOE, CR1, BIN1, CD33, TREM2, PICALM) (see 76 for review). Among them, preclinical and clinical studies indicate a strong association between reduced TREM2 and increased CD33 expression with increased Aβ accumulation (78). These data agree with several lines of evidence indicating that microglia hyperactivation is intimately associated with formation of amyloid plaques and AD progression. AD brain tissue displays upregulated levels of chemokines and chemokine receptors (79) released in response to microglia-mediated inflammatory response, which may be involved in plaque-associated inflammation and neurodegeneration.

The identification of microglia-related risk alleles represents a fundamental step toward risk prediction and preventive interventions. Despite the still unclear role of microglia in AD neurodegeneration, it has to be acknowledged that many disease-modifying treatments currently in clinical trials have been demonstrated to affect microglia inflammation. As described above, convincing evidence indicates that some Abs might clear Aβ plaques by activating microglia and Fcγ receptor (FcγR)–mediated phagocytosis. Interestingly, Abs with high affinity for FcγR are more likely to be associated with amyloid-related imaging abnormalities. Several small molecules showed encouraging results in the treatment of AD by acting on microglia (**Table 1**). Tramiprosate (Neurochem, Inc.) binds to soluble Aβ and prevents Aβ aggregation, favors soluble Aβ clearance, and inhibits the inflammatory response associated with amyloid build-up (80). A phase III trial showed safety and tolerability, but the study is now inactive.

More directly applicable to microglia, azeliragon (Pfizer, Inc., TransTech Pharma, Inc., vTv Therapeutics, Inc.) (**Table 1**) is a small-molecule inhibitor of receptor for advanced glycation end products (RAGE), a cell-surface receptor of the immunoglobulin superfamily. RAGE binding to advanced glycation end products causes inflammation and oxidative damage. RAGE is importantly implicated in AD. It binds Aβ (81), seems to be involved in the toxic effects of Aβ oligomers in neurons, and is upregulated in the brains of AD patients (82). In animal models, azeliragon treatment showed decreased brain Aβ levels and improved cognitive performances. Azeliragon is currently in phase III trials.

A recent attempt to treat AD by targeting microglia is based on clinical and preclinical evidence suggesting that the expansion of the microglial population during neurodegeneration is highly dependent on proliferation of resident cells (83–85). This observation led to the study of possible strategies to target the microglia proliferative activity [e.g., inhibition of the colony-stimulating factor 1 receptor (CSF1R)]. The association between neuroinflammation and AD suggests that NSAIDs might have therapeutic potential (**Table 1**). However, different compounds failed in clinical trials (ibuprofen and flurizan; Myriad Genetics, Inc.), probably because of pharmacodynamics limitations. Overall, no evidence seems to support the use of NSAIDs for AD treatment based on neuroinflammation as a target (reviewed in 86).

An emerging hypothesis is that the physiological role of amyloid is to provide antimicrobial peptide properties (87–89), and that AD pathophysiology may result from dysregulated innate immunity. The normal antimicrobial peptide activity of Aβ might protect the host from infection through nonspecific agglutination and "caging" of invading species utilizing oligomerization and fibrillization. Kumar et al. (90) report that Aβ is associated with increased survival in cell culture and in nematode and mouse models of infection. APP knockout mice exhibited higher mortality than wild-type mice after infection with *Salmonella typhimurium*, consistent with a protective role

for Aβ in innate immunity. The authors offer a model by which the heparin-binding motif aids in Aβ oligomer binding to carbohydrates in the cell wall of microbes, and growing protofibrils and fibrils not only prevent microbial adhesion but also entrap the organism with a protease-resistant amyloid network (90). In light of AD pathophysiology, these data suggest innate immune dysregulation leading to excessive Aβ deposition. Whether an "infection hypothesis" for AD pathology is substantiated remains to be determined, but this exciting emerging hypothesis might lead to additional therapeutic opportunities.

Targeting Metabolic Disorders

There is a growing body of evidence that metabolism and metabolic disorders can play a role in AD pathology. Indeed, research indicates that treating the whole body rather than the CNS alone may have advantages. The lipid-trafficking molecule ApoE4 is a major risk factor for the development of AD as well as type 2 diabetes (91), and several studies have linked obesity and energy regulation with AD (92–94). Reducing caloric intake and adhering to a "Mediterranean diet" appear to improve cognitive health (95, 96). However, the molecular pathways involved are not clear.

Insulin resistance may be a factor in neurodegeneration, and insulin receptors have been shown to be increased in AD brains, perhaps due to a compensatory mechanism from global insulin resistance (97). Insulin resistance can lead to hyperinsulinemia, thereby occupying insulin-degrading enzyme (IDE), which is essential for Aβ degradation and clearance (98). Based on this work, one would hypothesize that drugs targeting insulin signaling may promote cognitive improvement in models of AD. Indeed, in studies of a mouse model of AD and in a human trial, diabetes-modifying drugs caused cognitive improvement over control (99–101). Therefore, insulin signaling seems to be a novel therapeutic target for AD.

PERSPECTIVES

This review has provided a discussion of drug targets and potential disease-modifying therapeutics for AD. A resounding problem thus far has been the high failure rate of drugs in clinical trials, due in part to the complexities of measuring drug efficacy in a disease with a long prodromal phase, often-insidious onset, and rates of progression that may vary widely from individual to individual. In addition, clinical endpoints in AD trials tend to be subjective because of a lack of quantitative biomarkers of disease progression. Although progress in early diagnostic approaches has been made, there is an urgent need for accessible diagnostic assays that detect the earliest biomarkers of disease.

The complex multidomain molecular and cellular pathophysiological nature of AD, involving a causal and temporal hierarchy of Aβ aggregation, tau pathology, and neuroinflammation, is not fully understood. Recently, Jack et al. (102) proposed a descriptive classification scheme to be used as a framework whereby patients are classified based on the presence or absence of three key factors: Aβ (A), NFTs (T), and neurodegeneration (N), to be complemented by cognitive evaluation. The A/T/N classification system is not an individual diagnostic tool, but rather a biomarker classification scheme that allows the description and characterization of a wide range of patients, based on specific biomarkers. With an improved disease classification scheme, clinical-trial cohorts might be targeted more appropriately with drug candidates designed to ameliorate specific pathological mechanisms. Data from such trials might also help to distinguish the direct causes from secondary disease consequences in AD, especially as drug-discovery paradigms shift from a search for single-agent cures to stepwise improvements in therapeutic approaches, and even patient-specific precision treatments.

If there is one overriding lesson to be learned from AD drug-development efforts to date, it is that more basic knowledge about AD and other neurodegenerative diseases is still needed. Mustering and organizing the multidisciplinary resources needed to develop effective AD therapeutics will require unprecedented public–private partnerships and international collaboration.

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LITERATURE CITED

1. Winblad B, Amouyel P, Andrieu S, et al. 2016. Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol.* 15:455–532
2. Winblad B, Poritis N. 1999. Memantine in severe dementia: results of the 9M-Best Study (benefit and efficacy in severely demented patients during treatment with memantine). *Int. J. Geriatr. Psychiatry* 14:135–46
3. Pereira AC, Lambert HK, Grossman YS, et al. 2014. Glutamatergic regulation prevents hippocampal-dependent age-related cognitive decline through dendritic spine clustering. *PNAS* 111:18733–38
4. Goate A, Chartier-Harlin MC, Mullan M, et al. 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349:704–6
5. Sherrington R, Rogaev EI, Liang Y, et al. 1995. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375:754–60
6. Goate A, Hardy J. 2012. Twenty years of Alzheimer's disease-causing mutations. *J. Neurochem.* 120(Suppl. 1):3–8
7. Hardy JA, Higgins GA. 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256:184–85
8. Hardy J, Selkoe DJ. 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297:353–56
9. McCarthy JV, Twomey C, Wujek P. 2009. Presenilin-dependent regulated intramembrane proteolysis and gamma-secretase activity. *Cell. Mol. Life Sci.* 66:1534–55
10. Kopan R, Ilagan MX. 2009. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* 137:216–33
11. Searfoss GH, Jordan WH, Calligaro DO, et al. 2003. Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. *J. Biol. Chem.* 278:46107–16
12. Wong GT, Manfra D, Poulet FM, et al. 2004. Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J. Biol. Chem.* 279:12876–82
13. Doody RS, Raman R, Farlow M, et al. 2013. A phase 3 trial of semagacestat for treatment of Alzheimer's disease. *N. Engl. J. Med.* 369:341–50
14. Coric V, van Dyck CH, Salloway S, et al. 2012. Safety and tolerability of the gamma-secretase inhibitor avagacestat in a phase 2 study of mild to moderate Alzheimer disease. *Arch. Neurol.* 69:1430–40

15. Wolfe MS. 2007. gamma-Secretase modulators. *Curr. Alzheimer Res.* 4:571–73
16. Teranishi Y, Inoue M, Yamamoto NG, et al. 2015. Proton myo-inositol cotransporter is a novel gamma-secretase associated protein that regulates Aβ production without affecting Notch cleavage. *FEBS J.* 282:3438–51
17. Frykman S, Teranishi Y, Hur J-YY, et al. 2012. Identification of two novel synaptic γ-secretase associated proteins that affect amyloid β-peptide levels without altering Notch processing. *Neurochem. Int.* 61:108–18
18. Teranishi Y, Hur J-YY, Gu GJ, et al. 2012. Erlin-2 is associated with active γ-secretase in brain and affects amyloid β-peptide production. *Biochem. Biophys. Res. Commun.* 424:476–81
19. Kandalepas PC, Sadleir KR, Eimer WA, et al. 2013. The Alzheimer's beta-secretase BACE1 localizes to normal presynaptic terminals and to dystrophic presynaptic terminals surrounding amyloid plaques. *Acta Neuropathol.* 126:329–52
20. Ghosh AK, Gemma S, Tang J. 2008. beta-Secretase as a therapeutic target for Alzheimer's disease. *Neurotherapeutics* 5:399–408
21. Yan R, Vassar R. 2014. Targeting the beta secretase BACE1 for Alzheimer's disease therapy. *Lancet Neurol.* 13:319–29
22. Atwal JK, Chen Y, Chiu C, et al. 2011. A therapeutic antibody targeting BACE1 inhibits amyloid-beta production in vivo. *Sci. Transl. Med.* 3:84ra43
23. Arbel M, Yacoby I, Solomon B. 2005. Inhibition of amyloid precursor protein processing by beta-secretase through site-directed antibodies. *PNAS* 102:7718–23
24. Mawuenyega KG, Sigurdson W, Ovod V, et al. 2010. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 330:1774
25. Selkoe DJ. 2001. Clearing the brain's amyloid cobwebs. *Neuron* 32:177–80
26. Saido T, Leissring MA. 2012. Proteolytic degradation of amyloid beta-protein. *Cold Spring Harb. Perspect. Med.* 2:a006379
27. Saito T, Iwata N, Tsubuki S, et al. 2005. Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nat. Med.* 11:434–39
28. Jacobsen JS, Comery TA, Martone RL, et al. 2008. Enhanced clearance of Abeta in brain by sustaining the plasmin proteolysis cascade. *PNAS* 105:8754–59
29. Mueller-Steiner S, Zhou Y, Arai H, et al. 2006. Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. *Neuron* 51:703–14
30. Sun B, Zhou Y, Halabisky B, et al. 2008. Cystatin C-cathepsin B axis regulates amyloid beta levels and associated neuronal deficits in an animal model of Alzheimer's disease. *Neuron* 60:247–57
31. Schenk D, Barbour R, Dunn W, et al. 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173–77
32. Morgan D, Diamond DM, Gottschall PE, et al. 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982–85
33. Holmes C, Boche D, Wilkinson D, et al. 2008. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 372:216–23
34. Solomon B, Koppel R, Hanan E, et al. 1996. Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide. *PNAS* 93:452–55
35. Legleiter J, Czilli DL, Gitter B, et al. 2004. Effect of different anti-Abeta antibodies on Abeta fibrillogenesis as assessed by atomic force microscopy. *J. Mol. Biol.* 335:997–1006
36. Solomon B, Koppel R, Frankel D, et al. 1997. Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *PNAS* 94:4109–12
37. Frenkel D, Katz O, Solomon B. 2000. Immunization against Alzheimer's beta-amyloid plaques via EFRH phage administration. *PNAS* 97:11455–59
38. Moreth J, Mavoungou C, Schindowski K. 2013. Passive anti-amyloid immunotherapy in Alzheimer's disease: What are the most promising targets? *Immun. Ageing* 10:18
39. Bohrmann B, Baumann K, Benz J, et al. 2012. Gantenerumab: a novel human anti-Abeta antibody demonstrates sustained cerebral amyloid-beta binding and elicits cell-mediated removal of human amyloid-beta. *J. Alzheimers Dis.* 28:49–69

40. Haass C, Selkoe DJ. 2007. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat. Rev. Mol. Cell. Biol.* 8:101–12
41. Englund H, Sehlin D, Johansson AS, et al. 2007. Sensitive ELISA detection of amyloid-beta protofibrils in biological samples. *J. Neurochem.* 103:334–45
42. Sevigny J, Chiao P, Bussière T, et al. 2016. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature* 537:50–56
43. Hartl FU, Hayer-Hartl M. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295:1852–58
44. Arosio P, Michaels TC, Linse S, et al. 2016. Kinetic analysis reveals the diversity of microscopic mechanisms through which molecular chaperones suppress amyloid formation. *Nat. Commun.* 7:10948
45. Gupta R, Kapoor N, Raleigh DP, et al. 2012. Nucleobindin 1 caps human islet amyloid polypeptide protofibrils to prevent amyloid fibril formation. *J. Mol. Biol.* 421:378–89
46. Xu LQ, Wu S, Buell AK, et al. 2013. Influence of specific HSP70 domains on fibril formation of the yeast prion protein Ure2. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368:20110410
47. Muchowski PJ. 2002. Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? *Neuron* 35:9–12
48. Knowles TP, Vendruscolo M, Dobson CM. 2014. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell. Biol.* 15:384–96
49. Cohen SI, Vendruscolo M, Dobson CM, et al. 2012. From macroscopic measurements to microscopic mechanisms of protein aggregation. *J. Mol. Biol.* 421:160–71
50. Miura K, Titani K, Kurosawa Y, et al. 1992. Molecular cloning of nucleobindin, a novel DNA-binding protein that contains both a signal peptide and a leucine zipper structure. *Biochem. Biophys. Res. Commun.* 187:375–80
51. Lin P, Li F, Zhang YW, et al. 2007. Calnuc binds to Alzheimer's beta-amyloid precursor protein and affects its biogenesis. *J. Neurochem.* 100:1505–14
52. Wang Y, Mandelkow E. 2016. Tau in physiology and pathology. *Nat. Rev. Neurosci.* 17:5–21
53. Caillet-Boudin M-LL, Buée L, Sergeant N, et al. 2015. Regulation of human MAPT gene expression. *Mol. Neurodegeneration* 10:28
54. Ke YD, Suchowerska AK, van der Hoven J, et al. 2012. Lessons from tau-deficient mice. *Int. J. Alzheimer's Dis.* 2012:87327
55. Roberson ED, Scearce-Levie K, Palop JJ, et al. 2007. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 316:750–54
56. Leroy K, Ando K, Laporte V, et al. 2012. Lack of tau proteins rescues neuronal cell death and decreases amyloidogenic processing of APP in APP/PS1 mice. *Am. J. Pathol.* 181:1928–40
57. DeVos SL, Goncharoff DK, Chen G, et al. 2013. Antisense reduction of tau in adult mice protects against seizures. *J. Neurosci.* 33:12887–97
58. Santa-Maria I, Alaniz ME, Renwick N, et al. 2015. Dysregulation of microRNA-219 promotes neurodegeneration through post-transcriptional regulation of tau. *J. Clin. Investig.* 125:681–86
59. Barten DM, Fanara P, Andorfer C, et al. 2012. Hyperdynamic microtubules, cognitive deficits, and pathology are improved in tau transgenic mice with low doses of the microtubule-stabilizing agent BMS-241027. *J. Neurosci.* 32:7137–45
60. Zhang B, Carroll J, Trojanowski JQ, et al. 2012. The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. *J. Neurosci.* 32:3601–11
61. Jeganathan S, von Bergen M, Brutlach H, et al. 2006. Global hairpin folding of tau in solution. *Biochemistry* 45:2283–93
62. Wischik CM, Novak M, Thøgersen HC, et al. 1988. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *PNAS* 85:4506–10
63. Wegmann S, Medalsy ID, Mandelkow E, et al. 2013. The fuzzy coat of pathological human Tau fibrils is a two-layered polyelectrolyte brush. *PNAS* 110:21:E313–E321
64. von Bergen M, Friedhoff P, Biernat J, et al. 2000. Assembly of tau protein into Alzheimer paired helical filaments depends on a local sequence motif ((306)VQIVYK(311)) forming beta structure. *PNAS* 97:5129–34

65. Peeraer E, Bottelbergs A, Van Kolen K, et al. 2015. Intracerebral injection of preformed synthetic tau fibrils initiates widespread tauopathy and neuronal loss in the brains of tau transgenic mice. *Neurobiol. Dis.* 73:83–95
66. Clavaguera F, Hench J, Lavenir I, et al. 2014. Peripheral administration of tau aggregates triggers intracerebral tauopathy in transgenic mice. *Acta Neuropathol.* 127:299–301
67. Sanders DW, Kaufman SK, DeVos SL, et al. 2014. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron* 82:1271–88
68. Hochgräfe K, Sydow A, Matenia D, et al. 2015. Preventive methylene blue treatment preserves cognition in mice expressing full-length pro-aggregant human Tau. *Acta Neuropathol. Commun.* 3:25
69. Baddeley TC, McCaffrey J, Storey JM, et al. 2015. Complex disposition of methylthioninium redox forms determines efficacy in tau aggregation inhibitor therapy for Alzheimer's disease. *J. Pharmacol. Exp. Ther.* 352:110–18
70. Theunis C, Crespo-Biel N, Gafner V, et al. 2013. Efficacy and safety of a liposome-based vaccine against protein Tau, assessed in tau.P301L mice that model tauopathy. *PLOS ONE* 8:e72301
71. Kontsekova E, Zilka N, Kovacech B, et al. 2014. First-in-man tau vaccine targeting structural determinants essential for pathological tau-tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model. *Alzheimers Res. Ther.* 6:44
72. Pedersen JT, Sigurdsson EM. 2015. Tau immunotherapy for Alzheimer's disease. *Trends Mol. Med.* 21:394–402
73. Hanisch UK, Kettenmann H. 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10:1387–94
74. Franceschi C, Bonafe M, Valensin S, et al. 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908:244–54
75. Streit WJ, Sammons NW, Kuhns AJ, et al. 2004. Dystrophic microglia in the aging human brain. *Glia* 45:208–12
76. Wes PD, Holtman IR, Boddeke EW, et al. 2016. Next generation transcriptomics and genomics elucidate biological complexity of microglia in health and disease. *Glia* 64:197–213
77. Orre M, Kamphuis W, Osborn LM, et al. 2014. Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice. *Neurobiol. Aging* 35:1–14
78. Ulland TK, Wang Y, Colonna M. 2015. Regulation of microglial survival and proliferation in health and diseases. *Semin. Immunol.* 27:410–15
79. Ransohoff RM. 2009. Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. *Immunity* 31:711–21
80. Aisen PS, Gauthier S, Vellas B, et al. 2007. Alzhemed: a potential treatment for Alzheimer's disease. *Curr. Alzheimer Res.* 4:473–78
81. Yan SD, Chen X, Fu J, et al. 1996. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* 382:685–91
82. Lue LFF, Yan SD, Stern DM, et al. 2005. Preventing activation of receptor for advanced glycation endproducts in Alzheimer's disease. *Curr. Drug Targets CNS Neurol. Disord.* 4:249–66
83. Gómez-Nicola D, Fransen NL, Suzzi S, et al. 2013. Regulation of microglial proliferation during chronic neurodegeneration. *J. Neurosci.* 33:2481–93
84. Gomez-Nicola D, Perry VH. 2015. Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity. *Neuroscientist* 21:169–84
85. Li T, Pang S, Yu Y, et al. 2013. Proliferation of parenchymal microglia is the main source of microgliosis after ischaemic stroke. *Brain J. Neurol.* 136:3578–88
86. Miguel-Alvarez M, Santos-Lozano A, Sanchis-Gomar F, et al. 2015. Non-steroidal anti-inflammatory drugs as a treatment for Alzheimer's disease: a systematic review and meta-analysis of treatment effect. *Drugs Aging* 32:139–47
87. Torrent M, Pulido D, Nogués MV, et al. 2012. Exploring new biological functions of amyloids: bacteria cell agglutination mediated by host protein aggregation. *PLOS Pathog.* 8(11):e1003005
88. Kagan BL. 2011. Antimicrobial amyloids? *Biophys. J.* 100:1597–98
89. Kagan BL, Jang H, Capone R, et al. 2012. Antimicrobial properties of amyloid peptide. *Mol. Pharm.* 9:708–17

90. Kumar DKV, Choi SH, Washicosky KJ, et al. 2016. Amyloid-β peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci. Transl. Med.* 8:340ra72
91. Peila R, Rodriguez BL, Launer LJ, et al. 2002. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia Aging Study. *Diabetes* 51:1256–62
92. Hughes TF, Borenstein AR, Schofield E, et al. 2009. Association between late-life body mass index and dementia: the Kame Project. *Neurology* 72:1741–46
93. Kivipelto M, Ngandu T, Fratiglioni L, et al. 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch. Neurol.* 62:1556–60
94. Whitmer RA, Gunderson EP, Quesenberry CP, et al. 2007. Body mass index in midlife and risk of Alzheimer disease and vascular dementia. *Curr. Alzheimer Res.* 4:103–9
95. Martin B, Mattson MP, Maudsley S. 2006. Caloric restriction and intermittent fasting: two potential diets for successful brain aging. *Ageing Res. Rev.* 5:332–53
96. Scarmeas N, Luchsinger JA, Schupf N, et al. 2009. Physical activity, diet, and risk of Alzheimer disease. *JAMA* 302:627–37
97. Frölich L, Blum-Degen D, Bernstein HG, et al. 1998. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural Trans.* 105:423–38
98. Qiu WQ, Folstein MF. 2006. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. *Neurobiol. Aging* 27:190–98
99. Heneka MT, Sastre M, Dumitrescu-Ozimek L, et al. 2005. Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice. *Brain J. Neurol.* 128:1442–53
100. Pedersen WA, McMillan PJ, Kulstad JJ, et al. 2006. Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice. *Exp. Neurol.* 199:265–73
101. Risner ME, Saunders AM, Altman JF, et al. 2006. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenom. J.* 6:246–54
102. Jack CR Jr., Bennett DA, Blennow K, et al. 2016. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* 87:539–47

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