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Current and future treatment of amyloid diseases

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Abstract

There are more than 30 human proteins whose aggregation appears to cause degenerative maladies referred to as amyloid diseases or amyloidoses. These disorders are named after the characteristic cross-β-sheet amyloid fibrils that accumulate systemically or are localized to specific organs. In most cases current treatment is limited to symptomatic approaches and thus disease-modifying therapies are needed. Alzheimer's disease is a neurodegenerative disorder with amyloid β-peptide (Aβ) plaques and tau neurofibrillary tangles as pathological hallmarks. Numerous clinical trials have been conducted with vaccines and small molecules to target Aβ formation and aggregation and also enhance Aβ clearance; so far such clinical trials have been unsuccessful. Novel strategies are therefore required and here we will discuss the possibility of utilizing the chaperone BRICHOS to prevent Aβ aggregation and toxicity. Type 2 diabetes mellitus is symptomatically treated with insulin. However the disease is linked to the aggregation and progressive accumulation of islet amyloid polypeptide and oligomers of this peptide are cytotoxic. Several compounds have been shown to inhibit islet amyloid aggregation and cytotoxicity *in vitro*. Future animal studies and clinical trials have to be be conducted to determine their efficacy *in vivo*. Transthyretin (TTR) amyloidoses are a group of systemic degenerative diseases involving multiple organ systems and caused by TTR aggregation. Liver transplantation decreases the generation of misfolded TTR and improves the quality of life for a subgroup of this patient population. Compounds that stabilize the natively folded, non-amyloidogenic, tetrameric conformation of TTR have been developed and the drug tafamidis is available as treatment.

Keywords

Alzheimer's disease; amyloidosis; transthyretin; treatment; type 2 diabetes

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Introduction

Amyloid diseases, or the amyloidoses, are characterized by the deposition of cross-β-sheet amyloid fibrils consisting of misfolded and/or misassembled proteins [1–3]. The amyloid fibrils that are the pathological hallmark of these disorders can be either deposited systemically or localized to specific organs. The development of amyloidosis is often linked to aging and is associated with decreased quality of life and substantial suffering for both patients and their families. Alzheimer's disease (AD) is an example of localized cerebral amyloidosis and type 2 diabetes mellitus is an example of localized extracerebral amyloidosis; both diseases are associated with aging. Systemic forms of amyloid disease, also often linked to aging, are less common and include the transthyretin (TTR) amyloidoses. The origin of amyloidosis is either sporadic, i.e. from the normal protein sequence, or hereditary (familial), i.e. from a protein harboring one or more point mutations [4]. In addition there are infectious forms of amyloidosis, such as the transmissible spongiform encephalopathies caused by the aggregation of prion protein [5, 6].

In this review we will discuss the current and potential future treatment of AD, type 2 diabetes, and the TTR amyloidoses. The case to be made for disease-modifying therapies is compelling, especially in AD where only symptomatic treatment is available. Strategies to halt aggregation, i.e. the formation of oligomers, protofilaments, pores, and amyloid fibrils, include vaccination, use of inhibitors or modulators of proteases responsible for amyloid production, aggregation inhibitors, or native state stabilizers, and organ transplantation-mediated gene therapy. Different strategies must be considered depending on the mechanism of amyloidogenesis and amyloid disease etiology. Targeting the central nervous system (CNS) is always a challenge because the blood–brain barrier limits access of pharmacological agents to the brain.

AD

Age-dependent dementia, and in particular AD, affects an increasing number of persons worldwide as the population lives longer. AD is the most common form of dementia and is associated with a lower quality of life and considerable suffering not only for patients, but also for their families. AD is characterized by a progressive loss of synapses and neurons starting in the hippocampus and entorhinal cortex and spreading to other parts of the cortex. The loss of synapses correlates well with cognitive impairment, which is an early sign of the disease. Despite huge efforts from academia and industry, no disease-modifying drugs have been approved by the regulatory agencies for the treatment for AD. Approved agents are limited to drugs with symptomatic modes of action, including acetylcholine esterase inhibitors and one NMDA receptor agonist. Pathological hallmarks of AD include the formation of extracellular plaques consisting of amyloid β-peptide (Aβ) and intraneuronal tangles formed by hyperphosphorylated tau. Aβ is generated from the amyloid precursor protein (APP) by the subsequential cleavage by β- and γ-secretases. Active γ-secretase complexes consists of either presenilin (PS)1 or PS2, Nicastrin, Aph1, and Pen2. To date around 100 different substrates for γ-secretase have been identified. Longer forms of Aβ (x-42, x-43), in comparison to the most common x-40 forms, are most prone to aggregate

and to form toxic intracellular oligomers and fibrils, which are eventually deposited in extracellular amyloid plaques in the brain. Aβ is normally degraded by proteases such as neprilysin and insulin-degrading enzyme, and subsequently cleared from the brain via P-glycoprotein pumps of the the blood–brain barrier. In AD, the processes of Aβ generation and clearance are modified resulting in a shift in the Aβ42/40 ratio, synapse toxicity, neuronal degeneration, and formation of amyloid plaques [7]. In addition to Aβ toxicity and plaque formation, the progressive accumulation of hyperphosphorylated tau contributes to neuronal dysfunction and degeneration. An inflammatory response is triggered in AD and dysfunctional cholinergic signaling, impaired glucose metabolism, cholesterol metabolism, and mitochondrial dysfunction are also characteristics of thedegenerative phenotype [8, 9].

Based on the features of the pathogenic process, several different pharmacological treatment strategies have been tested in animals and in clinical trials or are currently under development.

Even though most efforts have focused on reducing Aβ brain concentration or Aβ clearance, several clinical trials of compounds affecting other targets are or have been conducted. Such targets include for example tau phosphorylation (tideglusib, GSK3β inhibitor, Zeltia Group, discontinued), inflammation (pioglitazone, Takeda Pharmaceutical Company, http://www.alzforum.org/therapeutics/pioglitazone, Phase 3), cholesterol (simvastatin, Merck, http://www.alzforum.org/therapeutics/simvastatin, Phase 4), oxidative stress (alpha-tocopherol, i.e. vitamin E), mitochondrial function (latrepirdine, Medivation Inc., discontinued), and neurotrophins (encapsulated cell implants releasing nerve growth factor [10]).

On-going clinical trials for AD-targeting Aβ

Several strategies for targeting Aβ clearance, production, or aggregation have been tested or are under current investigation. Here we will discuss some of the molecules currently in clinical trials (Table 1). It is important to note that lessons learned from previous clinical trials have resulted in new enrollment strategies. For example many clinical trials now include patients with early-stage AD and use a positive PET scan for the presence of Aβ-plaques as one of the inclusion criteria. Some placebo-controlled studies are designed to test the effects of therapeutic candidates on certain groups of genetically defined patients. For example the active vaccine CAD106 (Novartis Pharmaceuticals Co) will be tested in a Phase 3 study in APOEε4/ε4 carriers, i.e. individuals with an increased risk of developing AD. In the Dominantly Inherited Alzheimer's Network (DIAN) initiative, 416 familial AD mutation carriers (mutations in *APP, PSEN1 or PSEN2*) are enrolled in an observational cohort study, in which the passive immunotherapies gantenerumab (Hoffmann-La Roche) and solanezumab (Eli Lilly & Co) are being tested based on changes in biomarker read-outs (http://www.alzforum.org/news/conference-coverage/dian-plans-trial-number-two-goal-go-big). Within the DIAN initiative, combination therapy with both vaccination and a BACE1 inhibitor to simultaneously affect Aβ clearance and production is being tested; results will be presented during 2016.

Several active and passive immunotherapies have been or are being tested in order to enhance clearance of Aβ from the brain. As mentioned above, CAD106 is currently under

clinical evaluation and is an example of an active immunotherapy designed to avoid inflammatory T cell activation. The vaccine is derived from the N-terminal B cell epitope of Aβ and several copies of $A\beta_{1-6}$ are coupled to the bacteriophage Qβ virus-like particle. CAD106 has so far been tested for antibody response and tolerability in five different Phase 2 studies and has shown positive results (http://www.alzforum.org/therapeutics/cad106). A Phase 3 study will now be initiated in the USA as described above.

The passive immunotherapies gantenerumab and solanezumab are currently in Phase 3 trials. In addition, the immunoglobulin preparation Gamunex (Grifolds Biologicals Inc.) is being tested in a multicenter Phase2/3 study in Spain involving 350 persons with mild to moderate AD. Gantenerumab is a human IgG1 antibody that binds to Aβ fibrils encompassing N-terminal and central amino acids of Aβ. The Phase 3 trial was started in March 2014 and includes 1000 patients with mild AD. Patients will receive subcutaneous injections every 4 weeks for 100 weeks. ADAS-cog and ADCD-ADL are used as primary outcome measurements and a combination of biomarkers and clinical measures are used as secondary outcomes. The brain amyloid burden in a subgroup of 100 patients will be followed by PET florbetapir [Amyvid™]. The study will be completed in 2019 (https://clinicaltrials.gov/ct2/show/NCT02051608?term=gantenerumab&rank=3).

Solanezumab is a humanized monoclonal IgG1 antibody that binds to soluble monomeric Aβ (mid-domain). The rationale of treatment with this passive immunotherapy is to target the soluble pool of small toxic Aβ species. A large multicenter study in 2100 patients with mild AD started in July 2013 and results are expected in December 2016. Solanezumab has previously been shown to have a beneficial effect on cognition, growing over time, in this patient group.

Another strategy for ameliorating AD is to decrease the production of Aβ by inhibiting or modulating secretases. Several γ-secretase inhibitors were tested in a clinical setting. However, a number of adverse effects were reported and the trials were terminated mainly due to undesired inhibition of Notch cleavage. Currently only one γ-secretase modulator, EVP-0962 (FORUM Pharmaceuticals Inc.), is being evaluated in patients. EVP-0962 is an NSAID derivative reported to decrease Aβ42 levels in different cell types and Tg2576 mice. A Phase 2 study was started in November 2012 to investigate the safety, tolerability, and pharmacokinetics of EVP-0962, and its effects on cerebrospinal fluid (CSF) Aβ concentrations. A total of 52 healthy subjects and those with mild cognitive impairment or early AD were enrolled. The study has been completed but no results are currently available (https://clinicaltrials.gov/ct2/show/NCT01661673?term=EVP-0962&rank=1).

An alternative target is BACE1, which is responsible for the initial cleavage of APP, generating the γ-secretase substrate C99. Inhibition of BACE1 decreases the generation of C99, which in turn reduces the Aβ concentration. Several BACE1 inhibitors are currently being tested in clinical trials. Two examples are the small molecules AZD3293 (AstraZeneca) and verubecestat (Merck), which are both being evaluated in Phase 3 trials. A large Phase 2/3 study of AZD3293 (administered in tablet form) enrolling 1551 patients with mild AD was initiated in December 2014 and will run for 5 years (http://www.alzforum.org/therapeutics/azd3293-0). Verubecestat is being tested in another large worldwide Phase 3

study enrolling patients with prodromal AD with measureable cognitive deficits and positive PET-flutemetamol scans (http://www.alzforum.org/therapeutics/verubecestat).

Proteostasis network targeting is another approach under consideration as chaperone pathways have the ability to prevent peptide and protein aggregation, whereas activation of the proteasome or autophagy can clear aggregated proteins. Next we will further discuss the role of chaperones in misfolding disease.

BRICHOS as a defense mechanism against amyloid toxicity

In cells, chaperones have the important role of counteracting misfolding and aggregation of non-native proteins or intrinsically disordered proteins [11], and thus molecular chaperones likely play a central role in preventing protein misfolding diseases. In line with this, mutations in genes coding for chaperones have been shown to be responsible for different hereditary diseases. For example, mutations in the small heat-shock protein α-crystallin give rise to the early onset of cataracts, due to loss of chaperone function leading to aggregation of lens proteins [11, 12]. Targeting chaperones for treatment of neurodegenerative amyloid diseases, such as poly-Q diseases, Parkinson's disease and AD, is being investigated preclinically [13]. Using transgenic *Drosophila melanogaster* models, it has been shown that Hsp70 can suppress toxic effects in models of polyQ diseases and Parkinson's disease [14, 15]; however, no chaperone-targeting treatment has been pursued beyond the preclinical stage. In 2012, it was shown that mutations in the BRICHOS domain of lung surfactant protein C precursor protein (proSP-C) gave rise to a novel amyloid disease. Mutations in proSP-C BRICHOS abrogated its function as a chaperone, which otherwise prevented amyloid formation of mature SP-C [16]. Harnessing BRICHOS as a specific anti-amyloid chaperone has been shown to have the potential to prevent the toxicity associated with the process of Aβ fibril formation [17–19]] (see below).

The BRICHOS family—BRICHOS is a protein domain present in a variety of proteins related to lung disease, dementia, and amyloid and proliferative diseases [20]. So far, the most extensively studied BRICHOS domain is the one present in proSP-C, and its crystal structure was resolved in 2012 [16]. The name BRICHOS is derived from the proteins Bri, chondromodulin, and proSP-C, which all contain a BRICHOS domain, and other characterized protein families include tenomodulin and the gastrokines 1, 2, and 3. The sequence similarity is low between the different protein families; however, the BRICHOS domain has three strictly conserved amino acid residues and shares a similar predicted secondary structure [20, 21]. BRICHOS domain-containing proteins are type II transmembrane proteins with a similar architecture that all contain a region prone to form β-strands. Already at the time of discovery, the BRICHOS domain was suggested to have an intramolecular chaperone-like function [20, 21].

ProSP-C BRICHOS—The physiological role of the BRICHOS domain of proSP-C is to facilitate membrane insertion and prevent misfolding of the transmembrane (TM) part of the mature peptide SP-C. SP-C is part of the lung surfactant and its TM portion has an unusually high content of Val residues, which makes the TM helix discordant. Instead, it is predicted to adopt a β-strand conformation [22, 23]. The metastability of the peptide SP-C makes it prone

to misfold and form a β-sheet structure. This has been shown to occur in the case of mutations in the BRICHOS domain of proSP-C, which can lead to interstitial lung disease with amyloid inclusions formed by SP-C [16]. In line with this, poly-Val stretches have been shown to be less efficient in forming α-helical structure during translation of TM domains, a feature that is needed for ER membrane insertion [24, 25]

Anti-amyloid properties of the BRICHOS domain—The chaperone-like function of the proSP-C BRICHOS domain, preventing amyloid formation of the aggregation-prone peptide SP-C, might be a function common to the BRICHOS domain in all families [20, 21, 26, 27]. All the BRICHOS domain-containing proteins, except for proSP-C, comprise a C-terminal region with high β-sheet propensity that is predicted to form a strand-loop-strand β-hairpin conformation, which suggests that these regions could need a chaperone such as the BRICHOS domain to prevent aggregation.

The SP-C peptide is a metastable peptide, i.e. in its native state it forms an α-helix even though the amino acid sequence is predicted to form a β-sheet. The AD-associated peptide Aβ also contains a discordant helix and, when it is proteolytically cleaved from its precursor protein APP (reviewed in [28]), it is highly prone to misfolding, aggregation, and formation of amyloid fibrils [29, 30]. The Aβ peptide adopts a β-hairpin structure as the building block in the amyloid fibril [31], which could be a general target for the BRICHOS domain [27]. Indeed, the BRICHOS domains from both proSP-C and the dementia-associated Bri2 are efficient inhibitors of Aβ42 (but also Aβ40) amyloid formation [32–35]. Recently gastrokine-1 has also been shown to prevent Aβ40 aggregation. Moreover, the proSP-C BRICHOS domain prevents amyloid formation of medin, a peptide that forms amyloid in the aortic wall [32], suggesting that BRICHOS domains are natural anti-amyloidogenic chaperones that may be exploited for treatment strategies for amyloid diseases.

BRICHOS domains against Aβ aggregation and toxicity—Amyloid fibril formation of Aβ proceeds via a nucleated polymerization reaction that includes two types of nucleation events, primary and secondary nucleation [36–39]. The Aβ aggregation starts with a lag phase, followed by a rapid process whereby new fibrils are formed, and a plateau phase is reached at equilibrium [38]. The mechanism underlying BRICHOS domain inhibition of Aβ42 aggregation has been studied in detail using Thioflavin T fluorescence combined with mathematical models of the aggregation kinetics [36]. This approach revealed that the proSP-C BRICHOS domain specifically inhibits the secondary nucleation event, which catalyzes the formation of toxic oligomers when Aβ monomers interact with the fibrillar surface. The BRICHOS domain binds to the fibrils and efficiently blocks the sites for secondary nucleation, leading to a significant decrease in the formation of toxic oligomers during Aβ42 aggregation (Fig. 1) [40].

Reducing the amounts of Aβ42 monomers during Aβ formation has been shown to have marked effects both *in vitro* and *in vivo*. The ProSP-C BRICHOS domain is able to prevent Aβ-induced alteration of the excitatory/inhibitory balance in the hippocampal network, which leads to a protective effect on gamma oscillation power. The ProSP-C BRICHOS domain also prevents the increased toxic effects observed in gamma-oscillation experiments

using mouse hippocampal slices following the addition of minute amounts of Aβ42 fibrils as seeds to Aβ42 monomers [19, 41].

In the brains of transgenic *D. melanogaster* flies, the BRICHOS domain protects neurons against Aβ42 toxicity *in vivo* [17]. Co-expression of the proSP-C BRICHOS domain together with Aβ leads to an increase in soluble Aβ42, while flies expressing Aβ alone show a high degree of aggregated Aβ. In the brain of the fly, co-localization of the BRICHOS domain and Aβ42 was observed and, importantly, flies co-expressing Aβ42 and the proSP-C BRICHOS domain showed increased lifespan as well as increased locomotor activity, compared to flies expressing Aβ42 alone [17].

In a mouse model expressing a modified version of the Bri2 protein, in which the naturally occurring C-terminal Bri23 peptide has been replaced by the Aβ42 peptide (Bri2-Aβ42), Aβ deposition was slower with a decrease in oligomeric forms of Aβ42, compared with APP transgenic models. In addition, the Bri2-Aβ42 mice did not develop any cognitive impairment despite the fact that they eventually developed plaques [18]. It is possible that overexpressing Aβ42 in combination with Bri2 in the mouse brain results in the co-expressed BRICHOS domain delaying the aggregation of Aβ42 and efficiently reducing the Aβ42-related toxicity.

The recent results showing that BRICHOS domains inhibit the toxicity associated with Aβ fibril formation, even though amyloid is formed at a slower rate, hold promise for targeting this chaperone as a treatment not only for AD but also for other amyloid diseases. Events such as secondary nucleation that might take place on existing amyloid plaques, leading to toxicity toward neurons in the AD brain, could possibly be prevented with BRICHOS domains even at a stage where the disease has already started. One of the obstacles associated with treating neurodegenerative diseases is of course the blood–brain barrier, so a key challenge for exploiting BRICHOS domains as drugs would be determining how to deliver these domains, or mimics thereof, to the brain or finding a way to increase the activity of endogenous BRICHOS domains.

Type 2 diabetes mellitus

Similar to AD, type 2 diabetes is linked to the aggregation and progressive accumulation of islet amyloid. Our knowledge of amyloidogenesis *in vitro* has increased considerably over the last 30 years, but why proteins aggregate into amyloid in the human body is still an open question. We know from studies of serum amyloid A (AA) amyloid that there is an effective endogenous mechanism responsible for degradation of amyloid, which renders it less inert than previously believed, and studies on islet amyloid polypeptide (IAPP) and Aβ in particular have increased our understanding of small aggregates, fibrillar propagation, and cytotoxicity.

Diabetes currently affects almost 350 million persons worldwide and the majority suffer from type 2 diabetes, a progressive disease mainly characterized by beta cell dysfunction and insulin resistance [42, 43]. Beta cell dysfunction includes changes in insulin secretion,

conversion of prohormone into its biological active counterpart, and deposition of islet amyloid.

Islet amyloid in diabetes

IAPP was characterized in 1986 [44] as the main protein constituent of islet amyloid. IAPP is a polypeptide hormone that participates in blood glucose regulation together with insulin. Proinsulin and proIAPP are cleaved at dibasic residues by prohormone convertases PC2 and PC1/3 to become biologically active insulin and IAPP [45, 46], which are released simultaneously from the beta cells. IAPP exhibits auto/paracrine activity and acts as a modulator of insulin secretion.

Peripheral insulin resistance leads to a compensatory increase in insulin production and when sustained can result in chronic beta cell stress [47]. In addition to biologically active hormones, there is an increase in secretion of partially processed and unprocessed prohormone, with low or no biological function.

In studies of human proIAPP in cell lines with different expression patterns of prohormone convertases (PC2 and PC1/3) it was shown that proIAPP had a higher tendency to form intracellular amyloid. ProIAPP expression in cell lines deficient in both convertases resulted in formation of intracellular amyloid composed of proIAPP whereas expression of proIAPP in a beta cell line expressing both convertases and where proIAPP was cleaved into IAPP never resulted in formation of IAPP amyloid [48]. Therefore, it is possible that IAPP amyloid formation is seeded by proIAPP aggregates. To investigate whether proIAPP fibrils can act as a template for IAPP fibrillation, preformed proIAPP or IAPP fibrils were injected intravenously into transgenic mice expressing human IAPP. The results showed that preformed IAPP fibrils were more potent as seeds compared to preformed fibrils from proIAPP, and amyloid deposits were present in 24.0% and 15.4% of the islets, respectively. In negative control mice injected with fibrils composed of insulin C-peptide A chain, amyloid developed in two out of six mice and only and amyloid was only present in 4% of the islets. However, not only the number of engaged islets but also the amount of amyloid increased in mice injected with preformed fibrils from proIAPP/IAPP [49]. As the formation of intracellular IAPP amyloid is linked to cell death, it is possible that amyloid aggregates, which escape degradation, remain in the islets and instead becomes active seeds for further amyloid growth. Quantification of islet cells at autopsy indicated a 50–60% reduction in beta cells in patients with type 2 diabetes [50]. *In vitro* studies suggested that IAPP oligomers are cytotoxic whereas amyloid fibrils are harmless, but whether IAPP oligomers are formed *in vivo* remains to be proven [51]. When IAPP is allowed to form aggregates in the presence of beta cells, caspase 3 is activated and apoptosis is induced [52], but the cytotoxicity is abolished when mature IAPP fibrils are formed [53]. This indicates an initial protective effect of amyloid that is most likely transient. Within an islet, small amyloid deposits can occur at multiple sites and, through growth, the islet amyloid area expands and will ultimately occupy most of the islet. Insulin secretion in response to glucose is oscillatory and beta cells within a normal pancreatic islet are electrically coupled through gap junctions [54]. Loss of gap junctions leads to a loss of synchronized insulin secretion. Because

propagation of amyloid disrupts islet architecture, it is most likely that extracellular amyloid deposition will also affect islet cell function.

The pro-inflammatory cytokine IL-1β is associated with impaired insulin secretion, and administration of an IL-1β receptor antagonist can improve beta cell function in patients with type 2 diabetes. Recently, it was shown that the inflammasome can be activated by islet amyloid [55] and that amyloid-containing islets contain more macrophages than non-amyloid-containing islets [56]. The inflammasome consists of a protein complex that upon activation gives rise to a cascade of events including cleavage of procaspase-1 and subsequent generation of IL-1β. Despite extensive studies, the source of IL-1β in islet tissue is still debated, but chemical depletion of islet macrophages leads to an increase in amyloid load, pointing to macrophages residing in islets as the source of IL-1β [57]. From studies of AA amyloid resolution, it is clear that macrophages can degrade large amounts of amyloid in a short time period [58]. Therefore, it is intriguing that islet amyloid remains in islets where few or no beta cells remain. This amyloid is expected to be cleared as further growth of amyloid mass is prevented by low or no IAPP synthesis.

Amyloid as a molecular link between AD and diabetes

Despite differences in protein primary structure and biochemical properties, it is basically impossible to distinguish between amyloid from different proteins based on morphological appearance. Therefore, it is possible that amyloid fibrils comprising one protein can act as a nidus and seed further amyloid propagation of amyloid from a distinct protein, giving rise to amyloid fibrils composed of more than one type of protein. Type 2 diabetes has been identified as a risk factor for AD in epidemiological studies [59]. These two conditions have multifactorial pathogeneses, but both have local amyloid deposition in common. The amyloid proteins Aβ, which forms local amyloid in the brain of patients with AD, and IAPP exhibit almost a 50% amino acid sequence identity and it has been shown *in vitro* that fibrils composed of either peptide can seed or induce aggregation of the other peptide, supporting the concept of heterogeneous seeding [60]. Studies of the IAPP and Aβ interaction have also identified regions in which the peptides interact; these are often regions important for aggregation [61]. We have studied the *in vivo* interaction between IAPP and Aβ by injecting preformed Aβ fibrils corresponding to 20 μg peptide intravenously in transgenic mice expressing human IAPP. [49]. In pancreas recovered 10 months after injection, IAPP amyloid was present in five out of seven mice, and 15.2% of the islets were affected by IAPP amyloid in these five animals. The amount of injected and preformed Aβ fibrils was below the detection level but an increase in the number of affected islets suggests that initiation of IAPP aggregation was dependent on seeding. In control mice, islet amyloid affected only 4% of islets.

To determine whether IAPP and Aβ co-localize in plaques, pancreas sections from type 2 diabetes patients with amyloid and brain sections from AD patients with diffuse and dense plaques were analyzed using a proximity ligation assay to simultaneously detect IAPP and Aβ. This highly specific antibody-based system generates a positive signal when antibodies bind to their respective antigens in close proximity (within 40 nm). In brain sections, IAPP reactivity appeared evenly distributed throughout both diffuse and dense plaques. IAPP

reactivity was also present in the media of vessels with congophilic angiopathy. In addition, combined detection of proIAPP and Aβ generated a positive signal indicative of the presence of proIAPP in the brain (Fig. 2). In contrast to the brain, no co-localization of IAPP and Aβ could be detected in islet amyloid in the pancreas from patients with type 2 diabetes. The results suggest that IAPP and Aβ co-localize in the brain but do not identify the origin of the IAPP. It is known that IAPP is synthesized in various brain regions but misfolded IAPP and proIAPP may also be released from the pancreas and transported through the blood-–brain barrier and initiate seeding of Aβ in the brain.

Studies of islet amyloid and cytotoxicity suggest a crucial role for IAPP aggregation in the development of beta cell deficiency in type 2 diabetes. If cross-seeding of amyloid occurs *in vivo* and is important for the association between diabetes and AD, the need for lifestyle changes would further increase. There is still a lack of imaging technology to detect islet amyloid *in vivo*, and pancreatic biopsies are associated with high risks. However, in the future, the ability to measure islet amyloid non-invasively will enable the effects of treatment to be monitored, as currently one of the main concerns regarding islet amyloid treatment is when to start intervention. It takes many years to develop diabetes, and there is a marked reduction in beta cell loss prior to the occurrence of clinical symptoms. Several compounds have been shown to affect IAPP aggregation *in vitro* and also reduce IAPP-induced toxicity in various cell-based experiments. Different polyphenols have been shown to effectively inhibit amyloid formation and the green tea flavonoid epigallocatechin-3-gallate prevents IAPP toxicity [62] by directing aggregation to an off-pathway route that results in the formation of non-toxic amorphous aggregates [63].

Curcumin is another example of a polyphenol that prevents IAPP aggregation at low concentrations and partly protects beta cells from IAPP toxicity [64]. However, in studies in rat islets in which human IAPP was overexpressed, curcumin treatment lacked effect on amyloid formation and cytotoxicity [65].

SiRNA has also been employed for reduction of proIAPP in human isolated islets. Transduction with recombinant adenoviruses expressing siRNA resulted in a 75% reduction in proIAPP expression and, after culture for 10 days, reductions in amyloid and apoptotic cell number were detected [66].

TTR amyloidosis

TTR is another amyloidogenic human protein, whose aggregation appears to cause degenerative diseases involving multiple organ systems [1–3]. TTR is a tetrameric protein, comprising 127-amino acid β-sheet-rich protomers secreted into the bloodstream by the liver [67–69] and into the CSF by the choroid plexus [70, 71]. TTR transports ≈0.5 equivalents of *holo*-retinol-binding protein per TTR tetramer in the blood [72–75]. TTR also binds thyroxine (T_4); however, the T_4-binding sites at the weaker of the two dimer–dimer interfaces of TTR are largely unoccupied in human blood and CSF [70, 71, 76]. TTR likely has additional unknown functions, especially in the brain [77–79].

TTR is a normally structured protein that has to undergo rate-limiting partial denaturation in order to become amyloidogenic (i.e. tetramer dissociation followed by conformational changes within the monomer) [80, 81]. While genetic, pharmacological, pathological, and biochemical evidence in the field of TTR amyloidoses strongly support the amyloid hypothesis, i.e. the notion that the process of aggregation causes the loss of post-mitotic tissue in these disorders, the structure(s) of the toxic species and the mechanism of proteotoxicity remain unclear (this is the case for all human amyloid diseases) [82–85].

There are two main categories of TTR amyloid diseases: hereditary and acquired. The hereditary TTR amyloidoses are caused by an inherited tetramer-destabilizing TTR mutation [86, 87]. Most patients with hereditary or familial TTR amyloidosis are heterozygotes, meaning that their TTR tetramers are largely composed of a mixture of mutant and wild-type (WT) subunits. The heterotetramers, which are generally less stable than WT homotetramers, are more prone to dissociation, leading to more monomer misfolding and aggregation. While it is clear why mutations that destabilize the TTR tetramer cause TTR amyloid disease, it is perplexing that dissociation, monomer misfolding, and aggregation from the more stable WT homotetramers can also occur, leading to the development of acquired WT TTR amyloidosis in a subset of older individuals (predominantly males). Such WT TTR amyloidosis manifests mainly, but not exclusively, as a cardiomyopathy and appears to be the largest category of TTR aggregation-associated degenerative diseases; it is estimated that there are 250,000 WT TTR cardiomyopathy patients in the USA [88, 89]. Aging is a significant risk factor for the development of the WT TTR amyloidoses (as well as a subset of the cases of familial TTR amyloidosis). Aging-associated deficiencies in protein homeostasis (or proteostasis) are hypothesized to contribute to the demise of tissue that does not easily regenerate [90–93]. Much remains to be learned about why aging is a major risk factor for the onset of some of the TTR amyloidoses.

As mentioned above, the characteristic clinical heterogeneity seen in the TTR amyloidoses is only partly understood [94–97]. Predominant cardiac involvement is seen mainly in WT TTR amyloidosis (previously referred to as senile systemic amyloidosis) and with some of the mutations that lead to familial amyloid cardiomyopathy (FAC), e.g. the V122I TTR mutant in heterozygotes. These patients present most frequently with symptoms of cardiac insufficiency with preserved ejection fraction [98, 99]; however other presentations dominated by arrhythmias (e.g. atrial fibrillation) or chest pain mimicking ischemic heart disease are also possible [100]. Although historically referred to as a purely cardiac disease, the involvement of other organs in WT TTR amyloidosis has been increasingly recognized [95, 101]. In the predominantly neurological phenotypes, generally associated with hereditary TTR amyloidosis and referred to as familial amyloid polyneuropathy (FAP), the typical clinical presentation is characterized by a small fiber (sensory and autonomic) length-dependent neuropathy [102]; however this is not always the case. More rarely, patients present with a predominantly motor neuropathy, or marked upper limb involvement with some cases of carpal tunnel syndrome as the presenting manifestation [103]. Even more rarely, patients present with syndromes resembling motor neuron disease [103]. This variability in FAP clinical phenotype(s) is seen in patients with the same mutation and even within the same kindred. Some FAP patients present with marked involvement of other less common organs, such as the eye [104], the CNS [105], or the kidney [106]. If left

unchecked, the aggregation of most TTR sequences leads to compromised function of multiple organs and ultimately death occurs within 4–12 years after the onset of symptoms [107–113].

Our current understanding of the mechanism of TTR aggregation or amyloidogenesis is shown schematically in Fig. 3. According to this mechanism, dissociation of the TTR tetramer is the rate-limiting step in the aggregation of TTR. After dissociation, the natively folded monomer must undergo partial denaturation in order for thermodynamically favored aggregation to commence [80–82, 114–119]. The rate and extent of TTR aggregation depends on the concentration of the amyloidogenic TTR monomer [120–122]: aggregation is faster when the concentration of amyloidogenic TTR monomer is high and slower when it is low. Consequently, several strategies under development to treat the TTR amyloid diseases focus on reducing the concentration of amyloidogenic monomers.

Transplantation and amyloidosis

In the 1990s, liver transplantation was introduced as the only potentially curative treatment for TTR amyloidosis [123, 124]. Because 95% of plasma TTR is synthesized by the liver, the net result of liver transplantation is to exchange the less stable TTR heterotetramers in the patient's blood for more stable WT homotetramers. This substantially lowers the concentration of the amyloidogenic monomer present in the blood and thereby slows disease progression and extends lifespan. The early experience was very encouraging. In all transplanted patients, the variant TTR disappeared from the blood and the progress of the disease was halted in most, but not all, individuals. Today more than 2000 patients have undergone liver transplantation for this disorder; the majority of the patients harbor the Val30Met mutation, with a smaller number of patients harboring genes encoding one of over 60 other different mutations of the TTR protein. Overall, patient survival is excellent, and markedly different from the expected prognosis seen before liver transplantation was introduced. However, not all patients benefit from the procedure. Patients without the Val30Met mutation have a less favorable long-term survival as well as only approximately 60% stabilization of the disease compared to 80–90% for those with this mutation. Among patients with Val30Met, male patients with late-onset disease (after 50 years of age) have been identified as having an inferior long-term survival compared to other patients with this mutation. A major reason for this is the progression of cardiac amyloidosis in men with late-onset disease, in spite of liver transplantation. Compared to patients with end-stage liver disease undergoing liver transplantation, cardiac-related deaths are much more common among patients transplanted due to TTR amyloidosis (21% vs. 9%, according to data from the European Liver Transplant Registry). The progress of cardiac amyloidosis in some patients after liver transplantation, especially those without the Val30Met mutation, has supported a combination of liver and heart transplantation in some cases with specific mutations in order to make the transplant procedure worthwhile [125]. Another option to consider is that patients who slowly progress owing to cardiomyopathy and/or neuropathy after liver transplantation [126–130] should be considered for tafamidis (see below) and/or TTR mRNA-lowering therapy to prevent the onset of cardiomyopathy or the progression of polyneuropathy post-transplantation.

The duration of the disease has been found to be an important prognostic factor for survival after transplantation. A short duration was also important in order to stabilize the disease after liver transplantation. Another important factor for outcome after transplantation was found to be the nutritional status at the time of transplantation. Body mass index modified by taking into account albumin, according to the formula mBMI=weight (kg) divided by height 2 (m) × albumin concentration (g/L), is a useful tool to prognosticate outcome. With the introduction of pharmacological treatment in recent years, a decline in the number of liver transplants for TTR amyloidosis has been observed (Fig. 4).

Other transplantation modalities have been used in order to either cure or modify the disease course in patients with other types of amyloidosis. Stem cell transplantation (bone marrow transplantation) is potentially curative, but needs to be performed before the occurrence of severe organ damage, i.e. heart, liver, or kidney failure due to extensive deposition of amyloid. Kidney, heart, and liver transplantation has been performed in a small percentage of amyloidosis patients with relatively modest long-term survival due to the remaining underlying systemic disorder [131]. Kidney transplantation has mainly been performed in order to improve quality of life for patients suffering from amyloidosis. In patients with a slow progression of the amyloidosis, such as those with hereditary lysosome amyloidosis, excellent long-term survival with good renal function can be expected. For immunoglobulin light chain (AL) amyloidosis with a more rapid rate of progression, the role of kidney transplantation without curative treatment of the underlying disease may be more questionable [132]. Although not curative, heart or liver transplantation may be considered in order to rescue the life of a patient with AL amyloidosis in order to prepare for post-transplant curative treatment with either stem cell transplantation or modern chemotherapy (i.e. the current standards of care for AL amyloidosis patients). Patients with advanced liver disease or even splenic or hepatic rupture due to extensive deposition of amyloid tissue cannot tolerate advanced chemotherapy or stem cell transplantation [133, 134].

Leveraging an understanding of the molecular mechanism of TTR aggregation linked to pathology to develop the kinetic stabilizer strategy to ameliorate degeneration

As a complement or alternative to transplantation, emerging approaches to lower the total TTR plasma concentration include the use of antisense oligonucleotides (Ionis Pharmaceuticals, Inc, Carlsbad, CA 92008, USA) and RNA interference (RNAi) (Alnylam Pharmaceuticals Inc, Cambridge, MA 02142, USA). Both approaches act by lowering TTR mRNA levels [135–138]. Another strategy for reducing the concentration of aggregation-competent TTR (i.e. misfolded monomeric TTR) is to focus on preventing the conformational excursions from the native tetramer that renders TTR amyloidogenic (Fig. 3) [3, 115, 117, 139–141]. Stabilizing the natively folded, non-amyloidogenic, tetrameric conformation of TTR may be considered as a conservative approach for treating the TTR amyloidoses, because not all the native functions of TTR are understood, and it is still unclear which misfolded or misassembled TTR structure(s) mediates proteotoxicity [142–147].

The approach of stabilizing the native tetrameric conformation of TTR to ameliorate the TTR amyloidoses is supported by some very interesting and important human genetic

observations made by Coelho and co-workers [148, 149]. They reported the cases of compound heterozygous patients who express the V30M mutation on one *TTR* allele (normally associated with highly penetrant polyneuropathy), but do not develop polyneuropathy (or only manifest very mild phenotypes). Instead of expressing WT TTR from their second allele, these patients express a T119M *TTR* variant. This genotype largely results in the formation of TTR heterotetramers that exhibit a statistical distribution of V30M and T119M subunits [82, 150]. Subsequent biophysical studies revealed that T119M subunit incorporation into tetramers otherwise composed of disease-associated subunits proportionately reduces the rate of tetramer dissociation at neutral pH (Fig. 5a). This interallelic *trans*-suppression-based mechanism of TTR kinetic stabilization explains the inhibition of TTR aggregation and the slower development of mild FAP in the V30M/T119M compound heterozygotes [82, 115]. The dissociation rate of the T119M TTR homotetramer is ~25-fold slower than the dissociation rate of the WT TTR homotetramer, thus the T119M TTR homotetramer exhibits a much higher dissociation barrier than that of the WT TTR homotetramer [82, 115, 151]. Hence, T119M subunit inclusion into a tetramer raises the dissociative transition state energy (Fig. 5a), protecting these individuals from disease manifestations [82, 115, 148, 149].

The small-molecule therapeutic strategy envisioned by Kelly and co-workers, in which small molecules bind selectively to the unoccupied T_4-binding sites within TTR in plasma and the CSF, was also expected to increase the barrier to TTR tetramer dissociation, i.e. through kinetic stabilization of the TTR tetramer (Fig. 5b) [115, 117, 141, 152].

This pharmacological discovery of a small molecule that binds to the native state of TTR very tightly without or only very weakly binding to the dissociative transition state was referred to as the kinetic stabilizer strategy (Fig. 5b).

First the validity of this approach was demonstrated using T_4, a thyroid hormone precursor that is a natural TTR ligand. The binding of T_4 inhibited TTR amyloidogenesis *in vitro* [141]. This proof-of-principle experiment motivated screening [153–157] and structure-based drug design efforts [158, 159] to discover small molecules that kinetically stabilize the non-amyloidogenic TTR tetramer [115, 117]. Importantly, these molecules lack thyroid hormone activity and bind tightly and selectively to native TTR in human plasma over the more than 4000 proteins in blood plasma, including albumin that binds promiscuously to many small molecules and, by doing so, could prevent TTR kinetic stabilizers from binding to TTR. Kelly's group recently reported a subunit exchange assay, which is a practical approach to demonstrate that the kinetic stabilizer of interest is able to selectively stabilize TTR in human plasma over all the other blood proteins to which it could bind [160].

The synthetic chemistry efforts ultimately yielded >1000 small-molecule TTR kinetic stabilizers that are potent aggregation inhibitors. These kinetic stabilizers comprise several structural families, including bisaryloxime ethers, biphenyls, 1-aryl-4,6-biscarboxydibenzofurans, 2-phenylbenzoxazole, and biphenylamines [115, 117, 141, 152, 158, 159, 161–177]. Through screening efforts, the non-steroidal anti-inflammatory drugs diflunisal and flufenamic acid were found to kinetically stabilize TTR [165, 178, 179]. Most kinetic stabilizers bind to TTR with negative cooperativity, apparently resulting from

conformational changes within the tetramer upon binding to the first T_4-binding site [117, 169, 180]. This is notable because occupancy of only one T_4-binding site is sufficient to impart kinetic stabilization on the entire TTR tetramer [169].

Testing the kinetic stabilizer strategy in a TTR polyneuropathy clinical trial

Two molecules discovered by Kelly's group, tafamidis (Vyndaqel®), which is a fit-for-purpose TTR kinetic stabilizer fashioned by structure-based drug design (K_{d1} = 2 nM, K_{d2} = 200 nM) [152, 181], and diflunisal, a repurposed drug discovered by screening (K_{d1} = 75 nM, K_{d2} = 1100 nM) [165, 179, 182, 183], have been evaluated in independent, international, randomized, double-blind, placebo-controlled TTR polyneuropathy clinical trials [85, 184, 185]. In the tafamidis trial, both primary endpoints were met in the efficacy-evaluable population (n = 87) although they were just missed in the intent-to-treat population (n = 125), not as a consequence of treatment failure, but because more patients than expected in the intent-to-treat population underwent liver transplantation during the course of the trial. Nonetheless, these patients were classified as treatment failures in the conservative analysis employed [184]. The results of the diflunisal clinical trial demonstrated the ability of this drug to significantly slow the rate of neurological impairment and preserve quality of life in polyneuropathy patients, enabling diflunisal to be repurposed as a TTR kinetic stabilizer [185]. Even though diflunisal is a less potent TTR kinetic stabilizer than tafamidis, it efficiently kinetically stabilizes TTR at a dose of 250 mg BID because of its high oral bioavailability and its correspondingly very high plasma concentrations (≈300 μM–1 mM). Diflunisal slows renal blood flow and is therefore contraindicated for some TTR amyloidosis patients, e.g. those with congestive heart failure or renal insufficiency. The real world experience with tafamidis suggests that it arrests the progression of polyneuropathy in ≈60% of early-stage polyneuropathy patients, while reducing the progression rate in the remainder by as much as 80%; this is a much better performance than may have been predicted by the neurological impairment score monitored in the clinical trial [85, 184, 186].

Projected clinical applications of TTR kinetic stabilizers

As mentioned above, the TTR amyloidoses are a group of systemic degenerative diseases that exhibit heterogeneous clinical phenotypes. The most well-recognized organs/systems involved are the peripheral nervous system and/or the heart, but some patients show a rarer presentation, such as mononeuropathies of the upper limbs (e.g. carpal tunnel syndrome) [103] or cardiac arrhythmias (e.g. atrial fibrillation) [100]. The reasons for this clinical heterogeneity are not well understood. Nonetheless, it appears that TTR amyloidogenesis underlies all of the phenotypes and the available treatments for TTR polyneuropathy should be considered for these alternative clinical manifestations.

Furthermore, some recent findings indicate that TTR misfolding and aggregation may be responsible for a much wider spectrum of pathologies than previously appreciated. Tafamidis has an excellent safety record, therefore evaluating its utility in other related diseases seems appropriate. Appropriately powered, randomized, double-blind, placebo-controlled clinical trials with tafamidis would at least yield important new insights into the mechanism underlying these pathologies that are now linked to TTR aggregation.

Cardiac TTR amyloidosis—The population of patients with predominantly cardiomyopathy symptoms resulting from TTR aggregation is quite large; indeed this is likely the largest TTR amyloidosis population [99], estimated to be >0.25 million patients in the USA. New therapies and early diagnostic strategies are desperately needed for this cohort. The aggregation of WT and/or specific mutants of TTR is known to lead to a predominantly cardiomyopathy phenotype [99, 187]. However, it is now clear that cardiac involvement is found in most of the TTR amyloidoses, even if polyneuropathy is the reason patients initially seek medical attention. Historically, WT TTR aggregation-associated cardiomyopathy was treated by heart transplantation and familial cardiomyopathy was treated by combined heart and liver transplantation [188], if it was diagnosed early (i.e. before the clinical manifestations were too advanced to constitute a contraindication for surgery) [189, 190]. The severity of these cardiac diseases, combined with the apparent lack of significant side effects in neuropathy patients effectively treated with tafamidis, prompted cardiologists to use this new approach both for WT and mutant TTR cardiac amyloidosis [191, 192]. Encouraging results from a clinical study led Pfizer to initiate a 30-month clinical trial including >400 WT and familial TTR amyloidosis patients to test the effectiveness of 20 or 80 mg tafamidis in these populations versus placebo. Additionally, Alnylam is currently enrolling familial amyloid cardiomyopathy patients in an 18-month trial to evaluate their RNAi drug, with the Isis antisense oligonucleotides cardiomyopathy trial starting soon.

Iatrogenic TTR amyloidosis (domino liver transplantation recipients)—In a domino liver transplantation, a patient in dire need of a liver (e.g. with hepatic carcinoma or advanced cirrhosis) receives the liver from a familial TTR polyneuropathy patient who has received a WT/WT TTR encoding liver from a cadaveric donor. It is now established that a subset of the domino liver recipients go on to develop TTR aggregation-associated polyneuropathy after a few years [193–203]. These data suggest that domino recipients should also be considered for tafamidis or other pharmacological treatment to prevent the onset of neuropathy and subsequently cardiomyopathy, especially when young patients receive a domino liver to ameliorate a life-threatening disease [201].

CNS TTR amyloidosis—There is substantial evidence that TTR secreted by the choroid plexus can dissociate, misfold, and aggregate on the surface of the leptomeninges and in the leptomeningeal vessels [204] and also, more rarely, in the walls of cortical vessels and brain parenchyma [205]. Patients with CNS TTR amyloidosis can present with a variety of progressive CNS syndromes such as cerebellar ataxia, dementia, myelopathy, and hearing loss [105, 204–221]. Episodes of fluctuating consciousness often associated with focal neurological deficits and headache have also been described, in some cases with pathology-proven brain microbleeds [222, 223]. Recurrent subarachnoid hemorrhages with no associated aneurism have also been reported as a life-threatening clinical manifestation [224, 225]. Because tafamidis can ameliorate TTR aggregation-associated polyneuropathy [85, 184], determining whether it can penetrate the brain sufficiently to significantly kinetically stabilize TTR in the CSF is a priority. It seems unlikely that the RNAi or antisense (RNAse) strategies would be able to target the choroid plexus, unless directly administered there.

Prophylaxis for familial polyneuropathy and familial cardiomyopathy amyloidosis—It is clear from the TTR polyneuropathy clinical trial data that there is an outcome advantage in treating polyneuropathy patients with tafamidis as early as possible [85, 184]. In certain geographical areas, there are abnormally high numbers of carriers with mutations that cause familial amyloid polyneuropathy: in the north of Portugal, the estimated prevalence of the V30M mutation is one in 1000 individuals [226]; in certain areas in northern Sweden, the frequency of this same mutation is 4% [227]. Asymptomatic TTR disease-associated mutation carriers in these high-prevalence areas are followed annually in reference centers, where symptom appearance is monitored. Because there is evidence from pathology [228] and magnetic resonance imaging [229] suggesting that the amyloid deposition and nerve damage starts years before the patients develop symptoms, an argument could be made for prophylactic treatment with tafamidis and/or other therapies with good safety profiles. Assuming that the cardiomyopathy clinical trial results demonstrate efficacy, the same arguments can be made for prophylactic treatment of familial amyloid cardiomyopathy mutation carriers. In the USA, the frequency of V122I mutation is approximately 4% among African-Americans, and there are other rarer cardiomyopathy mutations that appear to have a high penetrance for disease development. The case for prophylactic treatment of TTR aggregation-associated polyneuropathy and cardiomyopathy will become even more compelling once surrogate or diagnostic biomarkers in asymptomatic carriers can be followed so that unnecessary treatment can be avoided, as disease penetrance is incomplete.

Perspectives on treatment of TTR amyloidosis

The aggregation of TTR appears to cause a surprisingly diverse array of pathologies. Structure-based design principles [117, 158, 159, 181] should be development of the drug tafamidis [152], a highly selective TTR kinetic stabilizer that slows TTR aggregation and the progression of TTR amyloid disease in patients with polyneuropathy by binding avidly to TTR to slow its dissociation, the rate-limiting step for TTR aggregation [85, 184]. We envision that diseases caused by TTR aggregation will be ameliorated by using small-molecule kinetic stabilizers [85, 184, 185], possibly in combination with drugs that lower the concentration of TTR mRNA [135, 136, 138], and conceivably in the near future in combination with drugs that enhance the capacity of the cellular proteostasis network to achieve proteome maintenance [90, 92, 230]. We speculate that the clinical applications of tafamidis and other therapies that prevent TTR aggregation will expand in parallel with the discovery of new clinical manifestations of TTR amyloidosis, many of which represent unmet medical needs.

Many patients are presently receiving treatment with registered or investigational drugs, as evidenced by the drop in the number of patients who have undergone liver transplantation for TTR amyloidosis the last few years (Fig. 4). It is most likely that pharmacotherapy will replace liver transplantation in the future, thus avoiding advanced surgery in this patient population. It is however of outmost importance to monitor these patients during pharmacological treatment in order to verify that the disease has stabilized. As mentioned above, disease duration before transplantation should be short in order to obtain the best

results after transplantation, and delaying the time of transplantation with unsuccessful pharmacotherapy will impair the outcome.

Conclusions

Protein misfolding and aggregation appear to drive the pathology in various amyloid diseases, although it remains unclear how the process of aggregation leads to the loss of post-mitotic tissue. These diseases are associated with reduced quality of life, suffering, and death. If not treated, most forms of amyloidosis are lethal. Therefore, much effort is being exerted to find new efficient treatments. In this review, we chose to describe current and potential future treatments for AD, type 2 diabetes, and the TTR amyloidoses. Treatments for the TTR amyloidoses are more advanced than for other amyloid diseases, as the possibility exists to transplant organs and provide pharmacological relief. Still some forms of TTR amyloidosis are not suitable for transplantation and thus evaluation of the effect of other treatments, such as tafamidis, is important. Indeed the introduction of tafamidis has decreased the number of liver transplantations performed.

Low insulin levels in type 2 diabetes have been controlled for many years with insulin injections. However, the accumulation of IAPP-forming plaques in the islets of Langerhans is not treatable and more efforts are needed in order to decrease cell demise. There are interesting links between type 2 diabetes and AD and we have described the presence of IAPP in the brain and the finding that IAPP can initiate seeding of Aβ in the brain, providing evidence of a direct molecular interaction. This is one example of how different forms of amyloidosis may affect each other.

To date, there are no effective disease-slowing or -modifying treatments for AD. Even though several vaccines, small molecules, and dietary supplements are in clinical trial, more studies on the molecular mechanisms underlying the complex AD pathogenic process are needed. Moreover, it is important to combine knowledge gathered from different types of amyloidosis as the process of amyloid formation essentially follows the same pattern.

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Conflict of interest statement

Dr. Kelly reports grants from National Institutes of Health, grants from Skaggs Institute for Chemical Biology, during the conduct of the study; personal fees from Pfizer, personal fees from Pfizer, outside the submitted work; In addition, Dr. Kelly has a patent US7214695 with royalties paid to Pfizer, a patent US8653119B2 with royalties paid to Pfizer, and a patent US7560488B2 issued.

Dr. Johansson reports a patent METHODS FOR TREATMENT OF ALZHEIMER'S DISEASE pending to AlphaBeta AB.

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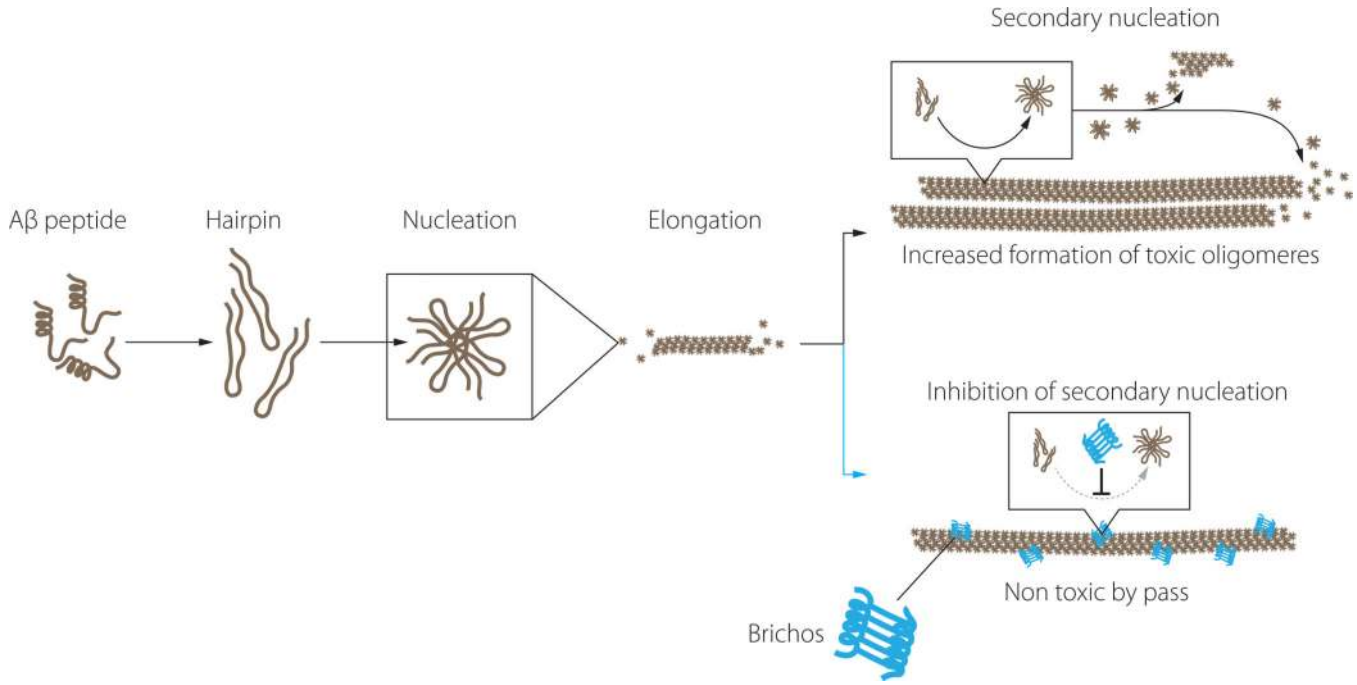


Fig. 1.

BRICHOS inhibition of Aβ fibril formation. BRICHOS binds to the surface of the Aβ fibril where it specifically protects the sites at which secondary nucleation events take place, thereby preventing the catalyzed formation of toxic oligomers [19].

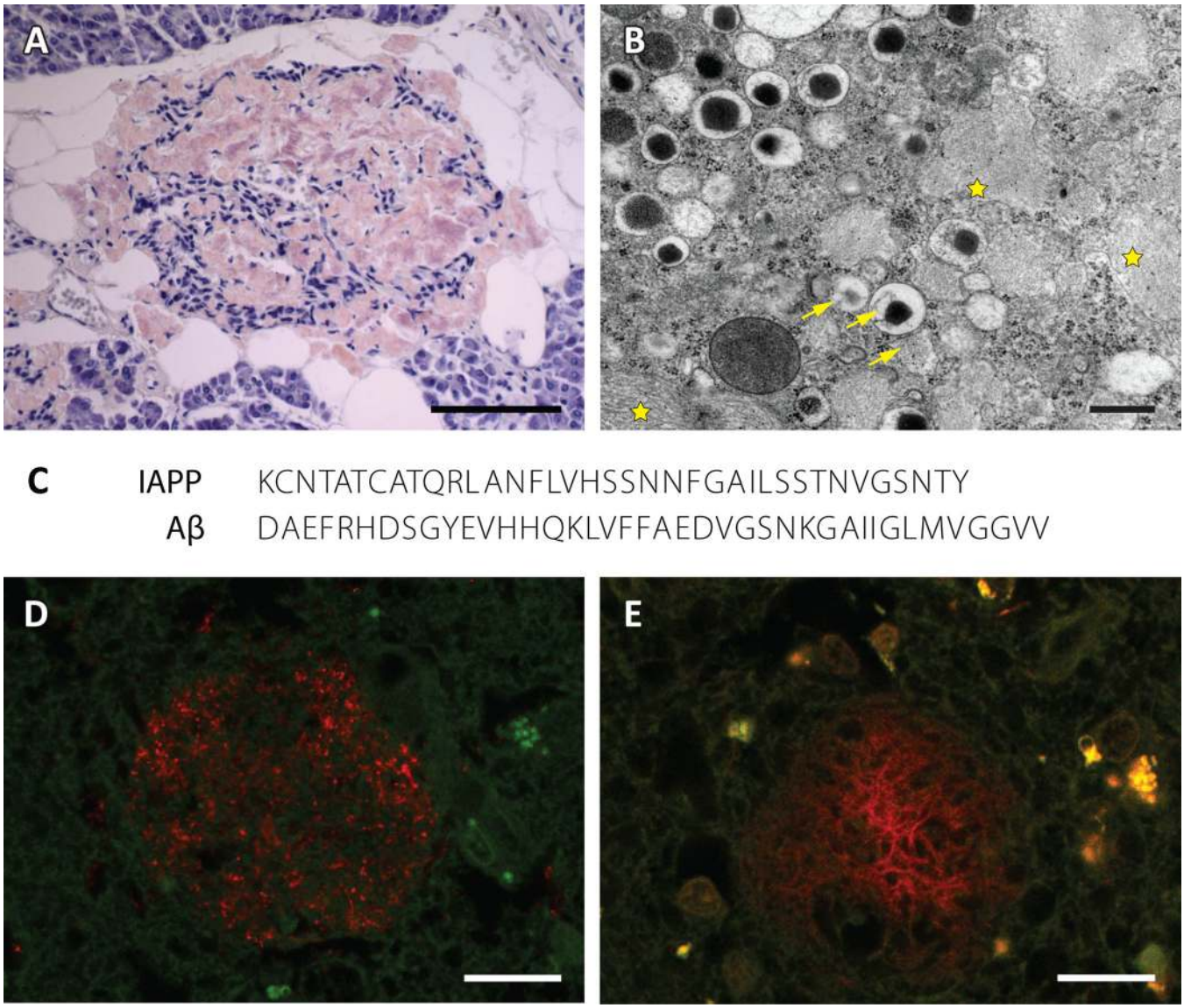


Fig. 2.

A) An islet with large amounts of amyloid with disrupted islet architecture. Bar 100 μm. B) Electron microscopy image of a beta cell with intracellular islet amyloid polypeptide (IAPP). Insulin constitutes the granule dense core while IAPP occupies the halo region. Arrow indicates proIAPP/IAPP fibrillar material, and asterisks show IAPP amyloid. Bar 250 nm. C) One-letter code of the amino acid sequences of human IAPP and Aβ1–40. D and E) Immunological detection of IAPP and Aβ in amyloid deposits in the brain of a patient with Alzheimer's disease. Proximity ligation assay (PLA) allows identification of co-localization when antigens are within 40 nm. Red fluorescent dots correspond to positive PLA signals (D). Consecutive section, with amyloid identified by Congo red staining (E). Bar 20 μm.

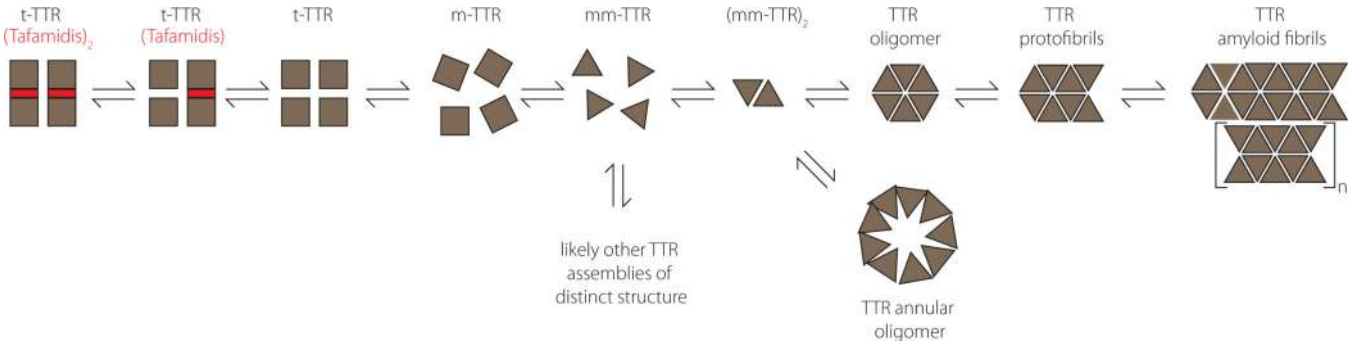


Fig. 3.

Schematic depiction of what is known about the mechanism of transthyretin (TTR) aggregation and how tafamidis (represented by filled rectangles) stabilizes the tetramer, preventing the dissociation of TTR, which is the rate-limiting step of TTR aggregation. It remains unclear how the process of aggregation leads to the loss of certain tissues, but it is clear that several different TTR aggregate structures are formed during aggregation. t=tetramer; m=monomer; mm=misfolded monomer.

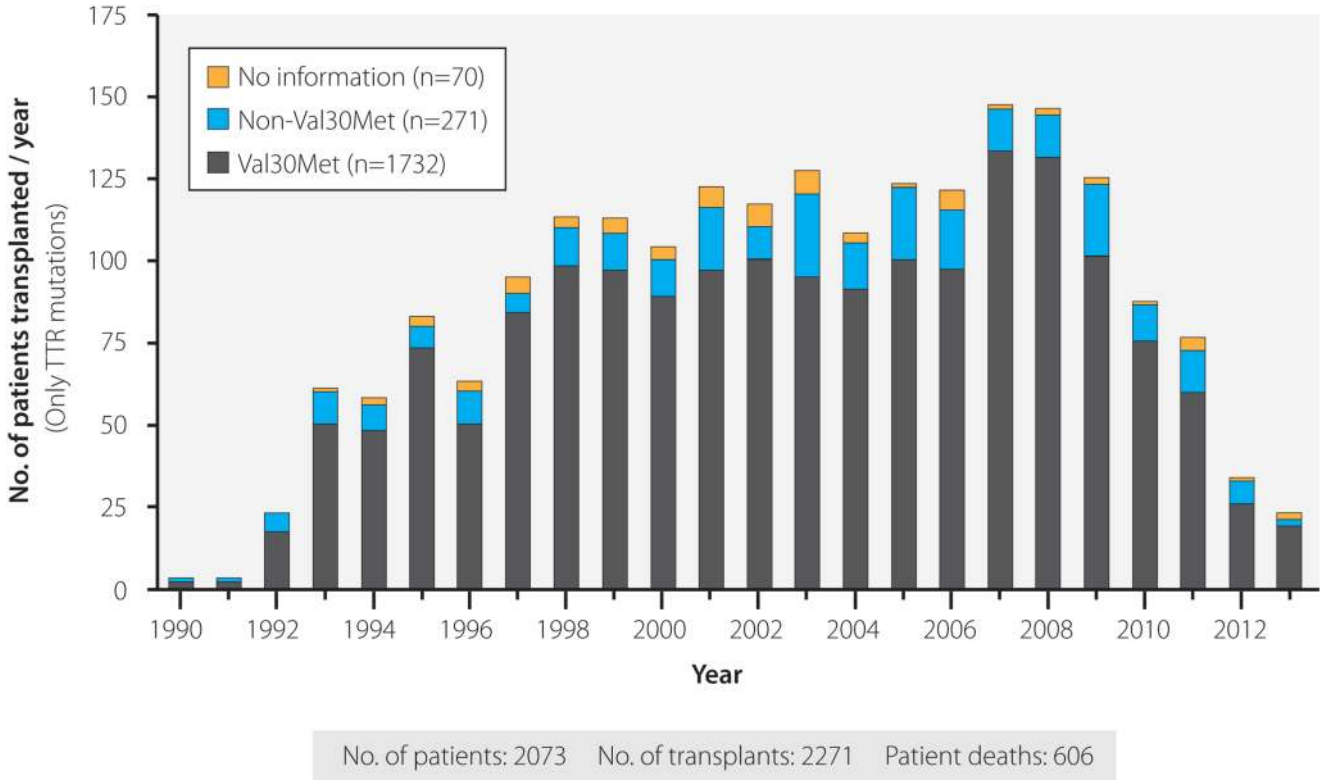
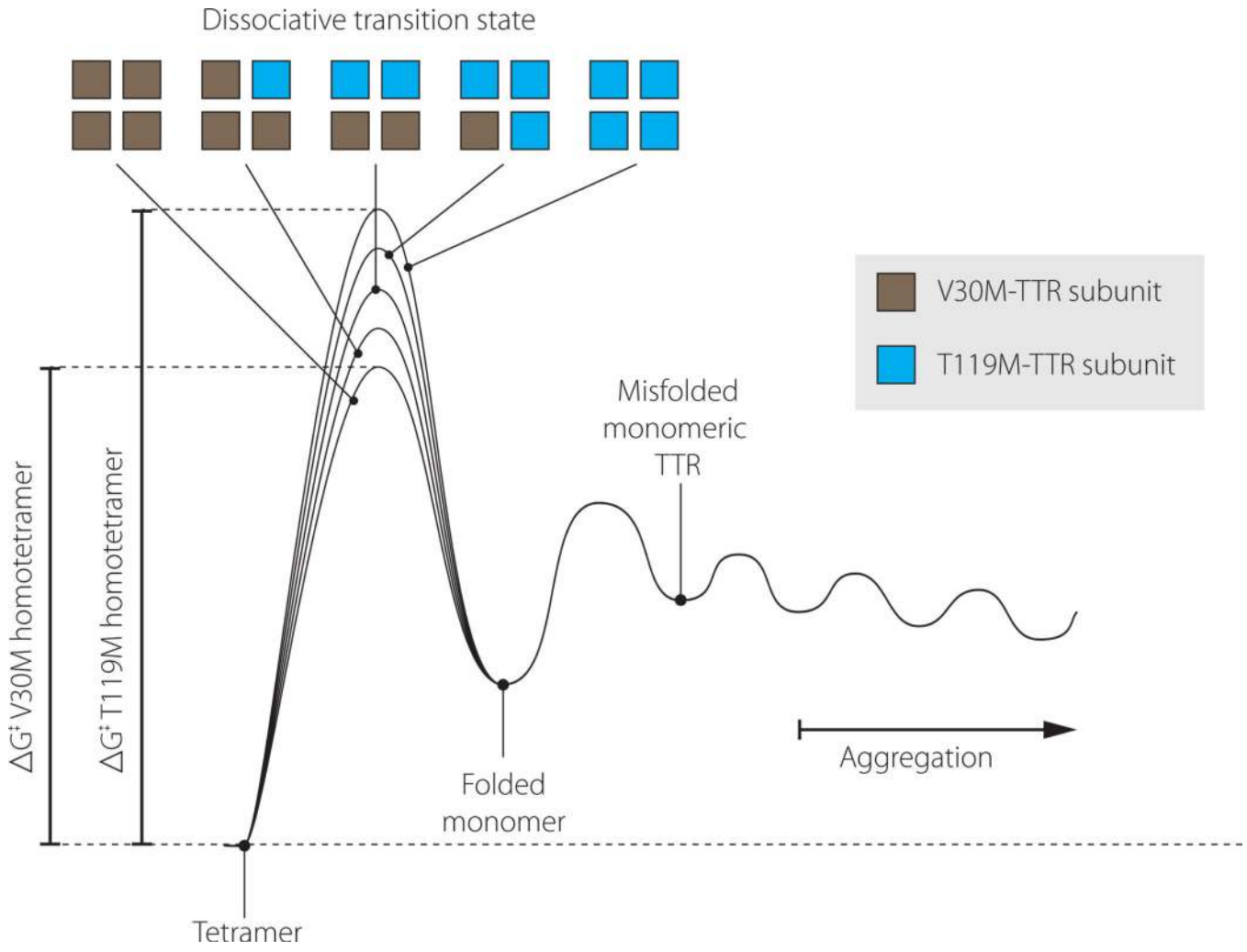


Fig. 4.

Number of patients transplanted due to transthyretin (TTR) amyloidosis per year and reported to the Familial Amyloidotic World Transplant Registry. The introduction of tafamidis and other drugs under clinical investigation have markedly reduced the transplant activity for this indication.



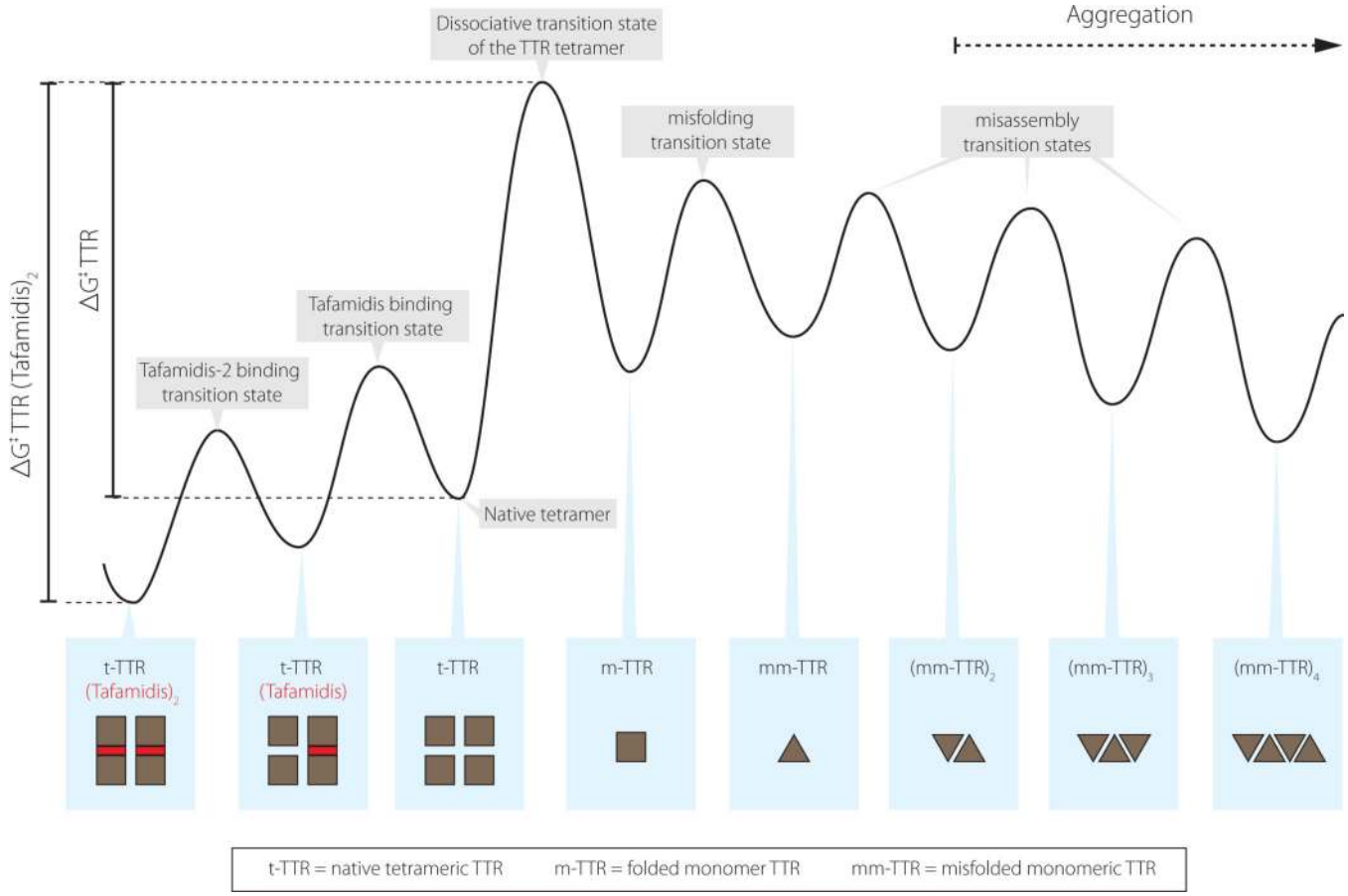


Fig. 5.

A) Mechanism of T119M interallelic trans-suppression of amyloidogenesis. Incorporation of T119M subunits into a transthyretin (TTR) tetramer otherwise composed of amyloidogenic protomers raises the barrier for dissociation proportional to the number of T119M subunits comprising the tetramer. Thus T119M subunit incorporation into the tetramer slows the rate-limiting step of TTR aggregation. B) Linear free energy diagram depicting what is known about the mechanism of TTR aggregation and how tafamidis (represented by filled rectangles) kinetically stabilizes the tetramer, slowing dissociation of TTR, which is the rate-limiting step of TTR aggregation. TTR aggregation is thermodynamically favorable, however the process can be stopped by eliminating the misfolded TTR monomer. t=tetramer; m=monomer; mm=misfolded monomer.

Table 1

Clinical trials for Alzheimer's disease: summary of ongoing amyloid-related clinical trials with active immunotherapy, passive immunotherapy, and small molecules

ACTIVE IMMUNOTHERAPY

Name	Synonyms	FDA Status	Company	Target Type	Therapy Type	Condition	No of patients	Finished
Affitope AD02		2	AFFiRiS AG	Amyloid-Related	Immunotherapy (active)	Alzheimer's Disease	194	2014 (terminated, results not posted)
CAD106		2	Novartis Pharmaceuticals Corporation	Amyloid-Related	Immunotherapy (active)	Alzheimer's Disease	1340	2023

PASSIVE IMMUNOTHEARPY

Name	Synonyms	FDA Status	Company	Target Type	Therapy Type	Condition	No of patients	Finished
AAB-003	PF-05236812	1	Janssen, Pfizer	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	52	2014 (completed, results not posted)
Aducanumab	BIIB037	1	Biogen	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	1350	2022
BAN2401		2	Biogen, Eisai Co., Ltd.	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	800	2018
Crenezumab	MABT5102A, RG7412	2	Genentech	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	72	2017
Gamunex	Intravenous Immunoglobulin, Human Albumin Combined With Flebogamma	2/3	Grifols Biologicals Inc.	Amyloid-Related, Inflammation	Immunotherapy (passive)	Alzheimer's Disease Apporoved for Immunodeficiency, chronic inflammatory demyelinating neuropathy	350	2016
Gantenerumab	RO4909832, RG1450	3	Chugai Pharmaceutical Co., Ltd., Hoffmann-La Roche	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	1000	2019
LY3002813	N3pG-Aβ Monoclonal Antibody	1	Eli Lilly & Co.	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	100	2016
MEDI1814		1	AstraZeneca	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	121	2016
Octagam®	Intravenous Immunoglobulin, NewGam	2, 2	Octapharma	Amyloid-Related, Inflammation	Immunotherapy (passive)	Alzheimer's Disease, Mild Cognitive Impairment	58	2010 (has results)
SAR228810		1	Sanofi	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	48	2015
Solanezumab	LY2062430	3	Eli Lilly & Co.	Amyloid-Related	Immunotherapy (passive)	Alzheimer's Disease	2052	2016

SMALL MOLECULE

Name	Synonyms	FDA Status	Company	Target Type	Therapy Type	Condition	No of patients	Finished
AZD3293	LY3314814 BACE inhibitor	2/3	AstraZeneca	Amyloid-Related	Small Molecule	Alzheimer's Disease	1551	2019
Azeliragon	PF-04494700, TTP488 RAGE inhibitor	3	Pfizer, TransTech Pharma, Inc.	Amyloid-Related, Inflammation	Small Molecule	Alzheimer's Disease	800	2018
BI 1181181	VTP 37948, BACE inhibitor	1	Boehringer Ingelheim, Vitae Pharmaceuticals	Amyloid-Related	Small Molecule	Alzheimer's Disease	36	2015 (terminated)
E2609	BACE inhibitor	2	Biogen, Eisai Co., Ltd.	Amyloid-Related	Small Molecule	Alzheimer's Disease	700	2018
EVP-0962	EVP 0015962 Gamma-secretase modulator	2, 2	FORUM Pharmaceuticals Inc. (was EnVivo)	Amyloid-Related	Small Molecule	Alzheimer's Disease, Mild Cognitive Impairment	52	2013 (completed, results not posted)
JNJ-54861911	BACE inhibitor	2	Janssen, Shionogi Pharma	Amyloid-Related	Small Molecule	Alzheimer's Disease	100	2014
Nasal insulin	Detemir, Levemir, Humulin, Novolin	2		Amyloid-Related, Other	Small Molecule, Other	Alzheimer's Disease, Mild Cognitive Impairment	30	2016

SMALL MOLECULE

Name	Synonyms	FDA Status	Company	Target Type	Therapy Type	Condition	No of patients	Finished
						Approved for Diabetes		
PQ912	Inhibitor of glutaminyl cyclase (QC), reduction of pGlu- Aβ generation	2	Probiodrug AG	Amyloid-Related, Inflammation	Small Molecule	Alzheimer's Disease	110	2016
Verubecestat	MK-8931, MK-8931-009, BACE inhibitor	3	Merck	Amyloid-Related	Small Molecule	Alzheimer's Disease	1500	2018

For further details see:
http://www.alzforum.org/therapeutics
https://clinicaltrials.gov

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