Amyloid-beta peptide and tau protein crosstalk in Alzheimer’s disease

Introduction

Most of the 50 million people living with dementia worldwide in 2018 suffered from Alzheimer’s disease (AD). AD is a major cause of disability and dependency among elderly people, so economic impact is huge, with an estimate of US$ 1,000,000 million just for the year 2018 (Alzheimer’s Disease International, 2018). AD is a neurodegenerative disease characterized by memory loss, cognitive impairment, and behavioral and psychological symptoms of dementia. The main histopathological hallmarks of AD include the extracellular accumulation of the amyloid-beta (Aβ) peptide in senile plaques and formation of neurofibrillary tangles (NFTs) by hyperphosphorylated tau protein. Mounting evidence supports the assumption that cerebral Aβ deposition begins decades before AD clinical onset and prior to cortical spreading of tau pathology. However, a complete understanding of the role of Aβ, from loss of synaptophysin to synaptic toxicity is still lacking. Similarly, it is accepted that tau hyperphosphorylation plays a critical role in neuron death; however, whether it is cause or consequence of disease development remains unknown. Although controversial, the amyloid cascade hypothesis (ACH) is the only hypothesis covering the multifactorial nature of AD (Karran and De Strooper, 2016; Selkoe and Hardy, 2016). The aim of this review is to discuss new findings on the interplay between Aβ and tau in the context of an updated view of the ACH and to summarize the state of the art in AD clinical trials that are the basis for finding subsequent anti-Aβ and anti-tau combined therapies.

Search Strategy and Selection Criteria

Literature search was performed on PubMed until November 2020. Only papers published in English were considered. The key words/terms were aggregation, Alzheimer, amyloid-beta, cascade hypothesis, dementia, crosstalk, interaction, immunotherapy, inflammation, neurodegeneration, and tau.

Amyloid-beta Peptide as the Alzheimer’s Disease Trigger: the Amyloid Cascade Hypothesis

Amyloid precursor protein (APP) is an ubiquitous single-pass transmembrane protein that contains an extracellular domain, a hydrophobic transmembrane domain, and an intracellular domain.
Kang et al. (1987). Under physiological conditions, soluble APP (sAPP) is involved in neurite outgrowth, synaptogenesis, synaptic plasticity, and cell survival through N-methyl-D-aspartate and gamma-aminobutyric acid receptors modulation, which maintain intracellular calcium homeostasis (Müller et al., 2017; Rice et al., 2019).

APP can be cleaved by a combination of different secretases, including two pathways (Figure 1A). In the non-amyloidogenic APP processing, the combined action of α- and γ-secretases releases sAPPα, P3 peptide (which corresponds to the APP intracellular domain, Aβ1-40, sequence) and the APP intracellular domain (De Strooper et al., 1998). The amyloidogenic processing of APP is initiated by β-secretase (or β-site APP-cleaving enzyme 1 (BACE1)), instead of α-secretase so sAPPα is released; next, γ-secretase generates Aβ peptides with different C-terminal residues and APP intracellular domain. This cleavage, at what is known as the β-site, can be also processing enhancer. One of the most remarkable FAD mutations is the Swedish mutation, a double mutation at the β-site (K670/N, M671L) that enhances APP processing by β-secretase and so generates increased levels of Aβ compared with control individuals. In contrast, Icelandic mutation (A673T), located at the β-site as well, lessens amyloidogenic APP processing and so protects against AD onset (Hardy et al., 2012). Some relevant mutations are found in APP gene located on the N-terminal part of the Aβ peptide or close to the γ-secretase site (Esquerda-Canals et al., 2017b).

The variety of mutations on PSEN genes encompasses missense mutations, small deletions and insertions, as well as genomic exon deletions, being PSEN1 mutations the most usual in FAD (Park et al., 2017; Bagyinszky et al., 2018; Giau et al., 2018a, b). Although hundreds of mutations on APP, PSEN1, and PSEN2 have been described, they only account for a small part of early-onset AD cases, about 5% of AD cases with on-set occurring before 65 years of age. However, when FAD early-onset AD cases are clustered with late-onset AD into what is called the sporadic form of the disease (SAD). Several years ago, it was observed that the serum of non-AD individuals shows auto-antibodies against Aβ, which indicates that some amyloidogenic processing also occurs in normal conditions. In addition, there is a different pharmacological role of BACE1, which generates Aβ peptides (as mentioned) and BACE2 (which generates both Aβ peptide and an N-truncated form, as mentioned). BACE1 is indispensable for proper astrogenesis, axonal growth and migration, myelination and remyelination, neuronal excitation, and synaptic plasticity; whereas BACE2 is involved in different processes, as glucose homeostasis and long-term potentiation, promoting survival of neurons, and promoting a neurogenic effect on neural progenitor cells. In addition, Aβ is involved in the repair of leaks in the blood-brain barrier, promotes recovery from injury, and acts as an antimicrobial and tumor suppressor peptide (Kent et al., 2020).

In 1992, taking into consideration FAD cases, together with the fact that location of APP gene on chromosome 21 leads Down syndrome individuals to suffer from AD (Macleod et al., 2015) by the time they reach middle to late age, the ACH was postulated (Hardy and Higgins, 1992) (Figure 2). ACH states that Aβ peptide deposition triggers tau hyperphosphorylation and aggregation in the form of NFTs, and these aggregates lead to inflammation, synaptic impairment, neuronal loss, and thus to cognitive decline and behavioral abnormalities. However, Aβ auto-antibodies and sAPPα identify a strong cause of loss and clinical symptoms in AD caused by Aβ auto-antibodies featuring SAD points to an impairment in Aβ clearance. In consonance, the most well-known genetic risk factor for developing SAD is the apolipoprotein E (APOE) gene, which encodes apolipoprotein E, and the risk specifically occurs when the APOE4 allele is present (Roda et al., 2019; Andrews et al., 2020).

The fact that several clinical trials for Aβ-immunotherapy-based drugs have failed make made some researchers to question whether the ACH explains the pathological AD by itself (Herrup, 2015). However, the decision on the accelerated approval of aducanumab (Adalimumab, Biogen) was recently made (https://www.fda.gov/drugs/news-events-human-drugs/adas-decision-approve-new-treatment-alzheimers-disease). An accelerated decision means that the drug is shown to have an effect on a surrogate endpoint that is reasonably likely to predict a clinical benefit to patients and there remains some uncertainty about the drug’s clinical benefit.

Different aggregation states of Aβ coexist in AD brains, monomers, oligomers, fibrils and amyloid plaques (Figure 3A). Experimental evidence suggests that plaques maintain a dynamic equilibrium with toxic oligomer species (Benilova et al., 2012). Monomeric Aβ is an intrinsically disordered protein; hence, Aβ does not adopt a single folded shape in aqueous environment but acquires an ensemble of conformations which are prone to aggregation in the form of Aβ oligomers (Ball et al., 2011). Aβ oligomers are the transient intermediate states between monomer and fibril formation. Both Aβ oligomers and fibrils are heterogeneous in structure and size and they are known to be the most toxic Aβ species (Benilova et al., 2012), particularly oligomers. Although solving the structure of the Aβ peptide from human samples has been quite challenging, the 3D-structure of both Aβ1-40 and Aβ1-42 fibrils has been solved in the last decade (Lu et al., 2013; Qiang et al., 2017). Aβ1-40 fibrils from one patient showed that Aβ1-42 peptide monomers aggregate into multiple of three oligomers (trimers, hexamers, nonamers and dodecamers), whereas Aβ1-40 termini (DAEFR, residues 1–5) are exposed to the solvent and hydrophobic C-termini are buried in the trimer core (Figure 3B; pdb 2M4J) (Lu et al., 2013). Because the concentration of Aβ1-42 is much higher than Aβ1-40, such multiple of three oligomers are found in most studies with animal models for AD, i.e., in the 3xTg-AD (Esteban et al., 2013). The N-terminus of Aβ1-40 fibrils shows a quite different structure, with two twisted protofilaments stacked in parallel and thus built of “LS”-shaped dimers, with the N-terminus slightly solvent-exposed and the C-terminus completely buried (Figure 3C; pdb 5QVO) (Gremer et al., 2017). How oligomers promote cell toxicity has not been completely unraveled. However, several molecular mechanisms as activation of metabotropic glutamate receptor 5, Caspase-3 activation, N-methyl-d-aspartate receptor interaction, and α7 nicotinic acetylcholine receptor upregulation have been proposed (Bezprozvanny, 2009).

Although amyloid deposits do not correlate with either disease progression or cognitive decline (Hanseeuw et al., 2019), their spatial and temporal emergence are well described (Braak and Braak, 1991). In a first stage, amyloid deposits occur in the frontal, temporal, and occipital lobes of the isocortex. In a second stage, they spread to all the isocortical association areas, except on primary sensory areas and motor field. Disperse amyloid deposits also occur in the frontal and parietal lobes. In this stage, the hippocampus is slightly affected. The final stage is characterized by depositions in primary isocortical areas and progressive spreading to the striatum, thalamus and hypothalamus.

Tau and Its Role in Neurodegeneration

As mentioned before, the lack of success in approving any anti-Aβ therapy up to now, as well as the poor correlation found between cognitive decline and the occurrence of amyloid plaques, have led the scientific community to question whether Aβ accumulation is actually triggering neurodegeneration in AD. The occurrence of tau NFTs relates to neurodegeneration and clinical symptoms (Hanseeuw et al., 2019) and, although Aβ may initiate a cascade of events, tau pathology is likely the effector molecule of neurodegeneration. Tau, apart from being a stabilizer of microtubules and being involved in their dynamics, plays a role in several physiological processes including myelination, axonal transport, neurogenesis, motor function, learning and memory, neuronal excitability, glucose metabolism, iron homeostasis, and DNA protection (Kent et al., 2020). It is important to mention that while Aβ accumulation is characteristic of AD, tau pathology also exists in a group of neurodegenerative diseases known as tauopathies, which are different to AD.
Figure 1 | Amyloid precursor protein (APP) processing.
(A) α- and γ-secretase act in the non-amyloidogenic pathway, whereas the activity of β-secretase (or BACE) instead of α-secretase defines the amyloidogenic pathway generating the Aβ peptide. BACE1 hydrolyses the β-site (M671-D672 bond) and BACE2 cleaves the β′-site (681Y-682E bond), rendering an N-t truncated form of the precursor (E11-E12), although also cleaves the β-site. Thus, β′-site cleavage is amyloidolytic rather than amyloidogenic. Finally, γ-secretase releases the Aβ peptide, or its N-t truncated form, after preferentially cleaving the V711-D712 and A713-T714 bonds rendering Aβ1-40 and Aβ1-42, respectively. (B) Target proteolytic sites in the Aβ peptide. (C) Some of the main FAD-related mutations in human APP. ACID: APP intracellular domain; Aβ: amyloid-beta peptide; BACE: beta-site APP cleaving enzyme; CTF: carboxyterminal fragments; FAD: familial Alzheimer’s disease.

Figure 2 | The amyloid cascade hypothesis (ACH).
Whether by an increase in production (FAD) or an impairment in clearance (SAD), Aβ peptide accumulates through an aggregation pathway populated by different species, i.e. monomers, oligomers, fibrils, and amyloid plaques. Aβ oligomers are the most toxic species, and to a lesser extent fibrils, causing calcium disruption, mitochondrial failure, inflammatory response, oxidative stress, transcriptional impairment, synaptic loss and, finally, neuronal death. In addition, Aβ oligomers induce the hyperphosphorylation of tau, which aggregates into oligomers that evolve to paired helical filaments and finally to NFTs. Tau oligomers are also toxic for neurons, with a strong effect on synapses, and, in turn, induce the formation of Aβ oligomers. In addition to this positive feedback between Aβ and tau aggregation, Aβ and tau cooperatively work to lead to neuronal death. Although still controversial, the ACH covers the multifactorial nature of AD. Aβ: Amyloid-beta peptide; FAD: familial Alzheimer’s disease; NFTs: neurofilament tangles; PHFs: paired helical filaments; SAD: sporadic Alzheimer’s disease.

Figure 3 | Amyloid fibril formation and three-dimensional structure.
(A) Aggregation of Aβ from the formation of different kind of oligomers to amyloid fibrils. (B) Aβ1-40 amyloid fibrils are composed by multiple ordered trimers. The N-termini are completely solvent-exposed, whereas the C-termini are buried in the hydrophobic core (PDB 2M4J) (Lu et al., 2013). Each color represents a monomer. (C) Aβ1-42 amyloid fibrils are made of dimers. The N-termini are slightly solvent-exposed, whereas the C-termini are completely buried in the interface between monomers (PDB 5OQV) (Gremer et al., 2017). Each monomer in the dimer shows higher β-content than in the trimer. Each color represents a monomer. Images were generated with the Swiss-PdbViewer software (Guex and Peitsch, 1997). Aβ: Amyloid-beta peptide; PDB: protein data bank.
The link between tau dysfunction and neurodegeneration was set through human genetics. Mutations in MAPT, the microtubule-associated protein Tau gene, give rise to an inherited form of Frontotemporal Dementia (FTD) and parkinsonism with abundant filamentous tau deposits in the brain, in the absence of Aβ deposits (Goedert, 2015). Six different isoforms of tau are expressed in the adult human central nervous system via alternative splicing of the MAPT gene, which comprises 16 exons and is found on chromosome 17q21.3 (Figure 4A). Regulated expression of exons 2 and 3 gives rise to tau isoforms with 0, 1, or 2 N-terminal inserts, whereas exclusion or inclusion of exon 10 leads to expression of tau isoforms with three (3R) or four (4R) microtubule-binding repeats (Connell et al., 2005). 3R or 4R isoforms are predominant in tau inclusions depending on the neurodegenerative disease. Progressive supranuclear palsy (PSP), corticobasal degeneration, argyrophilic grain disease, among others, exhibit overexpression of 4R isoforms; whereas Pick’s disease, and familial FTD and parkinsonism (MAPT mutations such as G272V and Q338R) are mainly characterized by tau inclusions rich in 3R tau isoforms (Irwin, 2016). In other diseases such as AD, Down syndrome or Chronic traumatic encephalopathy, both 3R and 4R isoforms are found in tau tangles. The N-terminal phosphorylation domain in tau, which triggers a signaling pathway that disrupts axonal transport, is exposed in all the isoforms. The extent of phosphorylation-dependent domain exposure and oligomerization is larger for tau aggregates composed of 4R isoforms than 3R isoforms. However, aggregates of all isoforms exhibit enough phosphorytase-activating domain exposure to impair axonal transport (Cox et al., 2016).

Tau is predominantly expressed in the central and peripheral nervous system, where it is most abundant in nerve cell axons. In the brain, tau is subject to several posttranslational modifications, including phosphorylation, acetylation, methylation, glycation, isomerization, O-GlcNac glycosylation, nitration, sumoylation, ubiquitination, and truncation (Morris et al., 2015), but the role all these modifications play in tau function remains unknown. The most studied tau posttranslational modification is phosphorylation, as despite the significant heterogeneity that exists between and within the different tauopathies, the deposited tau in pathological lesions is invariably highly phosphorylated (Noble et al., 2013). Phosphorylation of tau negatively regulates its ability to interact with microtubules, but whether phosphorylation is a trigger for aggregation remains to be proved. Although aggregated tau is heavily phosphorylated in the human brain, not all phosphorylated tau is aggregated (Goedert et al., 2017). Full-length tau assemblies into filaments through its repeats, with the N-terminal half and the C-terminus forming the fuzzy coat (Figure 4B; pdb 6HRE) (Falcon et al., 2018). The filament core contains the repeat sequences that are required to the binding of soluble tau to microtubules, suggesting that physiological function and pathological assembly are mutually exclusive (loss of function). Brains from different individuals with the same tauopathy show the same kind of tau filaments (Falcon et al., 2018), whereas each disease is characterized by its own unique tau fold (Scheres et al., 2020). Cross-seeding experiments have shown that Aβ oligomers induce tau oligomerization in vitro (Lasagna-Reeves et al., 2010), indicating that early abnormal Aβ oligomerization precedes tau aggregation. Interestingly, it has been reported that structure-based inhibitors of Aβ are able to avoid tau aggregation, suggesting that there is a common aggregation interface in both tau and Aβ (Griner et al., 2019). Although tau is mainly an intracellular protein, extracellular tau is present in the brain and interstitial fluid (Yamada et al., 2011), and it is thought to be secreted under physiological conditions. Microglial cells may propagate tau through exosome-dependent mechanisms (Asai et al., 2015). Spread of tau aggregated assemblies may seed and promote aggregation (Falcon et al., 2015) along different brain regions. In AD, deposition of tau aggregates follows a highly stereotyped pattern, beginning in the medial temporal cortex and hippocampus before propagation to other regions. Their stereotypical appearance in nerve cells underlies Braak’s stages of AD (Jellinger, 1998), according to which inclusions form first in subcortical regions, transentorhinal cortex, and entorhinal cortex (stages I and II). They then appear in the hippocampal formation and some parts of the neocortex (stages III and IV). Most of the neocortex (stages V and VI). Individuals at stages I and II are asymptomatic, some individuals at stages III and IV show signs of memory impairment, and those at stages V and VI suffer AD (Schöll et al., 2016).

### Amyloid-beta Peptide and Tau Protein Interplay: More Than Casual Partners

Currently it is becoming widely accepted that Aβ is the “trigger” and tau the “bullet” driving AD (Bloom, 2014). In addition, although tau load better relates to cognitive decline than Aβ load, as mentioned, it has recently been reported that the presence of both Aβ and tau is necessary for memory decline and AD. The most advanced stages of AD (Sperling et al., 2019). However, the mechanisms behind Aβ and tau interplay in AD remain elusive.

Recent data supports the hypothesis that there is a bidirectional interplay between Aβ and tau. Few years ago, it was demonstrated that addition of Aβ to human cells induces tau aggregation in the form of paired helical filaments-like filaments (Ferrari et al., 2003). Interestingly, cellular models have shown that over-expression of APP and PSEN1 increases not only Aβ, but also tau aggregation (Choi et al., 2014). A relation between Aβ and tau was also found in animal models, as in APPPS1 transgenic mice (APP KM670/671NL (Swedish), PSEN1 L166P) overexpressing Aβ also show an increase in CSF-tau levels. Furthermore, tau is able to facilitate Aβ aggregation, as tau deletion in APPPS1 transgenic mice ameliorated Aβ deposition (Peters et al., 2019); whereas the addition of human tau increased plaque size, supporting the hypothesis that there is a bidirectional interplay between Aβ and tau. However, the APPPS1 mouse model of AD does not develop NFTs, neither do other APP and/or PSEN models (Esquerda-Canals et al., 2017b). The only mouse model developing NFTs is the triple transgenic mouse model (3xTg-AD; PSEN1/PS1, APPsw, tauP301L) and this is so because one of the three transgenes is a mutated MAPT (tauP301L), found in familial FTD (Oddo et al., 2003).

In this mouse model, the only one where an evident neuron loss has been quantified even at the early stages of the disease. Interestingly, cellular density showed a strong correlation with intracellular Aβ burden in glutamatergic neuronal populations (Esquerda-Canals et al., 2017a). At the most advanced stages of the disease, it has been demonstrated that Aβ-immunotherapy not only decreases Aβ-burden but also affects tau load in this mouse model (Rodà et al., 2020). Similarly, active DNA Aβ immunization in 3xTg-AD mice reduced not only Aβ deposition but also tau pathology (Rosenberg et al., 2018).

The challenging modeling of AD in rats has been achieved with the line TgF344-AD expressing APPsw and PSEN1 transgenic mice (Cohen et al., 2011), and it is thought to be secreted under physiological conditions. Microglial cells may propagate tau through exosome-dependent mechanisms (Asai et al., 2015). Spread of tau aggregated assemblies may seed and promote aggregation (Falcon et al., 2015) along different brain regions. In AD, deposition of tau aggregates follows a highly stereotyped pattern, beginning in the medial temporal cortex and hippocampus before propagation to other regions. Their stereotypical appearance in nerve cells underlies Braak’s stages of AD (Jellinger, 1998), according to which inclusions form first in subcortical regions, transentorhinal cortex, and entorhinal cortex (stages I and II). They then appear in the hippocampal formation and some parts of the neocortex (stages III and IV). Most of the neocortex (stages V and VI). Individuals at stages I and II are asymptomatic, some individuals at stages III and IV show signs of memory impairment, and those at stages V and VI suffer AD (Schöll et al., 2016).

![Figure 4A](https://example.com/figure4a.png)

#### Figure 4A

Three-dimensional structure of tau NFTs and tau isoforms scheme.

(A) Six different isoforms of tau are expressed in the adult human central nervous system via alternative splicing pattern. The truncated expression of exons 2 and 3 gives rise to tau isoforms with 0, 1, or 2 N-terminal inserts whereas exclusion or inclusion of exon 10 leads to expression of tau isoforms with three (3R) or four (4R) microtubule-binding repeats. (B) Paired helical filaments from 3xTg-AD mouse brain shows the fuzzy core formed by aggregated repeats (residues G304-E330) (PDB 6HRE)(Falcon et al., 2018). Each color represents a monomer. Images were generated with the Swiss-PdbViewer software (Guex and Peitsch, 1997). AD: Alzheimer’s disease; MAPT: microtubule associated protein tau; PDB: protein data bank.
Another proposed link between Aβ and tau alterations is the C-terminus of hsp70-interacting protein, as it has been reported that Aβ accumulation decreases C-terminus of hsp70-interacting protein expression and increases tau levels. In addition, and Aβ-induced effects on tau are rescued by restoring C-terminus of hsp70-interacting protein levels (Oddo et al., 2008; Lyon and Milligan, 2019).

A common hallmark among neurodegenerative diseases is neuroinflammation. The innate immune system could be another link between amyloid and tau pathologies. Interestingly, both Aβ and tau proteins are capable of activating microglia (Asai et al., 2015). It is worth noting that the immune system is involved in the early synaptic loss induced by oligomeric species of Aβ (Hong et al., 2016). On the other hand, some studies suggest that microglia could play a pivotal role in tau spreading by making synapses more vulnerable to tau-induced synaptic impairment. Inhibition of exosome secretion by microglia prevents the spread of Aβ, suggesting that exosomes, play in prion-like spreading of tau throughout the brain (Small and Duff, 2008). Supporting this hypothesis, it has been shown that Aβ oligomers induce tau spreading by making synapses more vulnerable to tau-induced synaptic impairment. Inhibition of exosome secretion by microglia suppressed tau propagation to the dentate gyrus in a mouse model, recovering non-pathological excitability in this brain region. In contrast, extracellular soluble, but not intracellular, Aβ has been related with increased exosome-dependent tau spreading in both animal models and in neuropathological samples (Gomes et al., 2019). These observations support the important role microglia, and particularly microglial exosomes, play in prion-like spreading of tau throughout the brain (Asai et al., 2015). It is worth noting that the immune system is not only a mediator between Aβ or tau and neuronal death. Olson et al. showed that the immune system is not only a mediator but also a source of cytokines that precede neuronal death (Olson et al., 2019). In addition, the formation, triggering tau hyperphosphorylation and, subsequently, the formation of NFTs. Aβ oligomers are able to activate the inflammasome (Nod-like receptor family pyrin domain containing 3, NLRP3) that, in turn, regulates kinases, as glycogen synthase kinase 3 (GSK-3) and phosphatases, as protein phosphatase 2A, inducing tau phosphorylation (Ising et al., 2019) and evidence a clear link among Aβ, the immune system, and tau pathology.

Despite the evidence that the bidirectional interplay between Aβ and tau is widespread, the exact mechanisms that the Aβ and tau pathways use to affect our health are not clear. It is still unknown what factors could independently affect and trigger both Aβ and tau abnormalities (Small and Duff, 2008). This idea has been formulated as “The dual pathway hypothesis”, which states that there are common upstream triggers causing both Aβ and tau abnormalities (Small and Duff, 2008). Supporting this hypothesis, it has been shown that GSK-3 is necessary but not enough to induce tau pathology. Aβ plaque-bearing mouse models that do not overexpress tau need injection of misfolded AD-tau seeds to develop the three major types of AD-relevant tau pathologies: dystrophic neurites surrounding Aβ plaques (NP tau), neurofil threads and NFTs. These distinct tau pathologies show different temporal onset and functional consequences, with different activity of the major tau kinases, GSK-3 (Shaffer et al., 2008), and its inhibition reduces tau phosphorylation in vitro (Singh et al., 1995; Wagner et al., 1996), as well as in animal models (Uno et al., 2009). Thus, apoe or GSK-3 independent-Aβ alterations could enhance tau phosphorylation and aggregation, supporting the dual pathway hypothesis. Furthermore, it has been proposed that GSK-3 could be a link between Aβ and tau hyperphosphorylation (Medina and Avila, 2012). Regarding Aβ, it has been widely reported that apoE e4 enhances Aβ production and aggregation as well as impairs its clearance (Rodn et al., 2019). Recently, an interaction effect between apoE e4 and Aβ, rather than the sum of their independent effects, has been related to increased tau load in humans (Therriault et al., 2020). However, not only apoE e4 is a common trigger of Aβ and tau aggregation. Several metabolic diseases have been suggested to be related with neurodegeneration. In fact, diet-induced insulin resistance in animal models stimulates Aβ and tau aggregation, as well as neurodegeneration (Bharadwaj et al., 2017). Which are the exact mechanisms behind such a stimulation remains to be elucidated, but the occurrence of amyloidosis in orlistat amyloid polypeptide deposits in senile plaques suggests that a link between this pancreatic amyloid and AD could exist. Moreover, immune system dysregulation and oxidative stress, both present in type-2 diabetes, are involved in AD progression.

Thus, a large amount of evidence supports the assumption that Aβ peptide and tau protein are not casual partners. There is a link between both pathologies that cooperate to produce synaptic dysfunction, neuronal loss, and the eventual cognitive decline characterizing AD.

**Combined Therapies as a New Hope: Targeting Amyloid-beta and Tau**

Even though there is a bidirectional interaction and some factors could independently affect both molecules, Aβ oligomerization seems to precede tau hyperphosphorylation. The fact that Aβ-immunotherapy still raises some concerns is in agreement with the new idea of Aβ acting just as a switch-on. Once Aβ triggers the cascade of events that leads to neuronal death and dementia, it is no so important how these events can be prevented because the effector molecule driving to synaptic impairment and neuronal death is tau. This would explain why previous clinical trials for Aβ-immunotherapy enrolling mild to moderate AD patients, did not reach the end-points. Nowadays, active clinical trials for Aβ-immunotherapy enroll patients in early, even prodromal stages of the disease (see below).

In the last years, it has been reported that Aβ-immunotherapy reduces not only Aβ but also tau levels in animal models (Roda et al., 2020). Additionally, clinical trials (Rombouts et al., 2021) has shown that Aβ-immunotherapy is well tolerated, and the emerging clinical programs will start to study in the first line of Aβ immunotherapy (Blennow et al., 2012; Sevigny et al., 2016). In the last years, it has been reported that Aβ-immunotherapy reduces not only Aβ but also tau levels in animal models (Roda et al., 2020). Additionally, clinical trials (Rombouts et al., 2021) has shown that Aβ-immunotherapy is well tolerated, and the emerging clinical programs will start to study in the first line of Aβ immunotherapy (Blennow et al., 2012; Sevigny et al., 2016). In the last years, it has been reported that Aβ-immunotherapy reduces not only Aβ but also tau levels in animal models (Roda et al., 2020). Additionally, clinical trials (Rombouts et al., 2021) has shown that Aβ-immunotherapy is well tolerated, and the emerging clinical programs will start to study in the first line of Aβ immunotherapy (Blennow et al., 2012; Sevigny et al., 2016).
Aβ aggregation inhibitors

- β-Breakers
  - Tramiprosate (ZAPS, Alzhe Memory, ALZ-801 (Bellus Health Inc.))
  - Flecogamma (Grifols Biotech Inc.)
  - Octagam (Octapharma); Gammagard (Baxalta US Inc.; Grifols Biotech Inc.)
  - IgG fraction, polyclonal

Secretases inhibitors / modulators/activators

- β-Secretase (BACE) inhibitors
  - MK-8931 (Merck Sharp & Dohme Corp., N1-54861911 (Janssen), E2609 (Eisai Inc.), AZD3293 (LY3314814) (Eli Lilly and Co. & AstraZeneca)

- γ-Secretase inhibitor or modulator
  - Semagacestat (LY-450139) (Eli Lilly and Fluorizone (Tarenflurbil, MPE-7869) (Myriad Genetcs)

- α-Secretase activator
  - Etazolate (ETH 0202) (Exonhit)

Active immunotherapy

- Fibrillar Aβ (1–42), QS21 adjuvant
  - AN1792 (Elan and Wyeth)

- N-t Aβ (1–6) in viral particles
  - CAN106 (Novartis)

- N-t Aβ (1–14) + T-cell epitope, delivery system
  - UB-311 (United Neuroscience Ltd.)

Passive immunotherapy

- Humanized mAb, IgG1
  - Bapineuzumab (AAB-001) (Janssen & Pfizer)

- Anti-N-t (1–5)
  - Ponezumab (PF-04360365RN1219)

- Anti-C-t (30–40)
  - Anti-plasmatic Aβ (peripheral sink)
  - Solanezumab (LY206243) (Eli Lilly and Co. & Hoffmann–La Roche)

- Humanized mAb, IgG1
  - Anti-mid-region (16–24)
  - Fully human mAb, IgG1

- Anti-N-t (1–10) and-mid-region (23–25)
  - Binds fibrils

- Humanized mAb, IgG4
  - Crenzumab (MBTS5102A) (Genentech)

- Anti-mid-region (12–23)
  - Binds protofibrils

- Fully human mAb, IgG1
  - Aducanumab (BII8037) (Biogen Idec)

- Anti-N-t (3–6) and mid-region
  - Binds all aggregated forms
  - E22G “arctic” mutation (protofibrils)

- Intravenous IgGIV
  - Gammagard (Baxalta US Inc.); Octagam (Octapharma); Flebogamma (Grifols Biol. Inc)

- IgG fraction, polyclonal
  - Reduction in amyloid, cognitive improvement

| Therapeutic approach | Mechanism of action | Drugs (companies) | Clinical trials | Identifier |
|----------------------|---------------------|-------------------|----------------|------------|
| Aβ aggregation        | β-Breakers          | Tramiprosate      | Phase III did not reach primary outcomes, but had beneficial effect on APOE e4/4 patients with mild AD | NCT00314912 |
| inhibitors            |                     | Flecogamma        |                 |            |
|                      |                     | MK-8931           | Phase II did not reach primary outcomes | NCT00934050 |
| Secretases inhibitors | β-Secretase (BACE)  | Bapineuzumab (AAB-001) (Janssen & Pfizer) | No cognitive improvement | NCT00574132 |
| / modulators/activators |                    | Ponezumab (PF-04360365RN1219) | Phase II repurposed for CAA (1–40) | NCT01821118 |
|                      | γ-Secretase inhibitor| Semagacestat (LY-450139) (Eli Lilly and Fluorizone (Tarenflurbil, MPE-7869) (Myriad Genetcs) | Dangerous effects because their relation with cancer (+ for T-cell leukemia) | NCT00880412 |
|                      | α-Secretase activator| Etazolate (ETH 0202) (Exonhit) | No effect in Phase III | NCT00021723 |
| Active immunotherapy | Fibrillar Aβ (1–42), QS21 adjuvant | AN1792 (Elan and Wyeth) | Phase II discontinued (2002). 6% of patients with meningencephalitis. Clearance of plaques, decrease in cognitive decline. | NCT02565511 |
|                      | N-t Aβ (1–6) in viral particles | CAN106 (Novartis) | Phase II/III ongoing with cognitively healthy APOE e4/4 | NCT03531710 |
|                      | N-t Aβ (1–14) + T-cell epitope, delivery system | UB-311 (United Neuroscience Ltd.) | Phase II recently terminated | NCT00594568 |
| Passive immunotherapy| Humanized mAb, IgG1 | Bapineuzumab (AAB-001) (Janssen & Pfizer) | Phase III terminated (2012), mild to moderate AD. Fibrillar Aβ clearance (PET), CSF-p-tau reduction | NCT00105547 |
|                      | Anti-N-t (1–5) | Ponezumab (PF-04360365RN1219) | No cognitive improvement. 5% ARIA-E (APOE e4 carriers) | NCT02477800 |
|                      | Anti-C-t (30–40) | Solanezumab (LY206243) (Eli Lilly and Co. & Hoffmann–La Roche) | No benefit in Phase III, mild AD | NCT01760005 |
|                      | Anti-mid-region (16–24) | Solanezumab (LY206243) (Eli Lilly and Co. & Hoffmann–La Roche) | No cognitive improvement | NCT01821118 |
|                      | Anti-N-t (1–10) and-mid-region (23–25) | Gantenerumab (RO4909832, RG1450) (Hoffmann-La Roche) | Phase III (A4 study, asymptomatic) | NCT03439973 |
|                      | Binds fibrils | Gantenerumab (RO4909832, RG1450) (Hoffmann-La Roche) | Eight Phase III studies, prodromal and/or mild AD on going (i.e.) | NCT03439913 |
|                      | Humanized mAb, IgG4 | Crenzumab (MBTS5102A) (Genentech) | Phase II (DIAN-TU study, FAD asymptomatic) | NCT03444870 |
|                      | Anti-mid-region (12–23) | Crenzumab (MBTS5102A) (Genentech) | Phase III terminated (2019), mild to moderate AD | NCT03444870 |
|                      | Binds protofibrils | Crenzumab (MBTS5102A) (Genentech) | No cognitive improvement | NCT03977584 |
|                      | Fully human mAb, IgG1 | Aducanumab (BII8037) (Biogen Idec) | Accelerated approval by the FDA, June 7, 2021 | NCT03887455 |
|                      | Anti-N-t (3–6) and mid-region | Aducanumab (BII8037) (Biogen Idec) | Phase III just initiated, early AD | NCT04468659 |
|                      | Binds all aggregated forms | E22G “arctic” mutation (protofibrils) | Clears amyloid plaques, Cognitive improvement 10% ARIA-E (APOE e4 carriers) | NCT01760005 |
|                      | Intravenous IgGIV | Gammagard (Baxalta US Inc.; Octagam (Octapharma); Flebogamma (Grifols Biol. Inc) | All just completed Phase III, mild to moderate AD | NCT00818662 |

ClinicalTrials.gov accessed February 27, 2021. AD: Alzheimer’s disease; APOE: apolipoprotein E; ARIA-E: amyloid-related imaging anomalies-E; Aβ: amyloid-beta; BACE: beta-site APP cleaving enzyme; CAA: cerebral amyloid angiopathy; CSF: cerebro-spinal fluid; FAD: familial Alzheimer’s disease; IgG: immunoglobulin-G; mAb: monoclonal antibody; PET: positron emission tomography.
potential for near term approval (Tolar et al., 2020). Aducanumab and gantenerumab are fully human mAbs that target epitopes composed of the N-terminus and a central region of the Aβ peptide, whereas BAN2401 is a humanized mAb directed to an epitope on the fibrils made by the E22G “arctic” mutation.

Similarly, treatments to reduce tau aggregation or decrease tau levels are under development (Table 2), but not as advanced as for Aβ. A new therapeutic approach for treating AD or other tauopathies is the use of antisense RNAs interfering the expression of MAPT mRNA (Evers et al., 2015). Morpholinos are synthetic RNA analogs that are stable and specifically bind to the designed target mRNA. In neuroblastoma cell cultures, reductions in tau levels up to 80% were achieved by exon skipping inducing an out-of-frame deletion in tau mRNA (Sud et al., 2014). As mentioned before, six tau isoforms are found in the human brain (Figure 4A) and the ration between R3 (not including exon 10) and R2 isoforms (including exon 10) is impaired in several diseases such as PSP, Corticobasal Degeneration, Argyrophilic grain disease, Pick’s disease, and FTD-P, among others. Although this is not the case in AD, it is worth mentioning the capability of antisense oligonucleotides for controlling the skipping of exon 10 and therefore their therapeutic potential (Peacey et al., 2012).

In conclusion, the evidence of a bidirectional interplay between Aβ and tau better explains the ACH, which is currently complemented by the dual pathway hypothesis. Combined anti-Aβ and anti-tau therapies will be tested in the near future because Aβ-immunotherapy is quite advanced, with a recent accelerated approval for Adudhelm, and tau-immunotherapy is on the path. In addition, the determination of the three-dimensional structures of the fibrils made by Aββ42, Aββ40, and tau may be helpful in the design of new therapeutic molecules. Therefore, it is quite likely we are on the path to find a cure for this devastating disease.

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Alzforum (2020) AADvac1 (Axon Neuroscience SE) and several phase II trials for passive immunotherapy with gosuranemab, tilavonemab, semorinemab zagotenemab are ongoing (Alzforum, 2020). All these humanized mAbs have been obtained on an IgG4 scaffold and recognize the N-terminus of tau and hence extracellular tau, with the exception of zagotenemab that also recognizes a mid-region characterizing aggregated tau.

In this sense, a phase I clinical trials with the oligonucleotide NIO752 (Novartis) aimed to treat PSP is being initiated (NCT04539041) (NII, 2021). In the specific case of the treatment of AD, IONIS MAPTRx oligonucleotide is being tested in phase I/II for decreasing tau mRNA (NII, 2021). Another approach is the inhibition of tau aggregation that is being tested in a phase III clinical trial with methylene blue (Remer), already used for malaria. Concerning the enhancing of tau clearance, it must be taken into account that tau oligomers can exist in a variety of states including hyperphosphorylated forms, which can be both soluble and insoluble. In addition, the fact that they are six isoforms complicates the election of the best target. It remains to be determined (a) which of these oligomeric species of tau are causally involved in neurodegeneration, and (b) which trigger the beginning of the formation paired helical filaments filaments. Meanwhile, phase II trial for active immunotherapy with a fragment of tau (294–305) (AADvac1 (Axon Neuroscience SE) and several phase II trials for passive immunotherapy with gosuranemab, tilavonemab, semorinemab zagotenemab are ongoing (Alzforum, 2020). All these humanized mAbs have been obtained on an IgG4 scaffold and identify the N-terminus of tau and hence extracellular tau, with the exception of zagotenemab that also recognizes a mid-region characterizing aggregated tau.

| Table 2 | Tau therapeutics in clinical trials |
|---|---|---|---|
| Therapeutic approach | Mechanism of action | Drugs (companies) | Clinical trials | Identifier |
| Antisense RNA | MAPT mRNA splicing and/or silencing | NIO752 (Novartis) | Phase I registered for the treatment of PSP | NCT04539041 |
| | | IONIS MAPTRx (BiliB080, ISIS 814907) (Ionis Pharma, Inc.) | Phase I/II ongoing | NCT03186989 |
| Tau aggregation inhibitors | Several mechanism (i.e. for malaria, others) | Trx0237/LMTF-X/Methylene Blue (Remer) | Phase II ongoing | NCT03446001 |
| Active immunotherapy | Tau (294–305) + keyhole limpet hemocyanin, Al(OH)3, adjuvant. 66 copies of a synthetic, S396&S404-phosphorylated tau into liposomes. MPLA adjuvant | AADvac1 (Axon Neuroscience SE) | Phase II slowed neurodegeneration biomarkers | NCT02579252 |
| | | ACI-35 (AC Immune) | Elicited a weak immune response, and needed to be redesigned. Phase II ongoing. | NCT04445831 |
| Passive immunotherapy | Humanized mAb, IgG4 | Gosuranemab (BiliB092, IPN002) (Biogen) | Phase II just initiated, early AD | NCT0352557 |
| | Anti-Nt (15–24) | Binds e-tau | | |
| | Humanized mAb, IgG4 | Tilavonemab (ABBV-8E12, C2N-8E12) (AbbVie) | Phase II just initiated, early AD | NCT03712787 |
| | Anti-Nt (22–34), human | Binds e-tau | | NCT02880956 |
| | Humanized mAb, IgG4 | Semorinemab (RO7105705, Genentech, Inc.) | Phase II just initiated, prodromal to mild and moderate AD | NCT03828747 |
| | Anti-Nt (2–24), human | Binds e-tau | | |
| | Humanized mAb, IgG4 | Zagotenemab (LY3303560) (Eli Lilly Co) | Phase II just initiated, early AD | NCT03518073 |
| | Anti-Nt (7–9) and mid (312–322) | Binds aggregated-tau | | |

ClinicalTrials.gov accessed February 27, 2021. AD: Alzheimer’s disease; e-tau: extracellular tau; IgG: immunoglobulin; mAb: monoclonal antibody; MAPT: microtubule associated protein tau; mRNA: messenger ribonucleic acid; PSP: progressive supranuclear palsy.
