Initial Aβ seeds as therapeutic target for Alzheimer's disease

Dissertation

zur Erlangung des Grades eines
Doktors der Naturwissenschaften

der Mathematisch-Naturwissenschaftlichen Fakultät
und
der Medizinischen Fakultät
der Eberhard-Karls-Universität Tübingen

vorgelegt
von

Juliane Schelle
aus Berlin, Deutschland

June 2017

Tag der mündlichen Prüfung: 21.09.2017

Dekan der Math.-Nat. Fakultät:	Prof. Dr. W. Rosenstiel
Dekan der Medizinischen Fakultät:	Prof. Dr. I. B. Autenrieth
1. Berichterstatter:	Prof. Dr. M. Jucker
2. Berichterstatter:	Prof. Dr. P. Heutink
Prüfungskommission:	Prof. Dr. M. Jucker
	Prof. Dr. P. Heutink
	Dr. J. Neher
	Dr. D. David

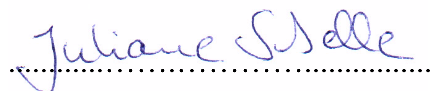
Erklärung

Ich erkläre, dass ich die zur Promotion eingereichte Arbeit mit dem Titel:

“Initial Aβ seeds as therapeutic target for Alzheimer’s disease”

selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Angabe einer Versicherung des Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

Tübingen, 30.09.2017



Unterschrift

Für meine Familie, Freunde und Hansi

"Erinnerung ist das Seil, ... das mich herauszieht aus dem Abgrund des Nicht-Seins."

"Auf der Suche nach der verlorenen Zeit" von Marcel Proust

Acknowledgements

First of all I want to thank Franziska Langer, who presented a poster during a lab visit, when I was applying for the Master program in Tübingen. Because of her my decision to study in Tübingen was enforced and I knew I would like to perform my master thesis in this lab. Mathias as my supervisor gave me the chance to do so two years later. I'm very grateful he prolonged my contract in order for me to start a PhD in his lab, during which he fully supported my project and me. Furthermore I want to express my gratitude to my Advisory Board members, Della David and Peter Heutink.

Throughout those years as a PhD student many people influenced my carrier, my work, my personality, but also my private life. Thanks to Sarah for supervising and introducing me into the complex topic at the beginning. Ulli, Jörg, Matthias Staufenbiel, Jonas, and the entire Jucker lab provided help when it came to stainings, mice, strange results or whatsoever. A big thank you goes to Marius, Lisa, and Stephan I spent a lot of time with discussing how to interpret MSD results. It is mainly Timo, Jay, Manuel, Anika, Renata, Angelos, Ann-Christin, and Mehtap I had the most fun with. I will never forget the bruises after paintball, the excitement during gocarting, skiing, or in Europapark, the bowling sessions, the funny nights at Chez Michel, the crazy Wasen experiences, and all the other great moments. In particular, thanks Timo for your technical help and the "Schweigefuchs" in San Diego; thanks Jay for your nice dance moves; thanks Manuel for unforgettable trips to Basel, Lausanne, and Strasbourg; thanks Anika for funny jokes; and the biggest thank you goes to Renata, Angelos, Ann-Christin, and Mehtap, who always had to deal with my emotions, doubts, anger, and tears. Thank you for always being at my side no matter if for laughing or crying!

Of course without my entire family and all of my friends this PhD would not have been possible. However, I want to emphasize my mum, who is the one person in the whole world, who always understands me, gives advise, and motivates me. Sorry, for all the work stories you had to listen to. I really appreciate it! Svenja, thank you for supporting me and being my best friend. A final thank you goes to the person, who has managed to make me forget my PhD and all the work, the person who can make me smile no matter what, and the person I love truthfully. Thank you for being you, Hansi!

Table of contents

1. Summary **1**

2. Synopsis **3**

2.1 Alzheimer's disease at a glance **3**

2.1.1 Characteristics of Alzheimer's disease 3

2.1.2 Aggregation of the Aβ peptide as key trigger of Alzheimer's disease 8

2.2 Inhibiting BACE1 to impede amyloid pathology **12**

2.2.1 Treatment strategies for Alzheimer's disease 12

2.2.2 Necessity of early biomarkers to detect and monitor pathological changes 18

2.3 Longevity of Aβ seeds and their therapeutic potential **22**

2.3.1 Prion-like properties of aggregated Aβ 22

2.3.2 Stability and durability of Aβ seeds 25

2.4 Targeting initial Aβ seeds for the prevention of Alzheimer's disease **28**

2.4.1 Clinical trials for Alzheimer's disease and possible reasons for recent failures 28

2.4.2 Delay of cerebral β-amyloidosis by short and early combination therapy 32

2.5 Concluding remarks and outlook **34**

2.6 References **36**

3. Publications **55**

3.1 Description of personal contribution **55**

3.2 Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition **56**

3.3 Persistance of Aβ seeds in APP null mouse brain **66**

3.4 Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds **74**

4. Appendix **96**

4.1 Abbreviations **96**

4.2 Curriculum vitae **99**

4.3 Bibliography **100**

1. Summary

Alzheimer's disease (AD) is the leading form of dementia interfering with daily life due to progressive memory loss and cognitive disabilities. With more than 45 million people suffering from dementia worldwide, AD is one of the costliest health conditions to society. Because of an increasing proportion of elderly people the number of individuals living with dementia is expected to more than triple by 2050. Although there are symptomatic treatments available that temporarily slow the worsening of clinical symptoms, a disease-modifying cure is still missing. Therefore, dementia, and AD in particular, is becoming a public health priority evoking worldwide efforts to delay or even prevent the disease from ever developing.

The amyloid cascade hypothesis explained in detail in chapter one of this thesis proposes that an impaired homeostasis of production and clearance of the amyloid-β (Aβ) peptide is the trigger initiating a sequence of pathogenic events causing AD. Aβ misfolding and aggregation leads to the accumulation of cerebral amyloid plaques, a typical hallmark of AD. As the pathology progresses, plaques continue to develop and grow, which is suggested to lead to the second disease characteristic, neurofibrillary tangles (NFTs), consisting of hyperphosphorylated tau proteins. Aβ as the driving force of this pathological cascade has been regarded as the most reasonable therapeutic target.

Therefore the second chapter is dedicated to different therapeutic approaches for AD with particular focus on the β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1), which is responsible for regulating the production of Aβ. To this end APP transgenic (tg) mice that mimic β-amyloidosis-related features of AD were treated with a potent BACE inhibitor for six months. In response to this long-term therapy, brain Aβ as well as plaque deposition were reduced to levels comparable to six months younger animals. Surprisingly, BACE inhibition also exhibited downstream effects preventing the pathology-dependent increase of tau in the cerebrospinal fluid (CSF). Thus, BACE inhibitors are valuable therapeutic agents and their effectiveness can be predicted by CSF tau measurements in clinical trials. These findings have been published in *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* (Schelle et al., 2017, *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, 13(6), pp.701-709).

The third part of this thesis investigated the stability of small Aβ aggregates (Aβ seeds), which share pathogenic properties with the prion protein implicated in transmissible spongiform encephalopathies. Whereas in Aβ seeds inoculated APP tg mice the presence of host-derived Aβ together with the exogenously applied seeds induced their propagation, the injected material was undetectable in *App* null mice 30 days post inoculation. However, reinoculation with brain extracts from *App* null mice inoculated with Aβ seeds up to six months harboring Aβ levels below detection, still induced cerebral β-amyloidosis in APP tg hosts. In conclusion, Aβ seeds can persist even in the absence of host Aβ and regain their pathogenic activity as soon as sufficient Aβ becomes available. This discovery indicates on the one hand that lowering Aβ production inhibits the formation of new Aβ seeds and on the other hand that therapeutic intervention is most effective when applied at early stages. These results have been published in *Nature Neuroscience* (Ye, Fritschi, Schelle et al., 2015, *Nature neuroscience*, 18(11), pp.1559-61).

Recent biomarker studies in familial AD subjects, which revealed that first pathological changes occur up to 25 years before clinical disease onset, supported this idea. Therefore, in the last part, pre-depositing APP tg mice were treated with a combinational therapy based on anti-Aβ immunization to remove Aβ seeds and a BACE inhibitor to block the production of soluble Aβ. Using two different APP tg mouse models results revealed that brain Aβ levels and plaque formation were reduced acutely after the treatment. Moreover, this short but early intervention delayed amyloid pathology after discontinuation of the treatment. Thus, targeting initial Aβ seeds by anti-Aβ combination therapy might be the most promising strategy to effectively prevent cerebral β-amyloidosis. These findings are now prepared for publication (Schelle et al., 2017).

In summary, this doctoral thesis highlights the importance of early therapeutic intervention with anti-Aβ drugs in order to prevent AD. Previous studies have shown that treatment of AD patients who have already developed irreversible neurodegeneration might be inadequate to stop the progression of this devastating disorder and could be one reason to explain recent failures of anti-amyloid agents in numerous clinical trials. The results presented in this doctoral thesis indicate that the treatment focus should be shifted toward earlier stages of AD and even toward primary prevention before symptom onset targeting initial Aβ seeds.

2. Synopsis

2.1 Alzheimer's disease at a glance

2.1.1 Characteristics of Alzheimer’s disease

26.11.1901: Alois Alzheimer im Gespräch mit Auguste D.

„Wie heißen Sie?“ – „Auguste.“

„Familienname?“ – „Auguste.“

„Wie heißt ihr Mann?“ – „Ich glaube Auguste.“

„Ihr Mann?“ – „Ach so, mein Mann.“

„Sind Sie verheiratet?“ – „Zu Auguste.“

„Frau D.?“ – „Ja, zu Auguste D.“

03.11.1906: Tübinger Chronik und Steinlach Bote
Amts- und Anzeigeblatt für den Oberamts-Bezirk Tübingen.

„Am Samstag Nachmittag 3 Uhr begannen im Hörsaale der hiesigen psychiatrischen Klinik die Verhandlungen der 37. Versammlung der süddeutschen Irrenärzte, zu welcher sich etwa 90 Teilnehmer eingefunden hatten... Über einen eigenartigen schweren Krankheitsprozess, der einen bedeutenden Schwund der Nervenzellen innerhalb von 4 $^{1}/_{2}$ Jahren verursachte, berichtete Privatdozent Dr. Alzheimer (München).“

11.01.1911: Über eigenartige Krankheitsfälle des späten Alters.
Von A. Alzheimer.

„Im Jahre 1906 habe ich einen Fall von Erkrankung des präsenilen Alters beschrieben, ... die damals noch unbekannt war. Hinsichtlich der klinischen Erscheinung war eigenartig eine rasch sich entwickelnde und in kurzer Zeit zu den tiefsten Graden fortschreitende Verblödung...“

Already in 1901 the neuropathologist Alois Alzheimer started to investigate his patient Auguste Deter, who suffered from a rapid loss of memory, disorientation in time and space, auditory hallucinations, and psychosocial impairment. Because she was only 56 years old when she died, she did not meet the inclusion criteria for senile dementia that had been known so far. Hence, senile dementia was excluded as a reason for her symptoms (Alzheimer, 1907). That is why in 1906 at a conference in Tübingen Alzheimer described, and published in 1907 and 1911, an unusual illness of the cerebral cortex, with unfamiliar clinical as well as pathological characteristics (Alzheimer, 1907; Alzheimer, 1911).

Nowadays Alzheimer's disease (AD) - named after the discoverer - is the most common neurodegenerative disease and the leading cause of dementia worldwide (Dartigues, 2009; Holtzman et al., 2011). Dementia is a general term for conditions characterized by a decline in memory or other cognitive skills due to neuronal loss leading to a person's inability to perform everyday activities (Alzheimer's Association, 2014). AD specifically is characterized by a progressive loss of episodic memory impeding recollection of recent events and activities, disorientation to time and place, and later the decline in executive functions, language, and emotional stability (Petersen et al., 1999; Alzheimer's Association, 2014). The global number of AD cases is estimated at 46.8 million with a new case of dementia arising somewhere in the world every four seconds (World Alzheimer Report, 2015). As life expectancy keeps rising and with increasing age being the greatest known risk factor for AD, the number of patients is expected to triple by 2050 (Reitz et al., 2011; Wimo, 2015; World Alzheimer Report, 2015). In fact, AD is not only a burden for the ever-increasing number of new patients worldwide, but has also tremendous consequences for their relatives, caregivers, as well as society (World Alzheimer Report, 2015). The global costs of dementia have increased by more than 35% between 2010 and 2015 and will soon escalate into a trillion dollar economic challenge (World Alzheimer Report, 2015). Therefore AD risk reduction, prevention, care, and cure should be made a public health priority.

Already more than 100 years ago, Alois Alzheimer microscopically and histologically analyzed Auguste Deter's brain post-mortem and reported two cardinal pathological alterations, namely extracellular plaques and bundles of neurofibrils (Alzheimer, 1911). Both hallmark lesions still remain a valid and definite clinical diagnosis for AD (Scheltens et al., 2016). The constituents of these plaques are aggregated forms of a small peptide named amyloid-β (Aβ), which forms fibrillar deposits (Glenner & Wong, 1984; Masters et al., 1985; Duyckaerts et al., 2009, Holtzman et al., 2011). Neurofibrillary tangles (NFTs) composed of abnormally hyperphosphorylated tau, a microtubule-associated protein, are found inside nerve cell bodies (dystrophic neurites) (Kosik et al., 1986, Grundke-Iqbal et al., 1986; Goedert et al., 1988; Goedert & Spillantini, 2006; Mandelkow & Mandelkow, 2012). Increased numbers of brain region-specific Aβ aggregates as well as NFTs are suggested to lead to the progression of AD (Braak & Braak, 1991; Thal et al., 2002; Jucker & Walker, 2013). Both assemblies show features characteristic of amyloid (Glenner & Wong, 1984; Masters et al., 1985; Duyckaerts et al., 2009; Eisenberg & Jucker, 2012). Amyloids are predominantly composed of β-sheet structures displaying a specific cross-β fiber diffraction

pattern. Furthermore they bind to the dye Congo Red, which exhibits anomalous colors, referred to as apple green birefringence, when examined between crossed-polarizers (Rambaran & Serpell, 2008; Sipe et al., 2014). Amyloids are usually unbranched and fibrillar in structure giving rise to toxic tissue deposits (Haass & Selkoe, 2007; Eisenberg & Jucker, 2012; Klein, 2013).

In 1992, John Hardy and Gerald Higgins described the most dominant model of AD pathogenesis to date. This amyloid cascade hypothesis postulates that an imbalance between the production and/or the clearance of Aβ peptides due to altered expression or processing of the β-amyloid precursor protein (APP) causes the deposition of Aβ in plaques followed by a sequence of pathogenic events leading to AD dementia (Hardy & Higgins, 1992; Hardy & Selkoe, 2002).

Aβ is produced by proteolytic cleavage of APP, a transmembrane protein with a single membrane-spanning domain (Glenner & Wong, 1984; Masters et al., 1985; Kang et al., 1987). Alternative splicing gives rise to different APP isoforms: APP_{695} is predominantly expressed in the central nervous system (CNS), whereas APP_{751} and APP_{770} are ubiquitously expressed in non-neuronal tissue (Kitaguchi et al., 1988; Ponte et al., 1988; Tanaka et al., 1988). Despite the involvement of APP in synaptic functioning and transmission as well as plasticity and memory formation, its exact physiological role is still under debate (Dawkins & Small, 2014). However, cerebral APP_{695} cleavage and processing can be divided into a non-amyloidogenic (non-pathogenic) and an amyloidogenic (pathogenic) pathway, both of which are illustrated in Figure 1. The non-amyloidogenic pathway is characterized by APP cleavage within the Aβ region by α-secretase and sequentially γ-secretase. This processing precludes the formation of the Aβ peptide and thereby interferes with amyloid deposition. The α-secretase belongs to the disintegrin and metalloprotease (ADAM) family (Sisodia, 1992; Lammich et al., 1999) and its APP cleavage releases soluble APP-α (sAPPα) externally from the endosome or cell membrane (Ghiso et al., 1989; Kojro & Fahrenholz, 2005; Jorissen et al., 2010). The remaining membrane-bound C-terminal fragment of 83 amino acid residues (C83 or CTFα) is subsequently cleaved by the γ-secretase. Two short non-amyloidogenic fragments termed p3 and APP intracellular domain (AICD) are produced (Haass & Selkoe, 1993). The γ-secretase is a multiprotein complex with its key biochemical components presenilin 1 (PS1) or presenilin 2 (PS2), glycosylated nicastrin (NCT), anterior pharynx-defective phenotype 1 (Aph-1), and presenilin enhancer 2 (Pen-2) (De Strooper, 2003;

Edbauer et al., 2003). Presenilins are aspartyl proteases and build the active site of the enzyme (De Strooper et al., 1998; Wolfe et al., 1999).

The amyloidogenic pathway is an alternative APP cleavage pathway resulting in Aβ generation. The β-site APP cleaving enzyme 1 (BACE1, β-secretase) initially cleaves APP at a position located 99 amino acids from the C-terminus (N-terminus of Aβ) (Vassar et al., 1999). Its highly sequence-specific cleavage results in the release of soluble APP-β (sAPPβ) into the extracellular space (Selkoe, 1991). BACE1 is a membrane-bound aspartyl protease (Vassar & Citron, 2000; Cole & Vassar, 2007, Dislich & Lichtenthaler, 2012) present in acidic cellular compartments like the Golgi apparatus and endosomes (Vassar et al., 1999). Other BACE1 substrates are important for the formation of voltage gated sodium channels and axon myelination (Wong et al., 2005; Hu et al., 2006). The remaining hydrophobic, membrane-spanning C-terminal region of APP consisting of 99 amino acid residues (known as C99 or CTFβ) becomes a substrate for the γ-secretase (Vassar et al., 1999; Haass et al., 2012). In contrast to the non-amyloidogenic pathway, the γ-secretase liberates AICD into the cytosol. In addition, various Aβ species are released into the extracellular space through progressive γ-secretase cleavage steps (between residues 37 and 43) (Vassar et al., 1999; Haass et al., 2012) after initially releasing $A\beta_{48}$ or $A\beta_{49}$ as two major precursors (Takami et al., 2009). The predominant Aβ species in healthy individuals is a peptide of 40 amino acid residues in length ($A\beta_{40}$) (De Strooper & Annaert, 2010), in contrast to the less abundant (approximately 10%) but more hydrophobic and presumably pathogenic 42 residue variant ($A\beta_{42}$) (Bitan et al., 2003; Yan & Wang, 2006). Consistently, longer Aβ species are more prone to aggregate than shorter ones (Burdick et al., 1992; Qi-Takahara et al., 2005). Because $A\beta_{42}$ fosters oligomerization, fibrillization, and plaque deposition, it is more amyloidogenic and neurotoxic than $A\beta_{40}$ (Jarrett et al., 1993; Suzuki et al., 1994; Younkin, 1998; Bitan et al., 2003). Furthermore AD patients often exhibit an increased $A\beta_{42:40}$ ratio enhancing synaptotoxicity and amyloid aggregation (Pauwels et al., 2012).

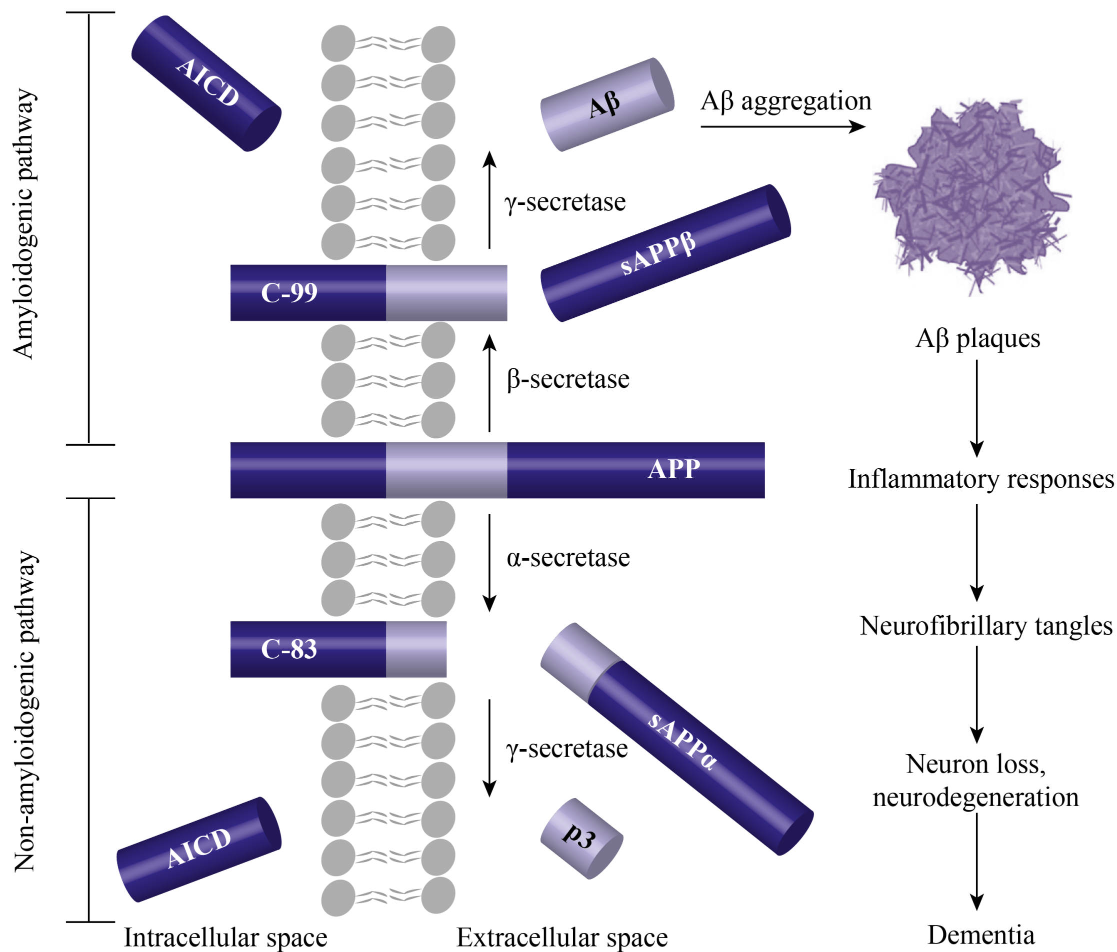


Figure 1: Sequential cleavage of the amyloid precursor protein (APP) and potential consequences of amyloid β (Aβ) peptide aggregation. During the non-amyloidogenic pathway APP is processed by the α-secretase giving rise to the membrane-bound C-terminal fragment C-83 and the soluble APPα (sAPPα). Sequential cleavage by the γ-secretase liberates p3 into the extracellular space and the APP intracellular domain (AICD). In contrast, the amyloidogenic pathway produces soluble APPβ (sAPPβ) and C-99 in response to β-secretase cleavage. The latter is further processed by the γ-secretase resulting in the release of AICD and extracellular Aβ molecules. According to the amyloid cascade hypothesis Aβ aggregation is suggested to trigger a sequence of major pathogenic events leading to dementia associated with Alzheimer's disease. However, Alzheimer's pathogenesis probably comprises parallel and circular interactions of risk factors rather than a linear pathological process (modified from Dislich & Lichtenthaler, 2012 and Hardy & Selkoe, 2002).

Recently a novel cleavage site at amino acid 504-505 of APP_{695} was discovered called η-cleavage (Willem et al., 2015). A membrane-bound matrix metalloproteinase, the η-secretase, cleaves APP and releases CTF-η and Aη of various sizes after further processing by α- or β-secretase (Willem et al., 2015). Although accumulations of the η-secretase and CTF-η found within dystrophic neurites might contribute to AD pathogenesis (Willem et al., 2015), AD causing mutations all affect Aβ, and not Aη, production and aggregation (Bekris et al., 2010), which will be described in more detail in the next chapter.

According to the amyloid hypothesis, aggregation of Aβ in brain regions responsible for memory and cognition is followed by the formation of intracellular NFTs (Kosik et al., 1986; Goedert & Spillantini, 2006) and many other structural and functional changes (Duyckaerts et al., 2009; Holtzman et al., 2011; Nelson et al., 2012). Those include aberrant neuronal network activities due to impaired synaptic function (Dekosky & Scheff, 1990), neuronal loss leading to brain atrophy (Jobst et al., 1994), and neuroinflammation accompanied by oxidative stress and thereby activation of astrocytes and microglia, the immune cells of the brain (Brun & Englund, 1981; Prinz & Mildner, 2011; Prokop et al., 2013). Many recent evidences have highlighted especially the immune system as a key influencer in increasing AD risk and accelerating AD progression (Naj et al., 2011; Jonsson et al., 2013; Huang et al., 2017). However, rather than a linear sequence of pathological events, multicausal, circular, and parallel pathways and risk factors lead to the manifestation of dementia. Nevertheless, all mentioned processes within this pathogenic cascade contribute to the progression of the pre-clinical/prodromal phase of AD to mild cognitive impairment (MCI), followed by the manifestation of mild (early stage), then moderate (middle stage), and finally severe (late stage) AD dementia (Chertkow et al., 2013).

2.1.2 Aggregation of the Aβ peptide as key trigger of Alzheimer's disease

The most convincing piece of evidence supporting the amyloid cascade hypothesis and the key role for Aβ deposition as the initiative event in AD pathogenesis comes from human genetic studies. AD can be divided into an early-onset (< 60 years) familial form (EOAD) and a late-onset (> 60 years) sporadic form (LOAD). Only less than one percent of AD patients carry a dominantly autosomal inherited mutation causing early-onset familial AD (FAD) (Bekris et al., 2010). In fact, all of those missense mutations cause dysfunctional Aβ homeostasis and occur in proteins involved in the generation of Aβ (Hardy & Selkoe, 2002; Selkoe & Hardy, 2016). On the other hand, mutations in the *microtubule associated protein tau* (*MAPT*) gene do not cause AD but frontotemporal dementia, parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy, and other tauopathies (Pagon et al., 1993; Goedert & Jakes, 2005).

Specific *App* gene mutations within and immediately flanking the Aβ region were identified in families suffering from EOAD such as those carrying the Dutch mutation (E693Q) (Van Broeckhoven et al., 1990), the London mutation (V171I) (Goate et al., 1991), the Indiana

mutation (V717F) (Murrell et al., 1991), the Swedish double mutation (K670N/M671L) (Mullan et al., 1992), and the Arctic mutation (E693G) (Kamino et al., 1992), named after the region of discovery. All these *App* mutations increase Aβ production or change the relative amount of the amyloidogenic $Aβ_{42}$ versus $Aβ_{40}$ peptide (Citron et al., 1992; Suzuki et al., 1994). Hence, Aβ aggregates and insoluble parenchymal plaques and/or cerebral hemorrhage with amyloidosis develop and affect normal brain functioning (Herzig et al., 2004; Goedert & Spillantini, 2006). In rare individuals, the *App* gene is duplicated, which also causes EOAD (Rovelet-Lecrux et al., 2006; Sleegers et al., 2006). Due to the *App* gene being located on chromosome 21 (Goldgaber et al., 1987; Robakis et al., 1987), Down's syndrome (trisomy of chromosome 21 and therefore also of the *App* gene) patients develop typical AD neuropathology because they produce excessive amounts of Aβ lifelong (Masters et al., 1985; Lemere et al. 1996). Moreover, amino acid substitutions in the *presenilin 1 (PSEN1)* or *2 (PSEN2)* genes cause the most common and most aggressive form of EOAD due to the relative elevation of $Aβ_{42}$ levels in the brain, which leads to profound Aβ deposition in mid-life (Jarrett et al., 1993; Borchelt et al., 1996; Citron et al., 1997; Selkoe, 2001). In contrast, an *App* missense mutation (A673T) at the second amino acid of the Aβ region has been identified precipitating a reduced Aβ production due to decreased APP cleavage by BACE1 (Jonsson et al., 2012). Consequently, not only the aggregation potency of this mutant Aβ form is lowered but mutation-carriers also have significantly decreased plasma Aβ and therefore the risk of developing AD is reduced (Jonsson et al., 2012; Benilova et al., 2014; Maloney et al., 2014; Zheng et al., 2015; Martiskainen et al., 2017), which confirms the critical involvement of Aβ in AD pathogenesis.

In contrast to the small proportion of FAD patients, in which one specific mutation causes the disease, the majority of cases manifest as the sporadic form, where many genetic and environmental factors could contribute to the disorder (Alzheimer Association Report, 2014). Although the causative triggers of the common sporadic form of AD are still unknown, genome-wide association studies (GWAS) and recent investigations about epigenetic mechanisms revealed certain risk factors (Reitz et al., 2011; Bettens et al., 2013). The strongest risk factor for developing LOAD is advanced age. Nevertheless, aging alone is not sufficient to cause the disease (Reitz et al., 2011). An increased risk of developing AD is the inheritance of one (fourfold increased risk) or two (twelvefold increased risk) copies of the *APOE ε4* gene encoding for the ε4 form (APOE4) of the apolipoprotein E (APOE)

(Schmechel et al., 1993; Strittmatter et al., 1993; Saunders et al., 1993; Corder et al., 1993; Raber et al., 2004). The APOE4 protein leads to chronically decreased brain clearance of Aβ, more amyloid deposits, and thereby contributes to AD risk (Rebeck et al., 1993; Holtzman et al., 2000; Castellano et al., 2011). Other risk genes for LOAD include *ABCA7* (ATP-binding cassette sub-family A member 7) involved in Aβ clearance (Hollingworth et al., 2011; Kim et al., 2013) and *SORL1* (sortilin-related receptor 1) (Rogaeva et al., 2007), *BIN1* (bridging integrator protein 1) (Seshadri et al., 2010), and *PICALM* (phosphatidylinositol-binding clathrin assembly protein) (Harold et al., 2009) as regulators of endosomal vesicle recycling and Aβ transportation. Furthermore *CR1* (complement receptor 1) (Lambert et al., 2009), *CD33* (member of the sialic-acid-binding immunoglobulin-like lectins family) (Bertram et al., 2008; Naj et al., 2011), and *TREM2* (triggering receptor expressed on myeloid cells 2) (Jonsson et al., 2013; Wang et al., 2015b), all involved in the phagocytic immune response to Aβ deposition, are other identified risk loci. Recently, a protective allele with reduced *SPI1* (spi-1 proto-oncogene) expression and therefore reduced AD risk was discovered (Huang et al., 2017). *SPI1* encodes for the microglial transcription factor PU.1, which was found to regulate many established AD risk genes, indicating a crucial role of microglia in the pathogenesis of AD (Huang et al., 2017). In addition to those genetic AD risk factors, smoking (Anstey et al., 2007; Rusanen et al., 2010), obesity (Kivipelto et al., 2005; Whitmer et al., 2008; Luchsinger et al., 2012), type 2 diabetes (Ahtiluoto et al., 2010; Cheng et al., 2011), low education (Stern et al., 1994), traumatic brain injury (Mortimer et al., 1991; Schofield et al., 1997; Lye & Shores, 2000), and other factors have been associated with an increased likelihood of developing AD.

In addition to the detailed analyses of different familial and sporadic AD cases that support the amyloid hypothesis (Citron et al., 1992; Cai et al., 1993; Suzuki et al., 1994; Hardy et al., 1998; Reitz et al., 2011), genetically modified rodents overexpressing mutant forms of human APP or presenilin were generated in order to partly mimic distinct features of AD pathology (Games et al., 1995; Hsiao et al., 1996; Sturchler-Pierrat et al., 1997; Lewis et al., 2001; Radde et al., 2006; Drummond & Wisniewski, 2017). Notably, wild type (wt) mice do not manifest any neuropathological signs of AD. This might be due to their short lifespans, missing or altered cofactors, environmental circumstances, or the three amino acids of the Aβ sequence that differ between mice and humans (Yamada et al., 1987; Walker & Jucker, 2017). Although APP transgenic (tg) mice do not express the entire AD pathology by lacking NFTs and extensive neuronal loss (Calhoun et al., 1998; Rupp et al., 2011), they recapitulate

age-related cerebral β-amyloidosis in the brain including parenchymal plaques and vascular deposition very similar to Aβ lesions characteristic for AD (Ashe & Zahs, 2010; Jucker, 2010). For example, APP23 tg mice overexpress the K670M/N671L-mutated human APP (APPSwedish) and start to form detectable Aβ deposits at six to eight months of age (Sturchler-Pierrat et al., 1997). APPPS1 tg mice co-express in addition to APPSwedish human mutated presenilin, which leads to the development of the first Aβ plaques after six weeks of age (Radde et al., 2006). Another tg mouse model that mimics at least partially sporadic AD overexpresses human wt APP (APP51 mice) and exhibits a much later onset of Aβ deposition (Bodendorf et al., 2002).

Two findings that might undercut the amyloid hypothesis of AD are the presence of Aβ plaques in some aged individuals without cognitive deficits (Braak & Braak, 1991), in line with the poor correlation of the Aβ load with the degree of clinical symptoms (Arriagada et al., 1992; Foley et al., 2015). In fact, the quantity of NFTs seems to correlate much better with cognitive impairment than plaque burden (Giannakopoulos et al., 2003). This potential discrepancy suggests that Aβ is not directly responsible for disease progression. Other causal contributions of Aβ to AD could be on the one hand that Aβ has to reach a certain threshold to cause pathogenic events or on the other hand that Aβ is the trigger of the disease process, which then becomes rather independent of Aβ (Karran et al., 2011). In one way or the other, Aβ aggregation is an early event that leads to downstream cellular and molecular brain alterations such as tangle formation, which then result in neuronal dysfunction and at a final stage in dementia (Selkoe & Hardy, 2016). Recent fluid and imaging biomarker studies in FAD patients, explained in more detail in chapter 2.2.2, revealed that Aβ changes occur before tau increases in the cerebrospinal fluid (CSF) and precede the onset of clinical symptoms by decades (Jack et al., 2010; Holtzman et al., 2011b; Bateman et al., 2012). Therefore non-demented people, who exhibit amyloid deposits post-mortem, could have been on the way to develop AD.

Taken together, human genetic as well as biomarker studies indicate that in AD pathogenesis Aβ accumulation is the driving force for downstream tauopathy and neurodegeneration. Although AD is a complex disease with multiple interactions among genetic, epigenetic, and environmental factors (Chouliaras et al., 2010; Barnes & Yaffe, 2011), Aβ aggregation seems to be the initial, underlying cause of AD and a reasonable target for therapeutic intervention.

2.2 Inhibiting BACE1 to impede amyloid pathology

In reference to:

Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition

Juliane Schelle, Lisa Häsler, Jens C. Göpfert, Thomas O. Joos, Hugo Vanderstichele, Erik Stoops, Eva-Maria Mandelkow, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker, Stephan A. Kaeser (2017). *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, 13(6), pp.701-709.

2.2.1 Treatment strategies for Alzheimer's disease

Current Food and Drug Administration (FDA) approved AD treatments focus only on the amelioration of symptoms rather then the prevention or slowdown of disease progression. Currently, AD patients undergo symptomatic therapies with acetylcholinesterase-inhibitors and a glutamate antagonist. Because the neurotransmitter acetylcholine (ACh) involved in learning and memory (Mitsushima et al., 2013) is depleted in AD brains (Davies & Maloney, 1976; Perry et al., 1977) and even interacts with Aβ directly (Garcia-Osta & Alberini, 2009; Puzzo et al., 2015), *Rivastigmine*, *Donepezil*, and *Galantamine* provide symptomatic relief due to inhibition of ACh clearance by the acetylcholinesterase (Román & Kalaria, 2006; Anand & Singh, 2013; Godyn et al., 2016). However, only a minority of treated patients shows modest and transient improvement of cognitive functions. Furthermore the utility of each of these drugs is limited because they are only effective in earlier pre-dementia stages by allowing the retention of ACh (Hansen et al., 2008). Another approved drug is *Memantine*, an uncompetitive N-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist (Winblad & Poritis, 1999; Chu, 2012). Decreased glutamate uptake from microglia cells can lead to glutamate-mediated excitotoxicity, which can be detrimental to cells (Prentice et al., 2015). *Memantine* prevents aberrant neuronal stimulation and shows symptomatic benefits in moderate to severe AD patients (Cummings, 2004). It either can be administered alone or in combination with an acetylcholinesterase inhibitor (Wang et al., 2015a). Another glutamatergic agent, *Riluzole*, is currently in phase II for the treatment of AD.

Although the four approved drugs, summarized in Table 1, became the standard of care for AD patients since they slow the decline in quality of life, but they do not modify the primary pathological processes underlying the disease. Hence, AD's therapy research shifted toward different targets namely anti-Aβ agents assuming Aβ aggregation as the initial driving force of the disease (Hardy & Selkoe, 2002; Golde et al., 2010). Multiple approaches targeting specific intermediates in the pathological pathway from Aβ monomer to amyloid deposit have gained scientific interest. In Table 1 the entire spectrum of therapeutic anti-Aβ agents in clinical trials is listed. Besides active and passive Aβ vaccinations to accelerate Aβ clearance, researchers focused on the β- and γ-secretase cleaving APP as therapeutic targets, especially after the discovery that Aβ is constantly produced and secreted by cells throughout life (Haass et al., 1992). Inhibitors of each enzyme should therefore decrease Aβ production and prevent plaque formation.

As the γ-secretase complex consists of four different substrates, it provides many binding sites for selective inhibitors. Although the identity of this enzyme has been known for a long time, it has been difficult to develop drugs that are able to penetrate the blood-brain-barrier (BBB) and selectively inhibit the amyloidogenic pathway of APP processing. Moreover, the γ-secretase cleaves 50 other substrates apart from APP such as voltage-gated sodium channel subunits and Notch receptors, which regulate cell proliferation and differentiation (Kopan & Ilagan, 2009; Citron, 2010; De Strooper & Annaert, 2010; Golde et al., 2011; Schor, 2011). Their inhibition led to severe side effects in numerous studies using γ-secretase inhibitors from Eli Lilly & Co (*Semagacestat*) and Bristol-Meyers Squibb (*Avagacestat*) including gastrointestinal bleedings, immunosuppression, and skin cancer (Searfoss et al., 2003; Wong et al., 2004; Fleisher et al., 2008; Coric et al., 2012; Doody et al., 2013). Hence, Notch-sparing γ-secretase modulators were designed and are currently tested in clinical trials because they selectively decrease the production of aggregation-prone $A\beta_{42}$ in favor of shorter Aβ species that may be less toxic (*EVP-0962*, FORUM Pharmaceuticals, Inc.) (Kukar et al., 2008; Wolfe, 2007; Golde et al., 2011; Karran et al., 2011). Interestingly, γ-secretase modulators such as cholestenoic acid, a cholesterol metabolite, also exist endogenously in the brain and might act as viable therapeutic targets as well.

Table 1: Therapeutic anti-Aβ agents in Alzheimer's disease

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

Updated summer 2017; BACE = β-site amyloid precursor protein cleaving enzyme (adapted from Graham et al., 2017)

While the safe use of γ-secretase modulators is still pending, the other rate-limiting secretase processing APP BACE1 that is known to be elevated in AD patients became the focus of therapeutic approaches (Ghosh et al., 2008; Citron, 2010; De Strooper & Annaert, 2010; Golde et al., 2011; Kandalepas et al., 2013). Already other members of the aspartic protease family could be successfully inhibited such as renin and human immunodeficiency virus (HIV) protease-1 (Nguyen et al., 2008). *BACE1* null mice overexpressing human APP lacked amyloid plaques (Luo et al., 2003) but showed sensorimotor impairments and spatial memory deficits (Kobayashi et al., 2008) mainly due to the involvement of BACE1 in the metabolism of myelination during development (Willem et al., 2006; Vassar et al., 2009; Hitt et al., 2010; Cai et al., 2012). Therefore research interest shifted more toward partial and transient BACE1 inhibition, which was sufficient to significantly reduce Aβ deposition in APP tg mice (McConlogue et al., 2007; Fukumoto et al., 2010; Eketjäll et al., 2013; Neumann et al., 2015). However, initial clinical trials turned out to be unsuccessful because of low oral bioavailability and low BBB penetration. Nevertheless partial BACE1 inhibition seemed to display less severe side effects than drugs targeting the γ-secretase. The development of more lipophilic compounds revealed better oral availability and good brain permeability but lacked high potency and accurate target selectivity for BACE1 over other enzymes such as BACE2 (Stachel et al., 2004; Cole et al., 2006; Baxter et al., 2007). BACE2 is a closely related β-secretase with significant structural homology compared to BACE1. In contrast to its homologue, BACE2 is not primarily expressed in the brain but in colon, kidney, and pancreas and is therefore not considered to be a major contributor to Aβ generation. For example, Novartis Pharmaceuticals developed a BACE inhibitor (*NB-360*) that was not selective for BACE1 but effectively reduced brain Aβ in rodents and dogs and blocked the progression of cerebral Aβ deposition in APP tg mice (Neumann et al., 2015). However, this potent BACE inhibitor induced hair depigmentation in mice suggesting that a potential therapeutic agent for AD should selectively target BACE1 (Shimshek et al., 2016). Ongoing phase II and III studies include selective small-molecule BACE1 inhibitors with satisfactory pharmacokinetics, including *E2609* from Biogen, Inc., Eisai Co., Ltd., *AZD3293* from Eli Lilly & Co., AstraZeneca, *JNJ-54861911* from Janssen Pharmaceutica, Shionogi, *Verubecestat* from Merck & Co, Inc., and also the processed and improved compound from Novartis Pharmaceuticals, Amgen, Inc. called *CNP520* (Godyn et al., 2016, Folch et al., 2016).

Because the alpha-secretase conducts the non-amyloidogenic pathway and prevents Aβ production, the elevation of α-secretase cleavage seems to be a viable option for treating AD (Kuhn et al., 2010). Indeed, in APP tg mice overexpression of ADAM10 acting as α-secretase reduced plaque deposition and improved cognitive impairment (Postina 2004; Lichtenthaler, 2011). Despite initial excitement, off-target effects of α-secretase modulators due to over 30 different substrates of the metalloprotease besides APP as well as underlying mechanisms need to be identified and understood first (Edwards et al., 2008; Lichtenthaler, 2011).

Not only the manipulation of APP processing is a rational and promising strategy to treat or even prevent AD but also immunological approaches enhancing Aβ clearance. Besides increased amyloidogenic APP processing and Aβ production, inefficient removal of Aβ peptides contribute to the pathology (Hardy & Selkoe 2002; Selkoe, 2001). In AD patients, Aβ clearance is altered due to reduced activity of Aβ-degrading enzymes such as neprilysin (Mawuenyega et al., 2010; Folch et al., 2016). Increasing the expression of those Aβ-degrading enzymes would be one therapeutic strategy and was already shown to increase Aβ clearance in APP tg mice (Mueller-Steiner et al., 2006). However, those approaches turned out to be difficult to translate into clinical trials. To this end, anti-Aβ immunotherapy was reported as an effective amyloid clearance mechanism. Active immunization stimulates the immune system to produce antibodies when exposed to synthetic $A\beta_{42}$ as antigen. Those immunization studies resulted in the clearance of Aβ plaques and improved cognitive performance in APP tg mice (Schenk et al., 1999; Morgan et al., 2000). However, a phase II clinical trial using active immunization with the full-length $A\beta_{42}$, called *AN-1792* (Janssen Pharmaceutica, Pfizer, Inc.), had to be stopped because of meningoencephalitis in 6% of immunized patients potentially caused by T-cell and microglia activation (Nicoll et al., 2003; Orgogozo et al., 2003; Holmes et al., 2008). Nevertheless, post-mortem analyses of the subjects enrolled in this trial revealed reduced plaque deposition in cortical brain regions (Nicoll et al., 2003; Nicoll et al., 2006).

Severe side-effects caused by active immunization led to passive immunization approaches, in which Aβ specific antibodies are applied activating clearance mechanisms in the brain such as F_c-receptor-mediated phagocytosis of microglia cells and subsequent peptide degradation (Bard et al., 2000). Peripheral administration of humanized monoclonal Aβ antibodies reduced or even prevented amyloid fibril formation *in vitro* (Solomon et al., 1996) and *in vivo* in mice (Bard et al., 2000; Pfeifer et al., 2002; Lee et al., 2006; Fuller et al.,

2015). Although antibodies struggle to cross the BBB, they appear to be highly specific, safe, and well tolerated (Graham et al., 2017). Nevertheless antibody infusion also induced cerebral hemorrhages at amyloid-laden vessels (Pfeifer et al., 2002) and neuronal hyperactivity (Busche et al., 2015). The first human studies used N-terminal Aβ antibodies, which induced amyloid-related imaging abnormalities (ARIA), including vasogenic edema and microhemorrhages in a couple of patients (Salloway et al., 2009; Sperling et al., 2011). For example, the N-terminal Aβ antibody *GSK933776* (GlaxoSmithKline PLC) (Leyhe et al., 2014) was discontinued due to the lack of clinical efficacy. Afterwards, antibodies with a more central epitope of Aβ were designed. *Solanezumab* (Eli Lilly & Co.) for example recognizes the mid-domain of Aβ, which becomes unavailable to the antibody during the process of Aβ aggregation, and therefore removes mainly small soluble Aβ species (Crespi et al., 2015). In contrast, *Crenezumab* (Genentech, Inc.) (Crespi et al., 2015) and *Gantenerumab* (Chugai Pharmaceutical Co., Ltd., Hoffmann-La Roche) (Bohrmann et al., 2012) recognize rather aggregated forms of Aβ and are currently tested in a phase II and III trial, respectively. In other clinical trials mild to moderate AD patients have been treated with *AAB-003* (Janssen Pharmaceutica, Pfizer, Inc.) binding fibrillar and soluble Aβ species, whereas early AD subjects have been infused with the Aβ protofibril specific antibody *BAN2401* (BioArtic Neuroscience AB, Biogen, Inc., Esai Co., Ltd.). Another anti-Aβ immunotherapeutic drug is the human monoclonal antibody *Aducanumab* (Biogen, Inc.) generated from healthy, aged donors. It binds a conformational epitope targeting aggregated forms of Aβ. In a phase I clinical trial *Aducanumab* decreased amyloid deposition and delayed cognitive decline in prodromal to mild AD patients, which is why *Aducanumab* is currently tested in a phase III trial (Sevigny et al., 2016).

In addition to the promising amyloid-β-directed strategies there are also other possibilities for therapeutic intervention. Tau-related agents are a reasonable strategy for the treatment of AD since the accumulation of tau protein in neurons is postulated to have severe consequences on cellular function (Hardy & Selkoe, 2002). Anti-tau immunotherapy (Godyn et al., 2016) and inhibitors of tau aggregation such as *Methylene Blue* (Schirmer et al. 2011; Stack et al., 2014) promote protein degradation and avert tau-associated cytotoxicity. Another reasonable therapeutic approach to reduce the risk of dementia includes anti-inflammatory drugs (Miguel-Alvarez et al., 2015). The critical role of microglia in the pathogenesis of AD was highlighted by GWAS, which discovered that genetic alterations modulating AD risk are

enriched in genomic regions that regulate the gene expression of microglial proteins (Huang et al., 2017). However, the best way to modulate the immune system without inducing toxic effects remains unclear. Although the effectiveness of non-pharmacological lifestyle changes including physical exercise, cognitive activity, energy restriction, and social engagement (Jedrziewski et al., 2014) in preventing the development of AD after diagnosis seems to be highly unlikely, the onset and the progression of the disease could possibly be delayed (Barnes & Yaffe, 2011). As AD is a multifactorial disease, a combination of disease-modifying therapeutics and lifestyle and environmental factors might be in favor of prevention.

2.2.2 Necessity of early biomarkers to detect and monitor pathological changes

A definite diagnosis for AD requires the analysis of post-mortem brain tissue revealing neuritic plaques and neurofibrillary tangles. However, physicians rely on different measures to diagnose AD clinically on individuals with cognitive impairments. Therefore, the patient's recent history of mental and behavioral deficits is evaluated according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V criteria) and of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA criteria). Physical and neuropsychological tests are combined with neuroimaging and biochemical biomarkers to specifically diagnose AD dementia. Biomarkers are objectively measurable, indicate or mirror either normal or pathological processes in the brain, and help to detect and diagnose diseases (Biomarkers Definitions Working Group, 2001). Furthermore they can be used to estimate the response to therapy and in the future potentially to individualize treatment (Zetterberg, 2017).

Brain atrophy as a measure of structural tissue loss and general neurodegeneration is assessed by magnetic resonance imaging (MRI) (Bobinski et al., 2000; Zarow et al., 2005). Prior to the development of pathology-specific Aβ tracers for positron emission tomography (PET), ^{18}F-fluorodeoxyglucose (FDG) PET was the most widely used imaging biomarker in AD based on altered cerebral glucose metabolism in AD patients (Chase et al., 1984; Foster et al., 1984; Minoshima et al., 1995; Mosconi et al., 2008). Nowadays, PET amyloid imaging is usually used to visualize brain Aβ deposition specifically (Klunk et al., 2004; Villemagne et al., 2011; Xu et al., 2016). Tracer molecules such as Pittsburgh compound B (PiB) or florbetapir

F 18 are injected intravenously, travel trough the blood to the brain, and attach to amyloid plaques (Klunk et al., 2004; Clark et al., 2011). Their radiant signal creates an informative picture about density and location of fibrillar Aβ deposits (Ikonomovic et al., 2008; Fleisher et al., 2011; Sojkova et al., 2011). Although amyloid can accumulate in age in cognitively healthy individuals (Driscoll et al., 2011), the presence of a positive amyloid scan in a symptomatic patient is a strong indicator of AD (Johnson et al., 2012).

In addition or alternatively to the very costly PET scans AD can be diagnosed by biochemical tests investigating specific fluid biomarkers such as Aβ and tau in the CSF. $A\beta_{42}$ peptides as major components of amyloid deposits (Masters et al., 1985) decline in the CSF of AD patients (Motter et al., 1995; Olsson et al., 2016). In line with the imaging biomarkers, CSF $A\beta_{42}$ is sequestered in growing brain aggregates, which is why CSF $A\beta_{42}$ levels inversely correlate with the plaque burden at autopsy (Strozyk et al., 2003; Tapiola et al., 2009). However, a better predictor of cognitive performance than amyloid alone is tau (Giannakopoulos et al., 2003). Abnormally phosphorylated tau proteins accumulate to neurofibrillary tangles within neuronal cells releasing tau as a consequence (Grundke-Iqbal et al., 1986). Increased concentrations of either total (t-tau) or phosphorylated tau (p-tau) species can be measured in the CSF of AD patients by sensitive enzyme-linked immunosorbent assays (ELISA) (Vandermeeren et al., 1993; Vigo-Pelfrey et al., 1995; Olsson et al., 2016). Indeed p-tau provides the highest specificity for AD (Zetterberg, 2017). However, those biochemical markers should only be used in combination in order to reliably diagnose AD (Zetterberg et al., 2003).

Although APP tg mice lack NFTs, the above-mentioned AD characteristic CSF biomarker changes can be replicated remarkably well (Maia et al., 2013). The development of a novel and highly sensitive sandwich immunoassay using the single molecule array (SIMOA) technology enabled our laboratory to reliably measure murine CSF tau even in pre-depositing or non-tg mice. In APPPS1 mice a correlation between brain Aβ reflecting cerebral β-amyloidosis and an increase in CSF tau could be shown. To further validate the causal relationship between CSF tau and β-amyloid pathology, APPPS1 mice were treated with a potent BACE inhibitor (*NB-360*) (Neumann et al., 2015) to suppress Aβ production and the influence on tau in the CSF was tested. As mentioned in chapter 2.2.1, *NB-360* application significantly reduces brain Aβ levels and plaque burden in APP tg mice after one month (Neumann et al., 2015). However, in this study *NB-360* was administered to early-depositing

APPPS1 mice and APP23 mice with moderate pathology for six months. In both mouse models brain Aβ as well as amyloid burden were significantly reduced after BACE inhibition compared to control littermates revealing levels similar to six months younger animals (Schelle et al., 2017). Hence, we published in *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* that *NB-360* is very effective in lowering brain Aβ levels and therefore prevents the progression of cerebral β-amyloidosis without clearing pre-existing Aβ plaques (Schelle et al., 2017). Strikingly, not only Aβ was affected by our *NB-360* treatment but also the age-dependent increase of CSF tau levels was completely prevented (Schelle et al., 2017). After short-term BACE inhibition in young APPPS1 and APP23 mice decreased CSF Aβ but unaltered CSF tau levels were observed and therefore any direct effects of soluble Aβ or the BACE inhibitor itself on CSF tau were excluded (Schelle et al., 2017). In conclusion, we have reported a mechanistic link between Aβ deposition and CSF tau suggesting CSF tau changes as potential marker to predict the efficiency of BACE inhibitors in clinical trials. Furthermore those findings indicate that the effects of BACE inhibition are not limited to Aβ, but include downstream pathogenic events highlighting the great potential of BACE as target for therapeutic AD intervention.

Although the treatment prevented further damage with regard to plaque burden and CSF tau levels, already present cerebral β-amyloidosis could not be reduced. Furthermore, the drug was administered continuously for the entire treatment period. For economical and practical reasons the prevention of the disease using only a short therapeutic paradigm applied at the critical time point of initial Aβ aggregation would be the goal to solve this health burden.

A longitudinal study in families with AD mutation-carriers and non-carriers shed light on the temporal sequence of pre-symptomatic pathophysiological changes in relation to the expected onset of AD symptoms based on the parental age of symptom-onset. It could be shown that $A\beta_{42}$ concentrations in the CSF already started to decline 25 years before the expected onset of the clinical disease (Bateman et al., 2012). Aβ deposits were detectable approximately 15 years before first symptoms arose (Bateman et al., 2012). A few years later, CSF tau increased to abnormally high levels, validating again that Aβ aggregation is an early event preceding tangle formation (Hardy & Selkoe, 2002; Bateman et al., 2012; Jack & Holtzman, 2013). Hence, even at the stage of early clinical disease, Aβ deposition already reached a plateau and other pathogenic processes such as tau pathology spread and microglia activation

have progressed to an advanced state (Bateman et al., 2012; Walker et al., 2013). Although only familial AD patients were included in this study, the time course of pathogenic events is suggested to be similar for the sporadic form of AD as well (Villemagne et al., 2013). The results of this study suggest that pathological changes precede the clinical manifestation of AD by up to two decades. Therefore, diagnostic tests as well as clinical trials should be moved toward earlier stages of AD or even toward studies in asymptomatic patients (Sperling et al., 2014a; Hsu & Marshall, 2017). In other words, Aβ has to be targeted earlier in the course of the disease before regular brain function is affected and irreversible neurodegeneration has occurred. However, to this end initial Aβ aggregation needs to be understood and characterized first in order to develop effective disease-modifying treatments.

2.3 Longevity of Aβ seeds and their therapeutic potential

In reference to:

Persistence of Aβ seeds in APP null mouse brain

Lan Ye*, Sarah K. Fritschi*, **Juliane Schelle**, Ulrike Obermüller, Karoline Degenhardt, Stephan A. Kaeser, Yvonne S. Eisele, Lary C. Walker, Frank Baumann, Matthias Staufenbiel, Mathias Jucker (2015). *Nature Neuroscience,* 18(11), pp.1559-1561. *contributed equally

---

2.3.1 Prion-like properties of aggregated Aβ

Despite different clinical manifestations, a diverse group of neurodegenerative diseases including AD, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and many others share one common pathogenic principle based on the seeded aggregation of specific proteins in characteristic patterns and locations (Prusiner, 2012; Jucker & Walker, 2013; Walker & Jucker, 2015). Templated protein misfolding and propagation has been hypothesized to initiate the pathological transition from a soluble to an aggregated state and is called the prion paradigm because it was first investigated in prion diseases. Prions (proteinaceous infectious particles) are infectious agents that consist of abnormally folded prion protein (PrP) (Prusiner, 1982). Prion diseases are transmissible spongiform encephalopathies including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), kuru, and fatal familial insomnia (FFI) in humans, scrapie in sheep, chronic wasting disease (CWD) of deer and elk, and bovine spongiform encephalopathy (BSE) in cows (Prusiner, 1998; Collins et al., 2004). In genetic as well as idiopathic forms the constantly generated prion protein (Chesebro et al., 1985; Oesch et al., 1985) misfolds and aggregates into PrP seeds (Jarrett & Lansbury, 1993; Prusiner, 1998; Prusiner, 2013). This induces a chain reaction forcing naïve PrP proteins into malformation proliferating to protofibrils and amyloid assemblies (Jarrett & Lansbury, 1993; Prusiner, 1998; Prusiner, 2013).

Recent findings suggest that this prion paradigm based on seeded corruption of naïve proteins underlies also other proteopathies including AD (Jucker & Walker, 2013). Although the

physiological function of the Aβ peptide is largely unknown, it is constantly produced in the brain (Haass et al., 1992) and holds the ability to adopt more aggregation-prone conformations. Amyloidogenic proteins such as Aβ seem to follow a nucleation-dependent polymerization process (Harper & Lansbury, 1997; Jarrett & Lansbury, 1993; Eisele, 2013). The single steps of this kinetic process are described in Figure 2. *In vitro* studies revealed that first of all the natively unfolded Aβ protein misfolds into a kinetically unfavorable conformation rich in β-sheet structure and after a series of intermediate states it might aggregate into a multimeric nucleus (seed) (Harper & Lansbury, 1997; Jarrett & Lansbury, 1993). This slow process of initial seed formation is called nucleation phase (lag phase). Through conformational conversion, other Aβ molecules are incorporated into the existing aggregate. Fragmentation of the progressively growing amyloid fibril generates more Aβ seeds leading to a self-propagating process as part of the so called rapid growth phase (Jarrett & Lansbury, 1993; Roychaudhuri et al., 2009; Knowles et al., 2011). The initial lag-phase preceding amyloid formation can be shortened by the addition of preformed Aβ seeds (Harper & Lansbury, 1997; Meyer-Luehmann et al., 2006).

Inoculation experiments support the nucleation-dependent polymerization of Aβ proteins by showing that Aβ deposition can also be induced *in vivo* following a prion-like mechanism. Young, pre-depositing APP tg mice were intracerebrally injected with minute amounts of brain homogenates from AD patients and controls (Kane et al., 2000; Walker et al., 2002; Meyer-Luehmann et al., 2006). After certain incubation time (lag phase) the animals developed cerebral β-amyloidosis in a time- and concentration-dependent manner (Meyer-Luehmann et al., 2006; Fritschi et al., 2014b). In contrast, extracts from control donors did not induce amyloid pathology (Kane et al., 2000; Meyer-Luehmann et al., 2006). To exclude the possibilities of induced Aβ lesions due to human-specific factors, brain material from aged APP tg donor mice as well as control mice were injected into the hippocampus of tg host mice (Meyer-Luehmann et al., 2006; Watts et al., 2011). Again, Aβ was observed to aggregate, self-propagate, and spread to neighboring and neuroanatomically connected brain regions similar to extracts from AD patients (Walker et al., 2002; Meyer-Luehmann et al., 2006; Eisele et al., 2009; Hamaguchi et al., 2012; Ye et al., 2015b) and prions (Fraser, 1982; Buyukmihci et al., 1983; Kimberlin & Walker, 1986; Rangel et al., 2014). Even Aβ seeds applied to the periphery of APP tg mice initiated plaque formation in the brain after a certain incubation time, ruling out brain injury or an immunological response at the injection site as

reasons for the induced cerebral β-amyloidosis (Eisele et al., 2010; Eisele et al., 2014). However, prion disease can be induced *de novo* in animals that usually would not develop the pathology (Prusiner, 1998; Soto, 2011). Therefore, APP tg rodents without Aβ deposits within the normal lifespan were inoculated with Aβ seeds and plaque deposition was induced. Hence, there was no acceleration of an ongoing process but soluble Aβ peptides were triggered to aggregate (Morales et al., 2012; Rosen et al., 2012). Like recombinant prion proteins (Legname et al., 2004), synthetic Aβ peptides have only poor potency to aggregate (Stöhr et al., 2012). Nevertheless, synthetic Aβ can induce Aβ deposition and amyloid plaques in a living cellular environment (Friedrich et al., 2010; Novotny et al., 2016). Another feature of prion proteins is the adoption of conformationally distinct amyloid strains (Prusiner, 1998; Collinge & Clarke, 2007), a property that could also be confirmed for Aβ seeds (Meyer-Luehmann et al., 2006; Heilbronner et al., 2013; Stöhr et al., 2014; Watts et al., 2014).

Despite all the similarities between prions and Aβ seeds, until now it seems unlikely that AD is infectious in terms of transmissibility from person to person under ordinary circumstances (Walker et al., 2016). However, the first evidence for prion-like seeding of Aβ in humans came from Jaunmuktane et al. in 2015. In this study, brains of eight people were investigated regarding Aβ pathology who were treated with growth hormones isolated from cadaveric human pituitary glands and died of CJD between 36 and 51 years of age (Jaunmuktane et al., 2015). Despite the young age and no present FAD mutation, half of the investigated hormone recipients showed significant amounts of cerebral β-amyloidosis (Jaunmuktane et al., 2015). This suggests a contamination of the 30 years before isolated and injected material not only with PrP prions but also with Aβ seeds (Jaunmuktane et al., 2015; Jucker & Walker, 2015). Even in people previously treated with cadaveric growth hormones who died of causes other than CJD Aβ pathology was found revealing its independence of the development of clinical CJD (Ritchie et al., 2017). However, the investigated subjects lacked cerebral tau pathology as well as clinical AD symptoms (Jaunmuktane et al., 2015; Ritchie et al., 2017). Whether those lesions would eventually have manifested later in life is unknown. But until the results of follow up studies of the surviving growth hormone recipients or other evidences emerge, AD is not proven to spread among humans in a sense that the disease-causing agent can be acquired from patients or environment and infect another person, like it is the case for prion diseases (Beekes et al., 2014; Jucker & Walker, 2015; Walker et al., 2016).

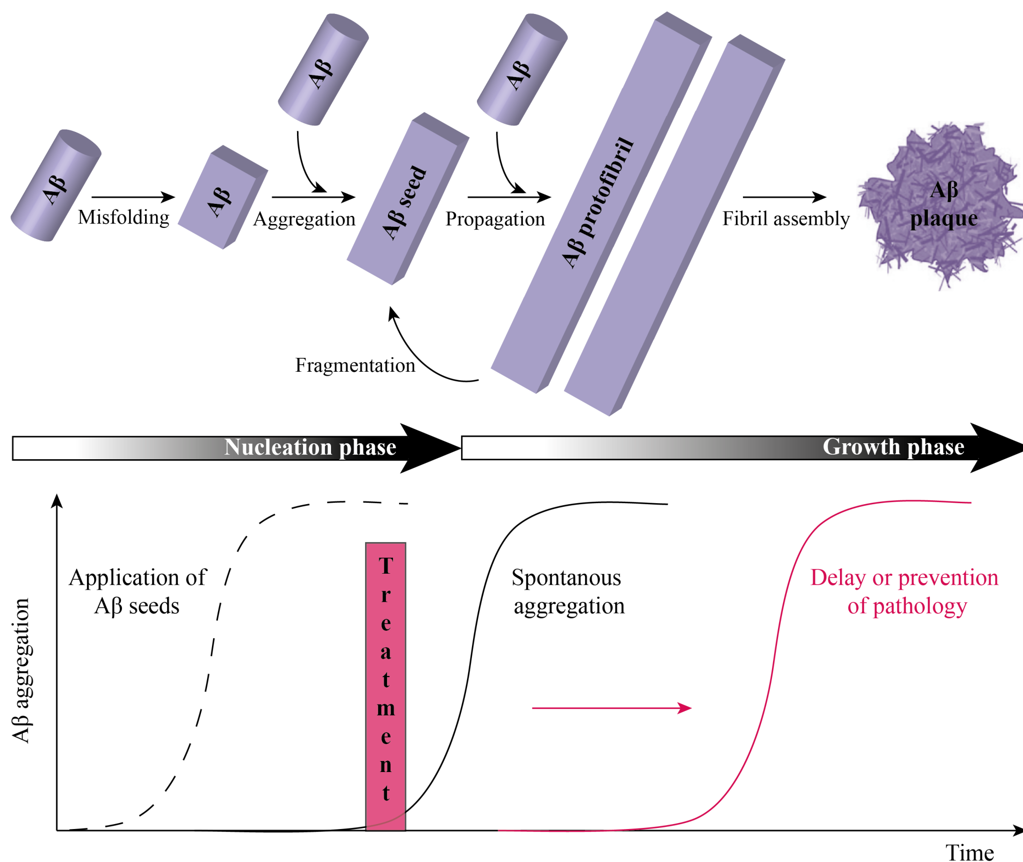


Figure 2: Pathological cascade of amyloid β (Aβ) accumulation. Misfolding of the Aβ peptide initiates the recruitment and malformation of natively folded Aβ monomers to form an oligomeric nucleus (Aβ seed). After this slow nucleation phase the Aβ seed self-propagates and forms amyloid protofibrils due to templated misfolding, which later accumulate into amyloid plaques. Within this rapid growth phase those fibrils can be fragmented again into smaller Aβ seeds. Addition of preformed seeds shortens the nucleation phase. Targeting initial Aβ seeds with anti-amyloid therapies might delay or even prevent cerebral β-amyloidosis (modified from Harper & Lansbury, 1997).

2.3.2 Stability and durability of Aβ seeds

Infectious PrP prions are known to vary in size and stability and to resist even harsh thermal and biochemical treatments (Appel et al., 2001; Tzaban et al., 2002; Silveira et al., 2005; Wiggins, 2009; Prusiner, 2013). As Aβ seeds seem to be the driving force of AD, the robustness and potency of different Aβ species to induce cerebral Aβ deposition is of tremendous interest. Comparable to prions, Aβ seeds tend to be very durable agents. This was postulated because boiling of Aβ seeds for five minutes still retained seeding capacity (Meyer-Luehmann et al., 2006). Even stainless-steel wires coated with dried Aβ-rich brain material implanted into brains of APP tg mice were able to induce plaque deposition (Eisele et al., 2009). Like prions (Pattison, 1965), Aβ seeds are resistant to inactivation by

formaldehyde, discovered by induced Aβ pathology in APP tg hosts inoculated with formaldehyde-fixed brain homogenates from AD patients or APP tg mice (Fritschi et al., 2014a). However, immunodepletion of Aβ-laden extracts as well as formic acid treatment resolving the tertiary protein structure prevented cerebral β-amyloidosis in inoculated APP tg mice (Meyer-Luehmann et al., 2006). Taken together those results suggest that Aβ seeds are extremely robust and Aβ-containing tissue or material should be handled with highest caution.

To assess the seeding capacity of Aβ seeds, brain extracts of APP tg mice were subjected to 100.000 x g ultracentrifugation and both supernatant and pellet fraction were injected in the hippocampus of pre-depositing APP tg mice. Although the supernatant fraction contained only less than 1% of total brain Aβ, the soluble fraction induced about 30% of the Aβ plaque load compared to the pellet fraction (Langer et al., 2011). Hence, small soluble Aβ seeds appear to be the most potent Aβ species. Further characterization involved digestion of Aβ seeds by proteinase K (PK). The degree of destruction of soluble Aβ seeds by PK extended the sensitivity of larger insoluble seeds to PK neutralization (Langer et al., 2011). Moreover, the potency of soluble Aβ species was investigated using a dilution series of soluble brain extracts rich in Aβ seeds. In fact, even a 10.000-fold diluted soluble AD brain material sample stimulated Aβ pathology in young APP tg hosts (Fritschi et al., 2014b). However, CSF from AD patients or aged APP tg mice containing soluble Aβ seeds was not proven to be seeding active (Fritschi et al., 2014b).

The persistence of those very active and potent cerebral Aβ seeds was further investigated in our recent study and published in *Nature Neuroscience* using different APP tg hosts (Ye et al., 2015a). APP23, wt, and *App* null (Calhoun et al., 1999) mice were injected into the hippocampus with brain extracts from aged APP tg mice. One, seven, and 30 days post inoculation (dpi) the hippocampi of those mice were assessed biochemically. One day post inoculation the injected human Aβ was present in all three murine hosts at similar levels, whereas at seven and 30 dpi human Aβ decreased below detection in *App* null and wt mice (Ye et al., 2015a). Although another study revealed that murine Aβ co-deposits in human APP overexpressing mice (Mahler et al., 2015), in this experiment the low levels of mouse Aβ in wt mice did not prevent the clearance of applied Aβ seeds. However, in APP23 mice propagation of the injected Aβ seeds could be detected (Ye et al., 2015a). Hence, cerebral β-

amyloidosis not only depends on the concentration of seeds applied but also on the availability of sufficient soluble Aβ from the host to be incorporated into the exogenously applied seeds. Reinoculations (second passage) of the hippocampi extracted from Aβ seed-injected *App* null mice into APP23 hosts were carried out and revealed residual seeding capacity. Already after four but even more prominent after eight months incubation time the *App* null 30 dpi hippocampal extract with no detectable Aβ induced amyloid deposition in an APP tg host (Ye et al., 2015a). Due to the unexpected results the experiment was repeated and the primary incubation time in the *App* null mice was extended up to six months. Again, the analyzed hippocampi revealed Aβ levels below detection. However, second passage inoculation of this hippocampal extract revealed robust induction of cerebral β-amyloidosis in APP tg mice (Ye et al., 2015a). In contrast, no induction was observed using wt extracts (Ye et al., 2015a). Consequently Aβ seeds in *App* null mice, despite the absence of replication and the presence of active clearance mechanisms, regained their pathogenicity and seeding activity as soon as host-derived Aβ became available again. The discovery of the extraordinary stability of Aβ seeds in the brain up to 180 days supports the idea that AD pathogenesis is initiated before Aβ deposition becomes detectable and explains the long silent lag phase seen in AD patients prior to symptom-onset (Bateman et al., 2012; De Strooper & Karran, 2016). Therefore, therapies for AD need to target those durable Aβ seeds at an early stage because apparently they are able to hide in yet unknown cellular compartments without loosing their toxicity. Ideally, the initial seed formation should be prevented at the first place.

2.4 Targeting initial Aβ seeds for the prevention of Alzheimer's disease

---

In reference to:

Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds

Juliane Schelle, Sarah K. Fritschi, Ulrike Obermüller, Lisa M. Häsler, Marius Lambert, Stephan A. Kaeser, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker (2017). Manuscript in preparation.

2.4.1 Clinical trials for Alzheimer's disease and possible reasons for recent failures

The fact that one out of nine people older than 65 years and one out of three for those over age 85 suffer from AD (Alzheimer's Association, 2014), has justified many scientific research approaches as well as clinical studies throughout the last decades. As mentioned in chapter 2.2.1, approved drugs for AD only relieve symptoms and do not change the course of the disease. Although we have learned a lot about AD pathophysiology and evidence for the lowering and neutralization of Aβ as a useful therapeutic approach has accumulated, many phase II and III clinical trials investigating disease-modifying agents have failed as displayed in Table 1 (Graham et al., 2017). This chapter is dedicated to potential causes for those clinical trial failures.

For example, the non-selective γ-secretase inhibitor *Semagacestat* (Eli Lilly & Co.) effectively lowered brain Aβ levels but induced serious side effects and worsened cognitive performance in a phase III trial due to missing substrate selectivity (Doody et al., 2013; De Strooper, 2014). Therefore, instead of stopping proteolysis, γ-secretase modulators are in favor now that shift the ratio of different Aβ species toward less toxic ones. Not only γ-secretase blockers, but also many BACE inhibitors such as *RG7129* (Roche), *LY2811376* (Eli Lilly & Co.), and *LY2886721* (Eli Lilly & Co.) failed to manage off-target effects and to pass toxicology studies.

Despite the disappointment about all failed clinical trials, numerous lessons can be learnt. Further critical examination of the failed antibodies as well as BACE inhibitor studies revealed that some of the enrolled subjects might not have had AD as indicated by negative

amyloid PET scans (Karran & Hardy, 2014; Salloway & Sperling, 2015). Fluid as well as imaging biomarkers including CSF Aβ, CSF t-tau and p-tau, PET amyloid, and structural imaging with MRI should be included to select only amyloid-positive trial subjects that presumably develop AD (Morris et al., 2009; Lim et al., 2013; Vos et al., 2013). This will improve the design of clinical studies and will increase the possibility to detect treatment effects in the future.

Furthermore appropriate clinical endpoints need to be selected. Recently, the FDA issued new guidelines for clinical trials in early AD. Although AD studies in demented people require both cognitive and functional outcome measures, only a single cognitive endpoint needs to be achieved for trials in preclinical AD (Kozauer & Katz, 2013).

In addition to the above-mentioned issues there are many other possible explanations for unsuccessful individual agents such as a lack of drug potency, insufficient compound safety, and the presence of co-pathologies. However, most likely disease severity, target engagement, and the advanced AD stage of selected subjects caused most of the recent failures in clinical AD trials. For example, *Verubecestat* (Merck & Co, Inc.), a small molecule non-selective BACE inhibitor, showed good tolerability without withdrawals due to side effects and CSF Aβ reductions of up to 90% in mild to moderate AD patients in a phase II/III EPOCH trial (Kennedy et al., 2016) but failed to significantly slow disease progression. Also the monocloncal antibody *Bapineuzumab* (Pfizer, Inc., Johnson & Johnson Pharmaceutical Company, Janssen Pharmaceutica, Elan Pharmaceuticals, Inc.) with its N-terminal Aβ epitope binding Aβ monomers, oligomers, and plaques significantly decreased PET amyloid signals as well as CSF p-tau levels (Rinne et al., 2010; Blennow et al., 2012) but induced severe side effects such as ARIA with edema and missed clinical endpoints in mild to moderate AD patients (Sperling et al., 2011). Thus, *Bapineuzumab* should not be used to treat patients with mild to moderate AD (Abushouk et al., 2017) similar to *Verubecestat*, which is currently tested in prodromal AD patients. Another example and the most recent disappointment in clinical AD trials is *Solanezumab* (Eli Lilly & Co.), described in chapter 2.2.1. No ARIA was evoked but also no cognitive benefit could be measured in moderate AD patients, leading to the discontinuation of this arm of the trial (Doody et al., 2014). In patients with only mild AD the same treatment showed slight improvements in cognitive tests over an 18-month period (Doody et al., 2014; Siemers et al., 2016) but failed to significantly decrease the rate of cognitive decline and CSF Aβ profile. As described in chapter 2.2.2, profound

brain alterations occur long before dementia can be diagnosed. These findings may explain why therapeutic intervention at a moderate AD stage failed in all previous clinical trials. The treatment initiated in symptomatic patients was probably too late to ameliorate symptoms because Aβ deposition and neuronal loss had already begun and progressed for many years (Graham et al., 2017). At this point, treatments may have become ineffective and additionally cause a lot of side effects. Eventually the efficacy and safety of therapeutics can be increased if tried on pre-symptomatic subjects positive for AD imaging and CSF biomarkers and therefore at high risk to develop AD. Such secondary prevention trials aim to treat the underlying pathophysiology in order to prevent cognitive symptoms from ever developing or at least delay the onset of the disease (Graham et al., 2017; Hardy & De Strooper, 2017).

Five large secondary prevention trials, depicted in Table 2, have started recruiting cognitively normal subjects and include: 1) the Alzheimer's Prevention Initiative (API) Autosomal-Dominant AD (ADAD) trial, 2) the API APOE4 trial, 3) the Dominantly Inherited Alzheimer Network (DIAN) Trials Unit (DIAN-TU), 4) the Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4) trial, and 5) the TOMORROW trial (Hsu & Marshall, 2017).

The API-ADAD and API-APOE4 trials have been enrolling cognitively healthy subjects who carry a *PSEN1* mutation or are homozygous for the APOE4 risk allele, respectively (Hsu & Marshall, 2017). The API-ADAD trial (Mullard, 2012) is using the Aβ specific antibody *Crenezumab* (Genentech, Inc.) described in chapter 2.2.1. The completion of the trial is estimated in 2020. The API-APOE4 trial is testing *CAD106* as an active amyloid vaccine (Winblad et al., 2012) as well as the BACE inhibitor *CNP520* (Novartis International AG, 2015) both developed by Novartis Pharmaceuticals.

The DIAN trials focus on families suffering from FAD carrying *PSEN1*, *PSEN2*, or *App* mutations (Mullard, 2012; Moulder et al., 2013; Hsu & Marshall, 2017). Cognitively normal individuals 15 years before the expected symptom onset, MCI, or mild AD subjects within those families are treated with *Solanezumab* (Eli Lilly & Co.) and/or *Gantenerumab* (Chugai Pharmaceutical Co., Ltd., Hoffmann-La Roche) (Ostrowitzki et al., 2012), the BACE inhibitor *JNJ-54861911* (Janssen Pharmaceutica, Shionogi), or placebo. Two-year follow up studies have been conducted assessing cognitive measures, neuroimaging, and CSF biomarkers.

The first prevention trials in pre-symptomatic subjects at high risk to develop LOAD based on abnormal amyloid PET scans are conducted in Australia and are called A4 and A5 study (Sperling et al., 2014b; Hsu & Marshall, 2017). With regards to the A4 trial, a total of 5000 individuals should receive monthly infusions of either *Solanezumab* (Eli Lilly & Co.) or placebo for slightly more than three years. The A5 trial is evaluating the efficiency of Janssen's BACE inhibitor in about 2000 people. Similar to the DIAN trials, cognitive tests will be the primary outcome measure followed by neuroimaging and biochemical biomarkers.

The TOMORROW trial is the only study that is not based on the amyloid hypothesis but rather focuses on the genetic enrichment of the *translocase of outer mitochondrial membrane 40 homolog* (*TOMM40*) gene, which is linked to *APOE ε4* gene (Hsu & Marshall, 2017). Both genes predict cognitive aging in late onset AD (Roses et al., 2010; Caselli et al., 2012). They plan to enroll 6000 cognitively normal individuals and treat them randomly with the mitochondrial targeting agent *Pioglitazone* (Takeda Pharmaceutical Company), which is an approved anti-diabetic drug (Geldmacher et al., 2011; Sato et al., 2011).

Table 2: Current secondary prevention trials in Alzheimer's disease

Trial	Intervention	Study sample	Location	Start	Duration
API-ADAD	*Crenezumab*	Presenilin-1 carriers	Colombia	2013	5 years
API-APOE4	*CAD106, CNP520*	APOE4 homozygotes	North America, Europe	2015	5 years
DIAN-TU	*Gantenerumab, Solanezumab*	Cognitively normal, MCI, or mild AD mutation carriers	U.S., Canada	2013	2 years
A4	*Solanezumab*	Cognitively normal people with abnormal amyloid PET	U.S., Canada, Australia	2014	3.25 years
TOMORROW	*Pioglitazone*	Cognitively normal people with genetic risk of TOMM40 and APOE4	Worldwide	2014	5 years

Updated summer 2017; A4 = Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease; AD = Alzheimer's disease; ADAD = Autosomal-Dominant AD; API = Alzheimer's Prevention Initiative; APOE4 = apolipoprotein E ε4; DIAN-TU = Dominantly Inherited Alzheimer Network Trials Unit; MCI = mild cognitive impairment; PET = positron emission tomography; TOMM40 = translocase of outer mitochondrial membrane 40 homolog (adapted from Hsu & Marshall, 2017)

2.4.2 Delay of cerebral β-amyloidosis by short and early combination therapy

The failed clinical trials using anti-amyloid drugs in mild to moderate AD patients suggest that treatment during or even at the beginning of the long prodromal phase is the most promising therapeutic approach (De Strooper & Karran, 2016). Since preclinical treatment clearly causes ethical and practical difficulties, tg animals should be used first in order to predict the outcome of certain treatment strategies. Indeed, animal data mirrored accurately the negative outcomes, namely the unsuccessful clearance of established plaques, in clinical trials testing *Solanuzumab* (Eli Lilly & Co.) (DeMattos et al., 2001). Also BACE inhibitors used experimentally in mice prevented the progression of plaque development but did not clear existing Aβ deposits (Fukumoto et al., 2010; Schelle et al., 2017) similar to the findings obtained in human subjects. In mice it appears to be fairly easy to determine the onset of plaque deposition and CSF biomarker changes (Sturchler-Pierrat et al., 1997; Radde et al., 2006; Maia et al., 2013; Maia et al., 2015). Because mixing Aβ seeds with Aβ specific antibodies reduced the induction of cerebral β-amyloidosis in inoculated APP tg hosts (Meyer-Luehmann et al., 2006) and Aβ seeds could be at least partially degraded in *App* null mice (Ye et al., 2015a), both anti-Aβ immunization and a reduction in Aβ generation by secretase inhibition appear to be rational therapeutic paradigms to reduce Aβ seeds.

Many studies that are explained in chapter 2.1.2 suggested that Aβ aggregation is the trigger of a sequence of pathogenic events, which once initiated become independent of Aβ and at a final stage cause AD dementia (Karran et al., 2011). In line with this, a recent aging study indicates that early Aβ seeds are the most active ones in terms of seeding capacity and display the greatest seeding potency when inoculated in young, pre-depositing APP tg mice (Ye et al., 2017). Hence, a combination therapy should be applied during this critical time window as indicated in Figure 2, when the first Aβ seeds emerge, clearing Aβ seeds with an Aβ specific antibody and blocking the production of soluble Aβ by BACE inhibition. Although BACE inhibitors are less expensive and have clearer endpoint measures than antibodies, a combination of both anti-Aβ approaches given at an early time point might provide better clinical outcomes. To prove that Aβ seeds can be inactivated by short and early Aβ immunotherapy, the plaque-clearing Aβ specific antibody *Beta-1* (*β1*) was injected (Pfeiffer et al., 2002; Meyer-Luehmann et al., 2006) into young, Aβ seed-inoculated APP23 mice once daily for five consecutive days. This short immunization was sufficient to

ameliorate the induction and propagation of Aβ aggregation four months later. Afterwards, the *β1* was combined with the potent BACE inhibitor *NB-360*, which was previously shown to effectively reduce Aβ in CSF and brain as well as to stop amyloid formation (Schelle et al., 2017). The combination of *NB-360* and *β1* was applied to young APP tg mice at a defined pre-depositing stage targeting first endogenous Aβ seeds. This short and early intervention of initial Aβ aggregation decreased Aβ seeds acutely but also delayed the onset of Aβ plaque pathology even after discontinuation of the treatment for several months. The findings could be replicated in APP51 mice overexpressing human wt APP, which suggests that targeting amyloid seeds shortly but at a pre-amyloid stage and thereby interfering with the initial seeding phase of amyloid formation by Aβ vaccination and BACE inhibition, might be an effective and promising therapeutic approach to prevent cerebral β-amyloidosis and eventually early- and late-onset AD.

2.5 Concluding remarks and outlook

Although effective disease-modifying drugs for the delay or even the prevention of AD still remain to be verified, many scientific studies and clinical trials have contributed to a basic understanding of the disease throughout the last decades. Since the publication of the amyloid hypothesis 25 years ago, many papers have revised the linearity of pathologenic events and have presented highly complex and interacting pathological pathways of proteostasis, lipid metabolism, vasculature, glial activation, and neuronal circuitries. However, the original sequence of pathogenic events still appears to hold its ground: 1) Aβ peptides aggregate and form Aβ seeds in a self-propagating nucleation dependent process; 2) inflammatory responses, tau pathology, synaptic dysfunctions, and neuronal loss follow as downstream events; 3) the combination of Aβ and tau pathologies characterizes AD dementia, despite the fact that almost everyone develops some tau pathology during aging, whereas only a subset of individuals show cerebral β-amyloidosis (Hardy & Higgins, 1992; Hardy & Selkoe, 2002; Selkoe & Hardy, 2016). Although the complex biology of the disease offers numerous flanks for attack by therapies, the previous chapters summarize the rationale behind Aβ as the most promising therapeutic target.

One of the presented studies showed that the initial trigger of the disease pathology can be targeted by effective BACE inhibition. Reducing the β-secretase activity prevented plaque formation and additionally affected downstream AD pathophysiology such as increasing CSF tau concentrations. Therefore BACE inhibitors not only serve as potent anti-amyloid drugs but their effectiveness can also be monitored in clinical trials by evaluation of tau levels in the CSF.

However, BACE inhibitors or other Aβ lowering agents applied to mild to moderate AD patients have resulted in negative clinical trial outcomes due to induced side effects and/or unaltered cognitive performance. The fact that pathological changes become measurable decades before first AD symptoms arise (Bateman et al., 2012), suggests the need for better characterization of this long pre-symptomatic period and earlier therapeutic intervention.

A recent paper from our laboratory confirms that the most seeding-active Aβ aggregates occur at early stages of the amyloid formation (Ye et al., 2017). In APP tg mice it could already be shown experimentally that targeting Aβ using a γ-secretase inhibitor prior to deposition has the most significant impact on cerebral β-amyloidosis compared to less

effective treatments at later stages (Das et al., 2012). However, lowering soluble Aβ generation to a certain level did not prevent amyloidosis completely and might not be sufficient to tackle Aβ seeds (Das et al., 2012; Schelle et al., 2017). The described characterization of Aβ seeds unveiled their tremendous durability as well as their ability to regain pathogenic activity after a silent phase of up to six months. Those experiments confirm the high therapeutic potential of targeting early Aβ seeds for AD treatment.

On that account, a combination of two anti-Aβ strategies seems to be a reasonable therapeutic approach. That is why the age of potential Aβ seed formation was determined in APP tg mice, which were then treated with a combination of a BACE inhibitor to turn off Aβ production and a non-selective Aβ antibody to reduce Aβ seeds during this critical time window. After discontinuation of the treatment brain Aβ levels as well as plaque burden were still significantly reduced compared to controls. However, comparing the levels to a 1.5 months younger control group revealed a shift in pathology by only the treatment period. Therefore amyloid pathology was successfully delayed, although only by the length of the treatment.

Hence, a β-secretase inhibitor should be combined with an antibody that selectively binds Aβ seeds over monomers. Applied at the beginning of Aβ seed formation this combinational therapy should prevent the development of plaques, downstream effects, and therefore AD. Furthermore, validated and standardized biomarkers should be included in each trial to help identify at-risk participants and synchronize AD measures. Asymptomatic individuals endangered to develop AD should be treated at the right time point with a combination of disease-modifying drugs such as BACE inhibitors and passive Aβ seed immunization strategies. Taken together, this early anti-Aβ seed-specific therapy interfering with the pathological cascade should enable us to prevent this devastating disorder, deescalate this burdensome health situation, and change people's lives.

2.6 References

Abushouk, A.I. et al., 2017. Bapineuzumab for mild to moderate Alzheimer's disease: a meta-analysis of randomized controlled trials. *BMC neurology*, 17(1), p.66.

Ahtiluoto, S. et al., 2010. Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. *Neurology*, 75(13), pp.1195–1202.

Alzheimer A., 1907. Über eine eigenartige Erkrankung der Hirnrinde. *Allgemeine Zeitschrift für Psychatrie und psychisch-gerichtliche Medizin*, 64: 146-8.

Alzheimer A., 1911. Über eigenartige Krankheitsfälle des späteren Alters. *Zeitschrift für die Gesamte Neurologie und Psychiatrie*, 4: 356-85.

Alzheimer's Association, 2014. 2014 Alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 10(2), pp.e47–92.

Anand, P. & Singh, B., 2013. Synthesis and evaluation of substituted 4-methyl-2-oxo-2H-chromen-7-yl phenyl carbamates as potent acetylcholinesterase inhibitors and anti- amnestic agents. *Medicinal chemistry (Shariqah (United Arab Emirates))*, 9(5), pp.694–702.

Anstey, K.J. et al., 2007. Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies. *American journal of epidemiology*, 166(4), pp.367–378.

Appel, T. et al., 2001. Heat stability of prion rods and recombinant prion protein in water, lipid and lipid-water mixtures. *The Journal of general virology*, 82(Pt 2), pp.465–473.

Arriagada, P.V. et al., 1992. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology*, 42(3 Pt 1), pp.631–639.

Ashe, K.H. & Zahs, K.R., 2010. Probing the biology of Alzheimer's disease in mice. *Neuron*, 66(5), pp.631–645.

Bard, F. et al., 2000. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nature medicine*, 6(8), pp.916–919.

Barnes, D.E. & Yaffe, K., 2011. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet neurology*, 10(9), pp.819–828.

Bateman, R.J. et al., 2012. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *The New England journal of medicine*, 367(9), pp.795–804.

Baxter, E.W. et al., 2007. 2-Amino-3,4-dihydroquinazolines as inhibitors of BACE-1 (beta-site APP cleaving enzyme): Use of structure based design to convert a micromolar hit into a nanomolar lead. *Journal of medicinal chemistry*, 50(18), pp.4261–4264.

Beekes, M. et al., 2014. Is there a risk of prion-like disease transmission by Alzheimer- or Parkinson-associated protein particles? *Acta neuropathologica*, 128(4), pp.463–476.

Bekris, L.M. et al., 2010. Genetics of Alzheimer disease. *Journal of geriatric psychiatry and neurology*, 23(4), pp.213–227.

Benilova, I. et al., 2014. The Alzheimer disease protective mutation A2T modulates kinetic and thermodynamic properties of amyloid-β (Aβ) aggregation. *The Journal of biological chemistry*, 289(45), pp.30977–30989.

Bertram, L. et al., 2008. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *American journal of human genetics*, 83(5), pp.623–632.

Bettens, K., Sleegers, K. & Van Broeckhoven, C., 2013. Genetic insights in Alzheimer's disease. *Lancet neurology*, 12(1), pp.92–104.

Biomarkers Definitions Working Group., 2001. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology and therapeutics*, 69(3), pp.89–95.

Bitan, G., Kirkitadze, M.D., et al., 2003. Amyloid beta -protein (Abeta) assembly: Abeta 40 and Abeta 42 oligomerize through distinct pathways. *Proceedings of the National Academy of Sciences of the United States of America*, 100(1), pp.330–335.

Blennow, K. et al., 2012. Effect of immunotherapy with bapineuzumab on cerebrospinal fluid biomarker levels in patients with mild to moderate Alzheimer disease. *Archives of neurology*, 69(8), pp.1002–1010.

Bobinski, M. et al., 2000. The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. *Neuroscience*, 95(3), pp.721–725.

Bodendorf, U. et al., 2002. Expression of human beta-secretase in the mouse brain increases the steady-state level of beta-amyloid. *Journal of neurochemistry*, 80(5), pp.799–806.

Bohrmann, B. et al., 2012. Gantenerumab: a novel human anti-Aβ antibody demonstrates sustained cerebral amyloid-β binding and elicits cell-mediated removal of human amyloid-β. *Journal of Alzheimer's disease : JAD*, 28(1), pp.49–69.

Borchelt, D.R. et al., 1996. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron*, 17(5), pp.1005–1013.

Braak, H. & Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica*, 82(4), pp.239–259.

Brun, A. & Englund, E., 1981. Regional pattern of degeneration in Alzheimer's disease: neuronal loss and histopathological grading. *Histopathology*, 5(5), pp.549–564.

Burdick, D. et al., 1992. Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *The Journal of biological chemistry*, 267(1), pp.546–554.

Busche, M.A. et al., 2015. Decreased amyloid-β and increased neuronal hyperactivity by immunotherapy in Alzheimer's models. *Nature neuroscience*, 18(12), pp.1725–7.

Buyukmihci, N., Goehring-Harmon, F. & Marsh, R.F., 1983. Neural pathogenesis of experimental scrapie after intraocular inoculation of hamsters. *Experimental neurology*, 81(2), pp.396–406.

Cai, J. et al., 2012. β-Secretase (BACE1) inhibition causes retinal pathology by vascular dysregulation and accumulation of age pigment. *EMBO molecular medicine*, 4(9), pp.980–991.

Cai, X.D., Golde, T.E. & Younkin, S.G., 1993. Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. *Science (New York, N.Y.)*, 259(5094), pp.514–516.

Calhoun, M.E. et al., 1998. Neuron loss in APP transgenic mice. *Nature*, 395(6704), pp.755–756.

Calhoun, M.E. et al., 1999. Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid. *Proceedings of the National Academy of Sciences of the United States of America*, 96(24), pp.14088–14093.

Caselli, R.J. et al., 2012. Longitudinal modeling of cognitive aging and the TOMM40 effect. *Alzheimer"s & dementia : the journal of the Alzheimer"s Association*, 8(6), pp.490–495.

Castellano, J.M. et al., 2011. Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Science translational medicine*, 3(89), p.89ra57.

Chase, T.N. et al., 1984. Regional cortical dysfunction in Alzheimer's disease as determined by positron emission tomography. *Annals of neurology*, 15 Suppl, pp.S170–4.

Cheng, D. et al., 2011. Type 2 diabetes and late-onset Alzheimer's disease. *Dementia and geriatric cognitive disorders*, 31(6), pp.424–430.

Chertkow, H. et al., 2013. Definitions of dementia and predementia states in Alzheimer's disease and vascular cognitive impairment: consensus from the Canadian conference on diagnosis of dementia. *Alzheimer's research & therapy*, 5(Suppl 1), p.S2.

Chesebro, B. et al., 1985. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. *Nature*, 315(6017), pp.331–333.

Chouliaras, L. et al., 2010. Epigenetic regulation in the pathophysiology of Alzheimer's disease. *Progress in neurobiology*, 90(4), pp.498–510.

Chu, L.W., 2012. Alzheimer's disease: early diagnosis and treatment. *Hong Kong medical journal = Xianggang yi xue za zhi*, 18(3), pp.228–237.

Citron, M., 2010. Alzheimer's disease: strategies for disease modification. *Nature reviews. Drug discovery*, 9(5), pp.387–398.

Citron, M. et al., 1997. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nature medicine*, 3(1), pp.67–72.

Citron, M. et al., 1992. Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature*, 360(6405), pp.672–674.

Clark, C.M. et al., 2011. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA*, 305(3), pp.275–283.

Cole, D.C. et al., 2006. Acylguanidines as small-molecule beta-secretase inhibitors. *Journal of medicinal chemistry*, 49(21), pp.6158–6161.

Cole, S.L. & Vassar, R., 2007. The Alzheimer's disease beta-secretase enzyme, BACE1. *Molecular neurodegeneration*, 2, p.22.

Collinge, J. & Clarke, A.R., 2007. A general model of prion strains and their pathogenicity. *Science (New York, N.Y.)*, 318(5852), pp.930–936.

Collins, S.J., Lawson, V.A. & Masters, C.L., 2004. Transmissible spongiform encephalopathies. *The Lancet*, 363(9402), pp.51–61.

Corder, E.H. et al., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science (New York, N.Y.)*, 261(5123), pp.921–923.

Coric, V. et al., 2012. Safety and tolerability of the γ-secretase inhibitor avagacestat in a phase 2 study of mild to moderate Alzheimer disease. *Archives of neurology*, 69(11), pp.1430–1440.

Crespi, G.A.N. et al., 2015. Molecular basis for mid-region amyloid-β capture by leading Alzheimer's disease immunotherapies. *Scientific reports*, 5, p.9649.

Cummings, J.L., 2004. Treatment of Alzheimer's disease: current and future therapeutic approaches. *Reviews in neurological diseases*, 1(2), pp.60–69.

Dartigues, J.F., 2009. Alzheimer's disease: a global challenge for the 21st century. *Lancet neurology*, 8(12), pp.1082–1083.

Das, P. et al., 2012. Transient pharmacologic lowering of Aβ production prior to deposition results in sustained reduction of amyloid plaque pathology. *Molecular neurodegeneration*, 7, p.39.

Davies, P.P. & Maloney, A.J.A., 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. *The Lancet*, 2(8000), pp.1403–1403.

Dawkins, E. & Small, D.H., 2014. Insights into the physiological function of the β-amyloid precursor protein: beyond Alzheimer's disease. *Journal of neurochemistry*, 129(5), pp.756–769.

De Strooper, B., 2003. Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex. *Neuron*, 38(1), pp.9–12.

De Strooper, B., 2014. Lessons from a failed γ-secretase Alzheimer trial. *Cell*, 159(4), pp.721–726.

De Strooper, B. & Annaert, W., 2010. Novel research horizons for presenilins and γ-secretases in cell biology and disease. *Annual review of cell and developmental biology*, 26, pp.235–260.

De Strooper, B. & Karran, E., 2016. The Cellular Phase of Alzheimer's Disease. *Cell*, 164(4), pp.603–615.

De Strooper, B. et al., 1998. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*, 391(6665), pp.387–390.

DeKosky, S.T. & Scheff, S.W., 1990. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Annals of neurology*, 27(5), pp.457–464.

DeMattos, R.B. et al., 2001. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 98(15), pp.8850–8855.

Dislich, B. & Lichtenthaler, S.F., 2012. The Membrane-Bound Aspartyl Protease BACE1: Molecular and Functional Properties in Alzheimer's Disease and Beyond. *Frontiers in physiology*, 3, p.8.

Doody, R.S. et al., 2014. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *The New England journal of medicine*, 370(4), pp.311–321.

Doody, R.S., Aisen, P.S. & Iwatsubo, T., 2013. Semagacestat for treatment of Alzheimer's disease. *The New England journal of medicine*, 369(17), p.1661.

Driscoll, I. et al., 2011. Lack of association between 11C-PiB and longitudinal brain atrophy in non-demented older individuals. *Neurobiology of aging*, 32(12), pp.2123–2130.

Drummond, E. & Wisniewski, T., 2017. Alzheimer's disease: experimental models and reality. *Acta neuropathologica*, 133(2), pp.155–175.

Duyckaerts, C., Delatour, B. & Potier, M.-C., 2009. Classification and basic pathology of Alzheimer disease. *Acta neuropathologica*, 118(1), pp.5–36.

Edbauer, D. et al., 2003. Reconstitution of gamma-secretase activity. *Nature cell biology*, 5(5), pp.486–488.

Edwards, D.R., Handsley, M.M. & Pennington, C.J., 2008. The ADAM metalloproteinases. *Molecular aspects of medicine*, 29(5), pp.258–289.

Eisele, Y.S., 2013. From soluble aβ to progressive aβ aggregation: could prion-like templated misfolding play a role? *Brain pathology (Zurich, Switzerland)*, 23(3), pp.333–341.

Eisele, Y.S. et al., 2009. Induction of cerebral beta-amyloidosis: intracerebral versus systemic Abeta inoculation. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31), pp.12926–12931.

Eisele, Y.S. et al., 2014. Multiple factors contribute to the peripheral induction of cerebral β-amyloidosis. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 34(31), pp.10264–10273.

Eisele, Y.S. et al., 2010. Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. *Science (New York, N.Y.)*, 330(6006), pp.980–982.

Eisenberg, D. & Jucker, M., 2012. The amyloid state of proteins in human diseases. *Cell*, 148(6), pp.1188–1203.

Eketjäll, S. et al., 2013. AZ-4217: A High Potency BACE Inhibitor Displaying Acute Central Efficacy in Different In Vivo Models and Reduced Amyloid Deposition in Tg2576 Mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 33(24), pp.10075–10084.

Fleisher, A.S. et al., 2008. Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. *Archives of neurology*, 65(8), pp.1031–1038.

Fleisher, A.S. et al., 2011. Using positron emission tomography and florbetapir F18 to image cortical amyloid in patients with mild cognitive impairment or dementia due to Alzheimer disease. *Archives of neurology*, 68(11), pp.1404–1411.

Folch, J. et al., 2016. Current Research Therapeutic Strategies for Alzheimer's Disease Treatment. *Neural plasticity*, 2016, p.8501693.

Foley, A.M. et al., 2015. Systematic review of the relationship between amyloid-β levels and measures of transgenic mouse cognitive deficit in Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 44(3), pp.787–795.

Foster, N.L. et al., 1984. Cortical abnormalities in Alzheimer's disease. *Annals of neurology*, 16(6), pp.649–654.

Fraser, H., 1982. Neuronal spread of scrapie agent and targeting of lesions within the retino-tectal pathway. *Nature*, 295(5845), pp.149–150.

Friedrich, R.P. et al., 2010. Mechanism of amyloid plaque formation suggests an intracellular basis of Abeta pathogenicity. *Proc Natl Acad Sci USA*, 107(5), pp.1942–1947.

Fritschi, S.K., Cintron, A., et al., 2014a. Aβ seeds resist inactivation by formaldehyde. *Acta neuropathologica*, 128(4), pp.477–484.

Fritschi, S.K., Langer, F., et al., 2014b. Highly potent soluble amyloid-β seeds in human Alzheimer brain but not cerebrospinal fluid. *Brain : a journal of neurology*, 137(Pt 11), pp.2909–15.

Fukumoto, H. et al., 2010. A noncompetitive BACE1 inhibitor TAK-070 ameliorates Abeta pathology and behavioral deficits in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(33), pp.11157–11166.

Fuller, J.P. et al., 2015. Comparing the efficacy and neuroinflammatory potential of three anti-abeta antibodies. *Acta neuropathologica*, 130(5), pp.699–711.

Games, D. et al., 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature*, 373(6514), pp.523–527.

Garcia-Osta, A. & Alberini, C.M., 2009. Amyloid beta mediates memory formation. *Learning & memory (Cold Spring Harbor, N.Y.)*, 16(4), pp.267–272.

Geldmacher, D.S. et al., 2011. A randomized pilot clinical trial of the safety of pioglitazone in treatment of patients with Alzheimer disease. *Archives of neurology*, 68(1), pp.45–50.

Ghiso, J. et al., 1989. Alzheimer's disease amyloid precursor protein is present in senile plaques and cerebrospinal fluid: immunohistochemical and biochemical characterization. *Biochemical and biophysical research communications*, 163(1), pp.430–437.

Ghosh, A.K., Gemma, S. & Tang, J., 2008. beta-Secretase as a therapeutic target for Alzheimer's disease. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*, 5(3), pp.399–408.

Giannakopoulos, P. et al., 2003. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology*, 60(9), pp.1495–1500.

Glenner, G.G. & Wong, C.W., 1984. Alzheimer"s disease and Down"s syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochemical and biophysical research communications*, 122(3), pp.1131–1135.

Goate, A. et al., 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*, 349(6311), pp.704–706.

Godyń, J. et al., 2016. Therapeutic strategies for Alzheimer's disease in clinical trials. *Pharmacological reports : PR*, 68(1), pp.127–138.

Goedert, M. & Jakes, R., 2005. Mutations causing neurodegenerative tauopathies. *Biochimica et biophysica acta*, 1739(2-3), pp.240–250.

Goedert, M. et al., 1988. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proceedings of the National Academy of Sciences of the United States of America*, 85(11), pp.4051–4055.

Golde, T.E., Petrucelli, L. & Lewis, J., 2010. Targeting Abeta and tau in Alzheimer's disease, an early interim report. *Experimental neurology*, 223(2), pp.252–266.

Golde, T.E., Schneider, L.S. & Koo, E.H., 2011. Anti-aβ therapeutics in Alzheimer's disease: the need for a paradigm shift. *Neuron*, 69(2), pp.203–213.

Goldgaber, D. et al., 1987. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science (New York, N.Y.)*, 235(4791), pp.877–880.

Graham, W.V., Bonito-Oliva, A. & Sakmar, T.P., 2017. Update on Alzheimer's Disease Therapy and Prevention Strategies. *Annual review of medicine*, 68, pp.413–430.

Grundke-Iqbal, I. et al., 1986. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences of the United States of America*, 83(13), pp.4913–4917.

Haass, C. & Selkoe, D.J., 1993. Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. *Cell*, 75(6), pp.1039–1042.

Haass, C. & Selkoe, D.J., 2007. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nature reviews. Molecular cell biology*, 8(2), pp.101–112.

Haass, C. et al., 1992. Amyloid beta-peptide is produced by cultured cells during normal metabolism. *Nature*, 359(6393), pp.322–325.

Haass, C. et al., 2012. Trafficking and proteolytic processing of APP. *Cold Spring Harbor perspectives in medicine*, 2(5), p.a006270.

Hamaguchi, T. et al., 2012. The presence of Aβ seeds, and not age per se, is critical to the initiation of Aβ deposition in the brain. *Acta neuropathologica*, 123(1), pp.31–37.

Hansen, C.P. et al., 2008. Novel acetylcholine and carbamoylcholine analogues: development of a functionally selective alpha4beta2 nicotinic acetylcholine receptor agonist. *Journal of medicinal chemistry*, 51(23), pp.7380–7395.

Hardy, J. & De Strooper, B., 2017. Alzheimer's disease: where next for anti-amyloid therapies? *Brain : a journal of neurology*, 140(4), pp.853–855.

Hardy, J.A. & Higgins, G.A., 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science (New York, N.Y.)*, 256(5054), pp.184–185.

Hardy, J. & Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)*, 297(5580), pp.353–356.

Hardy, J. et al., 1998. Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nature neuroscience*, 1(5), pp.355–358.

Harold, D. et al., 2009. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nature genetics*, 41(10), pp.1088–1093.

Harper, J.D. & Lansbury, P.T., 1997. Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annual review of biochemistry*, 66, pp.385–407.

Heilbronner, G. et al., 2013. Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice. *EMBO reports*, 14(11), pp.1017–1022.

Herzig, M.C. et al., 2004. Abeta is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis. *Nature neuroscience*, 7(9), pp.954–960.

Hitt, B.D. et al., 2010. BACE1-/- mice exhibit seizure activity that does not correlate with sodium channel level or axonal localization. *Molecular neurodegeneration*, 5, p.31.

Hollingworth, P. et al., 2012. Genome-wide association study of Alzheimer's disease with psychotic symptoms. *Molecular psychiatry*, 17(12), pp.1316–1327.

Holmes, C. et al., 2008. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *The Lancet*, 372(9634), pp.216–223.

Holtzman, D.M., 2011. CSF biomarkers for Alzheimer's disease: current utility and potential future use. *Neurobiology of aging*, 32 Suppl 1, pp.S4–9.

Holtzman, D.M. et al., 2000. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 97(6), pp.2892–2897.

Holtzman, D.M., Morris, J.C. & Goate, A.M., 2011. Alzheimer's disease: the challenge of the second century. *Science translational medicine*, 3(77), p.77sr1.

Hsiao, K. et al., 1996. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science (New York, N.Y.)*, 274(5284), pp.99–102.

Hsu, D. & Marshall, G.A., 2017. Primary and Secondary Prevention Trials in Alzheimer Disease: Looking Back, Moving Forward. *Current Alzheimer research*, 14(4), pp.426–440.

Hu, X. et al., 2006. Bace1 modulates myelination in the central and peripheral nervous system. *Nature neuroscience*, 9(12), pp.1520–1525.

Huang, K.-L. et al., 2017. A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheimer's disease. Accepted in *Nature neuroscience*.

Ikonomovic, M.D. et al., 2008. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain : a journal of neurology*, 131(Pt 6), pp.1630–1645.

Jack, C.R. & Holtzman, D.M., 2013. Biomarker modeling of Alzheimer's disease. *Neuron*, 80(6), pp.1347–1358.

Jack, C.R. et al., 2010. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. *Brain : a journal of neurology*, 133(11), pp.3336–3348.

Jarrett, J.T. & Lansbury, P.T., 1993. Seeding “one-dimensional crystallization” of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell*, 73(6), pp.1055–1058.

Jarrett, J.T., Berger, E.P. & Lansbury, P.T., 1993. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry*, 32(18), pp.4693–4697.

Jaunmuktane, Z. et al., 2015. Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. *Nature*, 525(7568), pp.247–250.

Jedrziewski, M.K. et al., 2014. The Impact of Exercise, Cognitive Activities, and Socialization on Cognitive Function: Results From the National Long-Term Care Survey. *American journal of Alzheimer's disease and other dementias*, 29(4), pp.372–378.

Jobst, K.A. et al., 1994. Rapidly progressing atrophy of medial temporal lobe in Alzheimer's disease. *The Lancet*, 343(8901), pp.829–830.

Johnson, K.A. et al., 2012. Brain imaging in Alzheimer disease. *Cold Spring Harbor perspectives in medicine*, 2(4), p.a006213.

Jonsson, T. et al., 2012. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature*, 488(7409), pp.96–99.

Jonsson, T. et al., 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. *The New England journal of medicine*, 368(2), pp.107–116.

Jorissen, E. et al., 2010. The disintegrin/metalloproteinase ADAM10 is essential for the establishment of the brain cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(14), pp.4833–4844.

Jucker, M., 2010. The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nature medicine*, 16(11), pp.1210–1214.

Jucker, M. & Walker, L.C., 2015. Neurodegeneration: Amyloid-β pathology induced in humans. *Nature*, 525(7568), pp.193–194.

Jucker, M. & Walker, L.C., 2013. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465), pp.45–51.

Kamino, K. et al., 1992. Linkage and mutational analysis of familial Alzheimer disease kindreds for the APP gene region. *American journal of human genetics*, 51(5), pp.998–1014.

Kandalepas, P.C. et al., 2013. The Alzheimer's β-secretase BACE1 localizes to normal presynaptic terminals and to dystrophic presynaptic terminals surrounding amyloid plaques. *Acta neuropathologica*, 126(3), pp.329–352.

Kane, M.D. et al., 2000. Evidence for seeding of beta -amyloid by intracerebral infusion of Alzheimer brain extracts in beta -amyloid precursor protein-transgenic mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(10), pp.3606–3611.

Kang, J. et al., 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*, 325(6106), pp.733–736.

Karran, E. & Hardy, J., 2014. A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Annals of neurology*, 76(2), pp.185–205.

Karran, E., Mercken, M. & De Strooper, B., 2011. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature reviews. Drug discovery*, 10(9), pp.698–712.

Kennedy, M.E. et al., 2016. The BACE1 inhibitor verubecestat (MK-8931) reduces CNS β-amyloid in animal models and in Alzheimer's disease patients. *Science translational medicine*, 8(363), p.363ra150.

Kim, Y. et al., 2013. Genome-scale analysis of ABC transporter genes and characterization of the ABCC type transporter genes in Magnaporthe oryzae. *Genomics*, 101(6), pp.354–361.

Kimberlin, R.H. & Walker, C.A., 1986. Pathogenesis of scrapie (strain 263K) in hamsters infected intracerebrally, intraperitoneally or intraocularly. *The Journal of general virology*, 67 (Pt 2), pp.255–263.

Kitaguchi, N. et al., 1988. Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. *Nature*, 331(6156), pp.530–532.

Kivipelto, M. et al., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Archives of neurology*, 62(10), pp.1556–1560.

Klein, W.L., 2013. Synaptotoxic amyloid-β oligomers: a molecular basis for the cause, diagnosis, and treatment of Alzheimer's disease? *Journal of Alzheimer's disease : JAD*, 33 Suppl 1, pp.S49–65.

Klunk, W.E. et al., 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Annals of neurology*, 55(3), pp.306–319.

Knowles, T.P.J. et al., 2011. Observation of spatial propagation of amyloid assembly from single nuclei. *Proc Natl Acad Sci USA*, 108(36), pp.14746–14751.

Kobayashi, D. et al., 2008. BACE1 gene deletion: impact on behavioral function in a model of Alzheimer's disease. *Neurobiology of aging*, 29(6), pp.861–873.

Kojro, E. & Fahrenholz, F., 2005. The non-amyloidogenic pathway: structure and function of alpha-secretases. *Sub-cellular biochemistry*, 38, pp.105–127.

Kopan, R. & Ilagan, M.X.G., 2009. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*, 137(2), pp.216–233.

Kosik, K.S., Joachim, C.L. & Selkoe, D.J., 1986. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, 83(11), pp.4044–4048.

Kozauer, N. & Katz, R., 2013. Regulatory innovation and drug development for early-stage Alzheimer's disease. *The New England journal of medicine*, 368(13), pp.1169–1171.

Kuhn, P.-H. et al., 2010. ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. *The EMBO journal*, 29(17), pp.3020–3032.

Kukar, T.L. et al., 2008. Substrate-targeting gamma-secretase modulators. *Nature*, 453(7197), pp.925–929.

Lambert, J.C. et al., 2009. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nature genetics*, 41(10), pp.1094–1099.

Lammich, S. et al., 1999. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proceedings of the National Academy of Sciences of the United States of America*, 96(7), pp.3922–3927.

Langer, F. et al., 2011. Soluble Aβ seeds are potent inducers of cerebral β-amyloid deposition. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(41), pp.14488–14495.

Lee, E.B. et al., 2006. Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. *The Journal of biological chemistry*, 281(7), pp.4292–4299.

Legname, G. et al., 2004. Synthetic mammalian prions. *Science (New York, N.Y.)*, 305(5684), pp.673–676.

Lewis, J. et al., 2001. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science (New York, N.Y.)*, 293(5534), pp.1487–1491.

Leyhe, T. et al., 2014. Modulation of β-amyloid by a single dose of GSK933776 in patients with mild Alzheimer's disease: a phase I study. *Alzheimer's research & therapy*, 6(2), p.19.

Lichtenthaler, S.F., 2011. α-secretase in Alzheimer's disease: molecular identity, regulation and therapeutic potential. *Journal of neurochemistry*, 116(1), pp.10–21.

Lim, Y.Y. et al., 2013. Cognitive consequences of high Aβ amyloid in mild cognitive impairment and healthy older adults: implications for early detection of Alzheimer's disease. *Neuropsychology*, 27(3), pp.322–332.

Luchsinger, J.A. et al., 2012. Central obesity in the elderly is related to late-onset Alzheimer disease. *Alzheimer disease and associated disorders*, 26(2), pp.101–105.

Luo, Y. et al., 2003. BACE1 (beta-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time. *Neurobiology of disease*, 14(1), pp.81–88.

Lye, T.C. & Shores, E.A., 2000. Traumatic brain injury as a risk factor for Alzheimer's disease: a review. *Neuropsychology review*, 10(2), pp.115–129.

Mahler, J. et al., 2015. Endogenous murine Aβ increases amyloid deposition in APP23 but not in APPPS1 transgenic mice. *Neurobiology of aging*, 36(7), pp.2241–2247.

Maia, L.F. et al., 2013. Changes in Amyloid-β and Tau in the Cerebrospinal Fluid of Transgenic Mice Overexpressing Amyloid Precursor Protein. *Science translational medicine*, 5(194), p.194re2.

Maia, L.F. et al., 2015. Increased CSF Aβ during the very early phase of cerebral Aβ deposition in mouse models. *EMBO molecular medicine*, 7(7), pp.895-903.

Maloney, J.A. et al., 2014. Molecular mechanisms of Alzheimer disease protection by the A673T allele of amyloid precursor protein. *The Journal of biological chemistry*, 289(45), pp.30990–31000.

Mandelkow, E.-M. & Mandelkow, E., 2012. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harbor perspectives in medicine*, 2(7), p.a006247.

Martiskainen, H. et al., 2017. Decreased plasma β-amyloid in the Alzheimer's disease APP A673T variant carriers. Accepted in *Annals of neurology*.

Masters, C.L. et al., 1985. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, 82(12), pp.4245–4249.

Mawuenyega, K.G. et al., 2010. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science (New York, N.Y.)*, 330(6012), p.1774.

McConlogue, L. et al., 2007. Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice. *The Journal of biological chemistry*, 282(36), pp.26326–26334.

Meyer-Luehmann, M. et al., 2006. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science (New York, N.Y.)*, 313(5794), pp.1781–1784.

Miguel-Álvarez, M. et al., 2015. Non-steroidal anti-inflammatory drugs as a treatment for Alzheimer's disease: a systematic review and meta-analysis of treatment effect. *Drugs & aging*, 32(2), pp.139–147.

Minoshima, S. et al., 1995. A diagnostic approach in Alzheimer's disease using three-dimensional stereotactic surface projections of fluorine-18-FDG PET. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, 36(7), pp.1238–1248.

Mitsushima, D., Sano, A. & Takahashi, T., 2013. A cholinergic trigger drives learning-induced plasticity at hippocampal synapses. *Nature communications*, 4, p.2760.

Morales, R. et al., 2012. De novo induction of amyloid-β deposition in vivo. *Molecular psychiatry*, 17(12), pp.1347–1353.

Morgan, D. et al., 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature*, 408(6815), pp.982–985.

Morris, J.C. et al., 2009. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Archives of neurology*, 66(12), pp.1469–1475.

Mortimer, J.A. et al., 1991. Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *International journal of epidemiology*, 20 Suppl 2, pp.S28–35.

Mosconi, L. et al., 2008. Multicenter standardized 18F-FDG PET diagnosis of mild cognitive impairment, Alzheimer's disease, and other dementias. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, 49(3), pp.390–398.

Motter, R. et al., 1995. Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Annals of neurology*, 38(4), pp.643–648.

Moulder, K.L. et al., 2013. Dominantly Inherited Alzheimer Network: facilitating research and clinical trials. *Alzheimer's research & therapy*, 5(5), p.48.

Mueller-Steiner, S. et al., 2006. Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. *Neuron*, 51(6), pp.703–714.

Mullan, M. et al., 1992. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nature genetics*, 1(5), pp.345–347.

Mullard, A., 2012. Sting of Alzheimer's failures offset by upcoming prevention trials. *Nature reviews. Drug discovery*, 11(9), pp.657–660.

Murrell, J. et al., 1991. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science (New York, N.Y.)*, 254(5028), pp.97–99.

Naj, A.C. et al., 2011. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nature genetics*, 43(5), pp.436–441.

Nelson, P.T. et al., 2012. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *Journal of neuropathology and experimental neurology*, 71(5), pp.362–381.

Neumann, U. et al., 2015. A novel BACE inhibitor NB-360 shows a superior pharmacological profile and robust reduction of amyloid-β and neuroinflammation in APP transgenic mice. *Molecular neurodegeneration*, 10(1), p.44.

Nguyen, J.-T. et al., 2008. Design of potent aspartic protease inhibitors to treat various diseases. *Archiv der Pharmazie*, 341(9), pp.523–535.

Nicoll, J.A.R. et al., 2006. Abeta species removal after abeta42 immunization. *Journal of neuropathology and experimental neurology*, 65(11), pp.1040–1048.

Nicoll, J.A.R. et al., 2003. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nature medicine*, 9(4), pp.448–452.

Novartis International AG, 2015. Novartis announces global partnership with Amgen to develop and commercialize pioneering neuroscience treatments. *Novartis media release*, pp.1–4.

Novotny, R. et al., 2016. Conversion of Synthetic Aβ to In Vivo Active Seeds and Amyloid Plaque Formation in a Hippocampal Slice Culture Model. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 36(18), pp.5084–5093.

Oesch, B. et al., 1985. A cellular gene encodes scrapie PrP 27-30 protein. *Cell*, 40(4), pp.735–746.

Olsson, B. et al., 2016. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet neurology*, 15(7), pp.673–684.

Orgogozo, J.-M. et al., 2003. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology*, 61(1), pp.46–54.

Ostrowitzki, S. et al., 2012. Mechanism of amyloid removal in patients with Alzheimer disease treated with gantenerumab. *Archives of neurology*, 69(2), pp.198–207.

Pagon, R.A. et al., 1993. MAPT-Related Disorders. *GeneReviews.*

Pattison, I.H., 1965. RESISTANCE OF THE SCRAPIE AGENT TO FORMALIN. *Journal of comparative pathology*, 75, pp.159–164.

Pauwels, K. et al., 2012. Structural basis for increased toxicity of pathological aβ42:aβ40 ratios in Alzheimer disease. *The Journal of biological chemistry*, 287(8), pp.5650–5660.

Perry, E.K. et al., 1977. Neurotransmitter enzyme abnormalities in senile dementia. Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. *Journal of the neurological sciences*, 34(2), pp.247–265.

Petersen, R.C. et al., 1999. Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology*, 56(3), pp.303–308.

Pfeifer, M. et al., 2002. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science (New York, N.Y.)*, 298(5597), p.1379.

Ponte, P. et al., 1988. A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. *Nature*, 331(6156), pp.525–527.

Postina, R. et al., 2004. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *The Journal of clinical investigation*, 113(10), pp.1456–1464.

Prentice, H., Modi, J.P. & Wu, J.-Y., 2015. Mechanisms of Neuronal Protection against Excitotoxicity, Endoplasmic Reticulum Stress, and Mitochondrial Dysfunction in Stroke and Neurodegenerative Diseases. *Oxidative medicine and cellular longevity*, 2015, p.964518.

Prinz, M. & Mildner, A., 2011. Microglia in the CNS: immigrants from another world. *Glia*, 59(2), pp.177–187.

Prokop, S., Miller, K.R. & Heppner, F.L., 2013. Microglia actions in Alzheimer's disease. *Acta neuropathologica*, 126(4), pp.461–477.

Prusiner, S.B., 2013. Biology and genetics of prions causing neurodegeneration. *Annual review of genetics*, 47, pp.601–623.

Prusiner, S.B., 2012. Cell biology. A unifying role for prions in neurodegenerative diseases. *Science (New York, N.Y.)*, 336(6088), pp.1511–1513.

Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. *Science (New York, N.Y.)*, 216(4542), pp.136–144.

Prusiner, S.B., 1998. Prions. *Proceedings of the National Academy of Sciences of the United States of America*, 95(23), pp.13363–13383.

Puzzo, D. et al., 2015. The keystone of Alzheimer pathogenesis might be sought in Aβ physiology. *Neuroscience*, 307, pp.26–36.

Qi-Takahara, Y. et al., 2005. Longer forms of amyloid beta protein: implications for the mechanism of intramembrane cleavage by gamma-secretase. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(2), pp.436–445.

Raber, J., Huang, Y. & Ashford, J.W., 2004. ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiology of aging*, 25(5), pp.641–650.

Radde, R. et al., 2006. Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO reports*, 7(9), pp.940–946.

Rambaran, R.N. & Serpell, L.C., 2008. Amyloid fibrils: abnormal protein assembly. *Prion*, 2(3), pp.112–117.

Rangel, A. et al., 2014. Distinct patterns of spread of prion infection in brains of mice expressing anchorless or anchored forms of prion protein. *Acta neuropathologica communications*, 2, p.8.

Rebeck, G.W. et al., 1993. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron*, 11(4), pp.575–580.

Reitz, C., Brayne, C. & Mayeux, R., 2011. Epidemiology of Alzheimer disease. *Nature reviews. Neurology*, 7(3), pp.137–152.

Rinne, J.O. et al., 2010. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet neurology*, 9(4), pp.363–372.

Ritchie, D.L. et al., 2017. Amyloid-β accumulation in the CNS in human growth hormone recipients in the UK. Accepted in *Acta neuropathologica*.

Robakis, N.K. et al., 1987. Chromosome 21q21 sublocalisation of gene encoding beta-amyloid peptide in cerebral vessels and neuritic (senile) plaques of people with Alzheimer disease and Down syndrome. *The Lancet*, 1(8529), pp.384–385.

Rogaeva, E. et al., 2007. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nature genetics*, 39(2), pp.168–177.

Román, G.C. & Kalaria, R.N., 2006. Vascular determinants of cholinergic deficits in Alzheimer disease and vascular dementia. *Neurobiology of aging*, 27(12), pp.1769–1785.

Rosen, R.F. et al., 2012. Exogenous seeding of cerebral β-amyloid deposition in βAPP-transgenic rats. *Journal of neurochemistry*, 120(5), pp.660–666.

Roses, A.D. et al., 2010. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. *The pharmacogenomics journal*, 10(5), pp.375–384.

Rovelet-Lecrux, A. et al., 2006. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nature genetics*, 38(1), pp.24–26.

Roychaudhuri, R. et al., 2009. Amyloid beta-protein assembly and Alzheimer disease. *The Journal of biological chemistry*, 284(8), pp.4749–4753.

Rupp, N.J. et al., 2011. Early onset amyloid lesions lead to severe neuritic abnormalities and local, but not global neuron loss in APPPS1 transgenic mice. *Neurobiology of aging*, 32(12), pp.2324.e1–6.

Rusanen, M. et al., 2010. Midlife smoking, apolipoprotein E and risk of dementia and Alzheimer's disease: a population-based cardiovascular risk factors, aging and dementia study. *Dementia and geriatric cognitive disorders*, 30(3), pp.277–284.

Salloway, S. & Sperling, R., 2015. Understanding Conflicting Neuropathological Findings in Patients Clinically Diagnosed as Having Alzheimer Dementia. *JAMA Neurol*, 72(10), pp.1106–1108.

Salloway, S. et al., 2009. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology*, 73(24), pp.2061–2070.

Sato, T. et al., 2011. Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease. *Neurobiology of aging*, 32(9), pp.1626–1633.

Saunders, A.M. et al., 1993. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*, 43(8), pp.1467–1472.

Schelle, J. et al., 2017. Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. *Alzheimer's & dementia: the journal of the Alzheimer's Association*, 13(6), pp.701–709.

Scheltens, P. et al., 2016. Alzheimer's disease. *The Lancet*, 388(10043), pp.505–517.

Schenk, D. et al., 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*, 400(6740), pp.173–177.

Schirmer, R.H. et al., 2011. "Lest we forget you--methylene blue...". *Neurobiology of aging*, 32(12), pp.2325.e7–16.

Schmechel, D.E. et al., 1993. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, 90(20), pp.9649–9653.

Schofield, P.W. et al., 1997. An association between head circumference and Alzheimer's disease in a population-based study of aging and dementia. *Neurology*, 49(1), pp.30–37.

Schor, N.F., 2011. What the halted phase III γ-secretase inhibitor trial may (or may not) be telling us. *Annals of neurology*, 69(2), pp.237–239.

Searfoss, G.H. et al., 2003. Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. *The Journal of biological chemistry*, 278(46), pp.46107–46116.

Selkoe, D.J., 2001. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *Journal of Alzheimer's disease : JAD*, 3(1), pp.75–80.

Selkoe, D.J., 2012. Preventing Alzheimer's disease. *Science (New York, N.Y.)*, 337(6101), pp.1488–1492.

Selkoe, D.J., 1991. The molecular pathology of Alzheimer's disease. *Neuron*, 6(4), pp.487–498.

Selkoe, D.J. & Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO molecular medicine*, 8(6), pp.595–608.

Seshadri, S. et al., 2010. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA*, 303(18), pp.1832–1840.

Sevigny, J. et al., 2016. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature*, 537(7618), pp.50–56.

Shimshek, D.R. et al., 2016. Pharmacological BACE1 and BACE2 inhibition induces hair depigmentation by inhibiting PMEL17 processing in mice. *Scientific reports*, 6, p.21917.

Siemers, E.R. et al., 2016. Phase 3 solanezumab trials: Secondary outcomes in mild Alzheimer's disease patients. *Alzheimer"s & dementia : the journal of the Alzheimer"s Association*, 12(2), pp.110–120.

Silveira, J.R. et al., 2005. The most infectious prion protein particles. *Nature*, 437(7056), pp.257–261.

Sipe, J.D. et al., 2014. Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, 21(4), pp.221–224.

Sisodia, S.S., 1992. Beta-amyloid precursor protein cleavage by a membrane-bound protease. *Proceedings of the National Academy of Sciences of the United States of America*, 89(13), pp.6075–6079.

Sleegers, K. et al., 2006. APP duplication is sufficient to cause early onset Alzheimer's dementia with cerebral amyloid angiopathy. *Brain : a journal of neurology*, 129(Pt 11), pp.2977–2983.

Sojkova, J. et al., 2011. In vivo fibrillar beta-amyloid detected using [11C]PiB positron emission tomography and neuropathologic assessment in older adults. *Archives of neurology*, 68(2), pp.232–240.

Solomon, B. et al., 1996. Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide. *Proceedings of the National Academy of Sciences of the United States of America*, 93(1), pp.452–455.

Soto, C., 2011. Prion hypothesis: the end of the controversy? *Trends in biochemical sciences*, 36(3), pp.151–158.

Sperling, R., Mormino, E. & Johnson, K., 2014a. The evolution of preclinical Alzheimer's disease: implications for prevention trials. *Neuron*, 84(3), pp.608–622.

Sperling, R.A. et al., 2011. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. *Alzheimer"s & dementia : the journal of the Alzheimer"s Association*, 7(4), pp.367–385.

Sperling, R.A., Rentz, D.M., et al., 2014b. The A4 study: stopping AD before symptoms begin? *Science translational medicine*, 6(228), p.228fs13.

Stachel, S.J. et al., 2004. Structure-based design of potent and selective cell-permeable inhibitors of human beta-secretase (BACE-1). *Journal of medicinal chemistry*, 47(26), pp.6447–6450.

Stack, C. et al., 2014. Methylene blue upregulates Nrf2/ARE genes and prevents tau-related neurotoxicity. *Human molecular genetics*, 23(14), pp.3716–3732.

Stern, Y. et al., 1994. Influence of education and occupation on the incidence of Alzheimer's disease. *JAMA*, 271(13), pp.1004–1010.

Stöhr, J. et al., 2014. Distinct synthetic Aβ prion strains producing different amyloid deposits in bigenic mice. *Proc Natl Acad Sci USA*, 111(28), pp.10329–10334.

Stöhr, J. et al., 2012. Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. *Proc Natl Acad Sci USA*, 109(27), pp.11025–11030.

Strittmatter, W.J. et al., 1993. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, 90(17), pp.8098–8102.

Strozyk, D. et al., 2003. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*, 60(4), pp.652–656.

Sturchler-Pierrat, C. et al., 1997. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proceedings of the National Academy of Sciences of the United States of America*, 94(24), pp.13287–13292.

Suzuki, N. et al., 1994. An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. *Science (New York, N.Y.)*, 264(5163), pp.1336–1340.

Takami, M. et al., 2009. gamma-Secretase: successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(41), pp.13042–13052.

Tanaka, S. et al., 1988. Three types of amyloid protein precursor mRNA in human brain: their differential expression in Alzheimer's disease. *Biochemical and biophysical research communications*, 157(2), pp.472–479.

Tapiola, T. et al., 2009. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Archives of neurology*, 66(3), pp.382–389.

Thal, D.R. et al., 2002. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology*, 58(12), pp.1791–1800.

Tzaban, S. et al., 2002. Protease-sensitive scrapie prion protein in aggregates of heterogeneous sizes. *Biochemistry*, 41(42), pp.12868–12875.

Van Broeckhoven, C. et al., 1990. Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). *Science (New York, N.Y.)*, 248(4959), pp.1120–1122.

Vandermeeren, M. et al., 1993. Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. *Journal of neurochemistry*, 61(5), pp.1828–1834.

Vassar, R. & Citron, M., 2000. Abeta-generating enzymes: recent advances in beta- and gamma-secretase research. *Neuron*, 27(3), pp.419–422.

Vassar, R. et al., 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science (New York, N.Y.)*, 286(5440), pp.735–741.

Vassar, R. et al., 2009. The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(41), pp.12787–12794.

Vigo-Pelfrey, C. et al., 1995. Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. *Neurology*, 45(4), pp.788–793.

Villemagne, V.L. et al., 2011. Amyloid imaging with (18)F-florbetaben in Alzheimer disease and other dementias. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, 52(8), pp.1210–1217.

Villemagne, V.L. et al., 2013. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet neurology*, 12(4), pp.357–367.

Vos, S.J.B. et al., 2013. Prediction of Alzheimer disease in subjects with amnestic and nonamnestic MCI. *Neurology*, 80(12), pp.1124–1132.

Walker, L.C. & Jucker, M., 2015. Neurodegenerative Diseases: Expanding the Prion Concept. *Annual review of neuroscience*, 38, pp.87–103.

Walker, L.C. & Jucker, M., 2017. The Exceptional Vulnerability of Humans to Alzheimer's Disease. *Trends in molecular medicine,* 23(6), pp.534–545.

Walker, L.C. et al., 2002. Exogenous induction of cerebral beta-amyloidosis in betaAPP-transgenic mice. *Peptides*, 23(7), pp.1241–1247.

Walker, L.C. et al., 2013. Mechanisms of protein seeding in neurodegenerative diseases. *JAMA Neurol*, 70(3), pp.304–310.

Walker, L.C., Schelle, J. & Jucker, M., 2016. The Prion-Like Properties of Amyloid-β Assemblies: Implications for Alzheimer's Disease. *Cold Spring Harbor perspectives in medicine*, 6(7).

Wang, J. et al., 2015a. Pharmacological treatment of neuropsychiatric symptoms in Alzheimer's disease: a systematic review and meta-analysis. *Journal of neurology, neurosurgery, and psychiatry*, 86(1), pp.101–109.

Wang, X. et al., 2015b. Genetic determinants of disease progression in Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 43(2), pp.649–655.

Watts, J.C. et al., 2011. Bioluminescence imaging of Abeta deposition in bigenic mouse models of Alzheimer's disease. *Proc Natl Acad Sci USA*, 108(6), pp.2528–2533.

Watts, J.C. et al., 2014. Serial propagation of distinct strains of Aβ prions from Alzheimer's disease patients. *Proc Natl Acad Sci USA*, 111(28), pp.10323–10328.

Whitmer, R.A. et al., 2008. Central obesity and increased risk of dementia more than three decades later. *Neurology*, 71(14), pp.1057–1064.

Wiggins, R.C., 2009. Prion stability and infectivity in the environment. *Neurochemical research*, 34(1), pp.158–168.

Willem, M. et al., 2006. Control of peripheral nerve myelination by the beta-secretase BACE1. *Science (New York, N.Y.)*, 314(5799), pp.664–666.

Willem, M. et al., 2015. η-Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature*, 526(7573), pp.443–447.

Wimo, A. et al., 2017. The worldwide costs of dementia 2015 and comparisons with 2010. *Alzheimer"s & dementia : the journal of the Alzheimer"s Association*, 13(1), pp.1–7.

Winblad, B. & Poritis, N., 1999. Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). *International journal of geriatric psychiatry*, 14(2), pp.135–146.

Winblad, B. et al., 2012. Safety, tolerability, and antibody response of active Aβ immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. *Lancet neurology*, 11(7), pp.597–604.

Wolfe, M.S., 2007. gamma-Secretase modulators. *Current Alzheimer research*, 4(5), pp.571–573.

Wolfe, M.S. et al., 1999. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature*, 398(6727), pp.513–517.

Wong, G.T. et al., 2004. Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *The Journal of biological chemistry*, 279(13), pp.12876–12882.

Wong, H.-K. et al., 2005. beta Subunits of voltage-gated sodium channels are novel substrates of beta-site amyloid precursor protein-cleaving enzyme (BACE1) and gamma-secretase. *The Journal of biological chemistry*, 280(24), pp.23009–23017.

World Alzheimer Report, 2015. The Global Impact of Dementia. *Alzheimer's Disease International*, pp.1–88.

Xu, L. et al., 2016. Prediction of Progressive Mild Cognitive Impairment by Multi-Modal Neuroimaging Biomarkers. *Journal of Alzheimer's disease : JAD*, 51(4), pp.1045–1056.

Yamada, T. et al., 1987. Complementary DNA for the mouse homolog of the human amyloid beta protein precursor. *Biochemical and biophysical research communications*, 149(2), pp.665–671.

Yan, Y. & Wang, C., 2006. Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. *Journal of molecular biology*, 364(5), pp.853–862.

Ye, L., Fritschi, S.K., et al., 2015a. Persistence of Aβ seeds in APP null mouse brain. *Nature neuroscience*, 18(11), pp.1559–61.

Ye, L., Hamaguchi, T., et al., 2015b. Progression of seed-induced Aβ deposition within the limbic connectome. *Brain pathology (Zurich, Switzerland)*, 25(6), pp.743–52

Ye, L. et al., 2017. Aβ seeding potency peaks in the early stages of cerebral β-amyloidosis. Accepted in *EMBO Reports*.

Younkin, S.G., 1998. The role of A beta 42 in Alzheimer's disease. *Journal of physiology, Paris*, 92(3-4), pp.289–292.

Zarow, C. et al., 2005. Correlates of hippocampal neuron number in Alzheimer's disease and ischemic vascular dementia. *Annals of neurology*, 57(6), pp.896–903.

Zetterberg, H., 2017. Applying fluid biomarkers to Alzheimer's disease. *American journal of physiology. Cell physiology*, p.ajpcell.00007.2017.

Zetterberg, H., Wahlund, L.-O. & Blennow, K., 2003. Cerebrospinal fluid markers for prediction of Alzheimer's disease. *Neuroscience letters*, 352(1), pp.67–69.

Zheng, X. et al., 2015. Amyloid β-Protein Assembly: Differential Effects of the Protective A2T Mutation and Recessive A2V Familial Alzheimer's Disease Mutation. *ACS chemical neuroscience*, 6(10), pp.1732–1740.

3. Publications

3.1 Description of personal contribution

I. *Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition*

Juliane Schelle, Lisa Häsler, Jens C. Göpfert, Thomas O. Joos, Hugo Vanderstichele, Erik Stoops, Eva-Maria Mandelkow, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker, Stephan A. Kaeser

Personal contribution: Experimental design and planning of the study (together with M.S, M.J. and S.A.K.); BACE inhibitor treatment of mice; CSF collection and tissue harvesting (together with S.A.K.); histology and immunohistochemistry; stereological analysis of plaque load; image acquisition and processing; statistical analysis (together with S.A.K.); figure design and preparation (together with S.A.K.); writing manuscript (together with M.S., M.J., and S.A.K. and the help of all the other authors).

Others: L.H., J.C.G, and S.A.K. performed the tau assay development and measurements. T.O.J., H.V., E.S., U.N. D.R.S., and M.S. provided crucial research reagents. M.S., M.J. and S.A.K. designed the study and with the help of all other authors prepared the manuscript.

II. *Persistence of Aβ seeds in APP null mouse brain*

Lan Ye*, Sarah K. Fritschi*, **Juliane Schelle**, Ulrike Obermüller, Karoline Degenhardt, Stephan A. Kaeser, Yvonne S. Eisele, Lary C. Walker, Frank Baumann, Matthias Staufenbiel, and Mathias Jucker *contributed equally

Personal contribution: Histological and morphological analysis (together with L.Y. and U.O.); light image acquisition and image processing (together with L.Y.).

Others: L.Y., S.K.F., and U.O. performed the experimental work. L.Y., S.K.F., U.O., K.D., S.A.K., F.B. and M.J. carried out the analysis. Experimental design and preparation of the manuscript was done by L.Y., S.K.F., Y.S.E., L.C.W., M.S. and M.J.

III. *Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds*

Juliane Schelle, Sarah K. Fritschi, Ulrike Obermüller, Lisa M. Häsler, Marius Lambert, Stephan A. Kaeser, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker

Personal contribution: Experimental design and planning of the study (together with S.K.F., S.A.K., M.S., and M.J.); stereotactic injections of brain extract (together with S.K.F.); passive Aβ immunization; BACE inhibitor treatment; brain tissue processing; immunohistochemical analyses; quantification of Aβ plaque load (together with S.K.F.); statistical analyses; figure design and preparation; writing manuscript.

Others: S.K.F., U.O., L.H., M.L., and S.A.K. performed the experimental work. U.N., D.R.S., and M.S. provided crucial research reagents. S.K.F., S.A.K., M.S., and M.J. designed the study.

3.2 Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition

Juliane Schelle, Lisa Häsler, Jens C. Göpfert, Thomas O. Joos, Hugo Vanderstichele, Erik Stoops, Eva-Maria Mandelkow, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker, Stephan A. Kaeser

Published in:

Alzheimer's & dementia: the journal of the Alzheimer's Association 2017, 13(6), pp.701–709

Alzheimer's & Dementia 13 (2017) 701-709

Featured Article

Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition

Juliane Schelle[a,b,c], Lisa M. Häsler[a,b,d], Jens C. Göpfert[d], Thomas O. Joos[d], Hugo Vanderstichele[e], Erik Stoops[e], Eva-Maria Mandelkow[f,g], Ulf Neumann[g], Derya R. Shimshek[h], Matthias Staufenbiel[a], Mathias Jucker[a,b,*], Stephan A. Kaeser[a,b,*]

[a]*Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany*
[b]*DZNE, German Center for Neurodegenerative Diseases, Tübingen, Germany*
[c]*Graduate School of Cellular and Molecular Neuroscience, University of Tübingen, Tübingen, Germany*
[d]*Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*
[e]*ADx NeuroSciences, Gent, Belgium*
[f]*DZNE, German Center for Neurodegenerative Diseases, Bonn, Germany*
[g]*CAESAR Research Center, Bonn, Germany*
[h]*Novartis Institutes for BioMedical Research, Neuroscience, Basel, Switzerland*

Abstract

Introduction: The inhibition of the β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) is a main therapeutic approach for the treatment of Alzheimer's disease (AD). We previously reported an age-related increase of tau protein in the cerebrospinal fluid (CSF) of amyloid β (Aβ) precursor protein (APP) transgenic mice.
Methods: APP transgenic mice were treated with a potent BACE1 inhibitor. CSF tau and CSF Aβ levels were assessed. A novel high-sensitivity tau sandwich immunoassay was developed.
Results: We demonstrate that long-term BACE1 inhibition prevents CSF tau increase both in early-depositing APP transgenic mice and APP transgenic mice with moderate Aβ pathology.
Discussion: Our results demonstrate that BACE1 inhibition not only reduces Aβ generation but also downstream AD pathophysiology. The tight correlation between Aβ aggregation in brain and CSF tau levels renders CSF tau a valuable marker to predict the effectiveness of BACE1 inhibitors in current clinical trials.
© 2016 the Alzheimer's Association. Published by Elsevier Inc. All rights reserved.

Keywords: Alzheimer's disease; Biomarker; CSF; Tau; BACE1 inhibitor; Treatment

1. Introduction

The microtubule-associated protein tau (tau) and amyloid β (Aβ) peptides are the main components of neurofibrillary tangles and amyloid plaques, respectively, that in turn are the pathologic hallmarks of Alzheimer's disease (AD). Moreover, tau and Aβ levels in the cerebrospinal fluid (CSF) are well-established biomarkers for AD progression [1]. While CSF Aβ—in particular the species ending with amino acid 42 ($Aβ_{42}$)—decreases with disease progression, total and phosphorylated tau increase. We previously showed that transgenic mouse models overexpressing human amyloid precursor protein (APP tg) replicate these AD CSF biomarker changes remarkably well [2]. While the CSF $Aβ_{42}$ decrease was presumably caused by sequestration of Aβ42 to growing amyloid plaques, the observation of increased (murine) tau in CSF of APP tg mice was more surprising because they lack neurofibrillary tangles and overt neuron loss [2].

A widely followed therapeutic concept in AD is the reduction of Aβ production through inhibition of β-site

*Corresponding authors. Tel.: +49 7071 29 81947; Fax: +49 7071 29 4521.
E-mail address: mathias.jucker@uni-tuebingen.de (M.J.), stephan.kaeser@uni-tuebingen.de (S.A.K.)

http://dx.doi.org/10.1016/j.jalz.2016.09.005
1552-5260/© 2016 the Alzheimer's Association. Published by Elsevier Inc. All rights reserved.

amyloid precursor protein-cleaving enzyme 1 (BACE1). A number of BACE1 inhibitors (BACEi) are currently being tested in clinical trials [3]. Their pharmacodynamic activity is primarily assessed by the reduction of Aβ levels in the CSF after short-term treatment. Long-term application is typically monitored by the suppression of amyloid plaque formation as shown in preclinical studies [4–6].

To test for downstream effects of BACE1 inhibition and to support the hypothesis of a causal relationship between CSF tau and β-amyloidosis, we treated APP tg mice with a potent BACEi (NB-360; Novartis, Basel, Switzerland) [6]. In addition, we developed a novel tau assay with very high sensitivity. Our results demonstrate that the administration of NB-360 for 6 months did not only block Aβ aggregation and deposition but also completely abolished the age-dependent increase of CSF tau in two different APP tg mouse lines. These findings are consistent with a linkage between amyloid increase and tau dysfunction and demonstrate downstream effects of BACE1 inhibition on tau. Thus, CSF tau levels cannot only be used to monitor AD progression but might also become an indispensible marker to predict the efficiency of BACEi in clinical trials.

2. Methods

2.1. APPPS1 mice

Male and female APPPS1 mice between 1.5 and 19 months of age and age-matched nontransgenic littermates were bred at the Hertie Institute for Clinical Brain Research (Tübingen, Germany). APPPS1 mice have been generated and are maintained on a C57BL/6J background and co-express human K670M/N671L–mutated APP and L166P-mutated presenilin 1 under the control of a neuron-specific Thy1 promoter element [7]. They develop first Aβ plaques after 6 weeks of age and show no gender effect. All mice were kept under specific pathogen-free conditions. The experimental procedures were undertaken in accordance with the veterinary office regulations of Baden-Württemberg (Germany) and approved by the local animal care and use committees.

2.2. APP23 mice

Male heterozygous APP23 mice (15- to 21.5-month-old) [8] were all bred at the Hertie Institute for Clinical Brain Research. APP23 mice express the K670M/N671L–mutated human APP under control of the neuron-specific Thy1 promoter element at about sevenfold over endogenous (murine) APP. The mice were initially generated on a B6D2 background but have since been bred with C57BL/6J mice over more than 20 generations. APP23 mice have been reported to develop plaques beginning at 6 to 8 months of age, and plaque development is faster in females than in males [8,9]. Therefore, only male animals were used for this study. All mice were kept under specific pathogen-free conditions. The experimental procedures were conducted in accordance with the veterinary office regulations of Baden-Württemberg (Germany) and were approved by the local animal care and use committees.

2.3. BACEi treatment of APP transgenic mice

For the long-term treatment, 1.5-month-old male and female APPPS1 as well as 15-month-old male APP23 mice were fed with food pellets containing the BACEi NB-360 [6,10] for 27 and 29 weeks, respectively. The selected dose (0.5 g inhibitor/kg food pellets) yielded brain exposure similar to the 100 μmol/kg oral dose previously described [6]. Average plasma and brain levels of NB-360 over 24 hours for this dose were 1.2 and 4.8 μM, respectively. Age-matched control mice received control pellets without the drug for the same time period. For the short-term experiment, male and female APPPS1 mice (2 months of age) and male APP23 mice (3 months of age) were fed with NB-360 or control pellets for one week. Food pellets were available ad libitum until the mice were killed. Animals were monitored weekly during the entire treatment time. There were no differences in food consumption and body weight between BACEi-treated mice and control mice.

2.4. CSF collection and tissue harvesting

For CSF collection, we adapted and refined protocols that were published previously [2,11]. Briefly, CSF was collected within a fixed time period of 4 hours to minimize circadian CSF Aβ variations [12]. The mouse was deeply anesthetized with ketamine (100 mg/mL) and xylazine (10 mg/mL) and kept on a heating pad during the whole procedure to avoid hypothermia. Surgery was done under a dissecting microscope. The skin was opened by an incision from the bregma to the occipital bone and fixed by means of a colibri retractor. Underlying tissue and neck muscles were separated bluntly and kept apart using a retraction system (Fine Science Tools) to expose the cisterna magna with the overlaying dura. For the collection, the mouse was kept in a horizontal position and the head was bent over (approximately 45°) but not fixed. The dura was carefully perforated with a 30-gauge needle, and the CSF was collected with a GELoader Tip (Eppendorf) shortened by about 2 cm at the tip. CSF samples were immediately centrifuged at 2000*g* for 10 minutes at room temperature, assessed macroscopically for blood contamination, aliquoted (5 μL) into polypropylene tubes (Eppendorf), snap-frozen in liquid nitrogen, and stored at −80°C until use. After CSF collection, mice were perfused with ice-cold sterile phosphate-buffered saline (PBS). The brain was removed and one hemisphere was snap-frozen in dry ice or liquid nitrogen and stored at −80°C until use. The other hemibrain was fixed in 4% paraformaldehyde with 0.1 M PBS, pH 7.6, for 48 hours at 4°C, immersed in 30% sucrose in PBS for an additional 48 hours at 4°C, snap-frozen in 2-methylbutane, and stored at −80°C.

Mass spectrometry analysis of CSF samples for hemoglobin was done in the past to refine the CSF collection technique.

However, in the present study, no influence of rarely detected small blood pellets on CSF tau levels was found.

2.5. *Biochemical analysis of brain tissue*

Forebrains (hemibrains without cerebellum) from APP23 and APPPS1 mice were homogenized at 10% (wt/vol) in homogenization buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, and protease/phosphatase inhibitor cocktail from Thermo Scientific) at 4°C using 7 mL lysing tubes with 2.8-mm ceramic beads and a tissue homogenizer (Precellys Bertin, Montigny-le-Bretonneux, France), twice at 5500 rpm for 10 seconds. The homogenized brain tissue was aliquoted and stored at −80°C until use. For Aβ measurements, the homogenates were extracted as follows: aliquots were thawed on ice, mixed 1:3.2 with cold formic acid (FA) (minimum 96% purity; Sigma, St. Louis, MO, USA), sonicated for 35 seconds at 4°C, and spun at 25,000*g* at 4°C for 1 hour. The supernatant was collected as the "FA-soluble fraction" and equilibrated (1:20) in neutralization buffer (1 M Tris base, 0.5 M Na_2HPO_4, 0.05% NaN_3).

2.6. *Aβ measurement in CSF and brain extracts*

$Aβ_{40}$ ($Aβ_{x–40}$) and $Aβ_{42}$ ($Aβ_{x–42}$) concentrations in CSF and brain extracts from APP transgenic mice were determined with an electrochemiluminescence-linked immunoassay using the V-PLEX Aβ Peptide Panel 1 (6E10) Kit (Meso Scale Discovery, Gaithersburg, MD, USA). CSF was diluted 1:14 in buffer (Diluent 35, Meso Scale Discovery) and analyzed according to the manufacturer's instructions. FA-soluble total brain extracts were diluted up to 1:300 in buffer (Diluent 35, Meso Scale Discovery) before measurement. Data analysis used MSD DISCOVERY WORKBENCH software 2.0. Internal reference samples were used as controls in every plate.

2.7. *Ultrasensitive sandwich immunoassay for tau measurements*

For measurement of murine tau, a bead-based immunoassay was developed using Single Molecule Array (Simoa) technology (Quanterix, Lexington, MA, USA). A large set of anti-tau antibodies had initially been checked for reactivity with murine tau, running 10% (wt/vol) brain homogenates from tau-deficient (B6.129X1-*Mapt*^{tm1Hnd}) [13], human tau transgenic (C57BL/6J-Tg[Thy1-MAPT*P301S]) [14], and wild-type (C57BL/6J) mice on Western blots (12% Bis-Tris PAGE; Thermo Scientific). Murine tau reactive antibodies were tested in a customized bead-based multiplex assay (Luminex, Austin, TX, USA) to select suitable antibody pairs. All antibodies were tested as capture and detection antibodies, and the most promising pairs (based on initial assessment of standard curve behavior and parallelism experiments) were transferred and further evaluated on the Simoa platform (Quanterix). The capture antibodies were immobilized on paramagnetic microparticles and the detection antibodies were biotinylated following standard procedures. Recombinant murine tau-430 was used as calibrator protein. ADx202 (ADx NeuroSciences, Gent, Belgium) for capturing and 77E9 (BioLegend, San Diego, CA, USA) for detection turned out to be the best performing antibody pair. Both antibodies bind to the proline-rich domain of tau, recognizing the region 218–225 (ADx202) and 185–195 (77E9) of the human sequence (or 207–214 and 174–184 of the murine sequence, respectively).

The assay was thoroughly validated before application in sample screening. Assessment of sensitivity and assay dynamic range revealed a lower limit of detection at 0.34 pg/mL and lower and upper limit of quantification at 0.69 and 600 pg/mL, respectively (repeated measurement in independent assays; criteria: recovery 75%–125%, CV $<$ 25%). CV of intra-assay precision was 6.9% (n = 20); inter-assay precision (low-, mid-, and high-concentration sample) showed a CV between 6.6% and 21.5% (triplicates over four independent assay runs). Parallelism analyses of pooled CSF from C57BL/6J wild-type mice showed 115%, 107%, 101%, and 77% recovery of endogenous tau when the final dilution was 20, 40, 80, and 160 times, respectively. (The average of all recalculated concentrations was considered as the 100% reference value.) Thus, murine CSF samples were measured in duplicates at a final dilution of 1:80. Specificity of the assay was confirmed with samples from tau null mice [13]. Spike-in recovery in native mouse samples was 121% to 132% throughout the assay range.

Samples were processed using a two-step assay protocol and measured on a HD-1 Analyzer (Quanterix). A weighted ($1/Y^2$) four-parametric logistics was used for curve fitting and calculation of tau protein content in mouse samples. All CSF tau measurements were done within a time window of 4 months. During this time, the assay performance was stable according to the standard curve and quality control (QC) samples (i.e., within a range of +/− 2 SDs).

Because of the limited amount of mouse CSF, diluted and spiked mouse blood plasma was used for internal assay QC. In each run, three QC samples at low, mid, and high concentrations of murine tau were measured in duplicates at a final dilution of 1:8. To exclude potential matrix effects on parallelism of blood samples, pooled EDTA plasma from C57BL/6J mice was assessed and revealed 101%, 97%, 100%, and 102% recovery of endogenous tau at the final dilution of 4, 8, 16, and 32 times, respectively.

2.8. *Histology and immunohistochemistry*

After freezing, fixed brains were cut into serial 25-μm-thick coronal sections using a freezing-sliding microtome. The sections were collected in tissue cryoprotection solution (35% ethylene glycol, 25% glycerol in PBS) and stained immunohistochemically according to previously published protocols using anti-Aβ polyclonal antibody CN5, a follow

up version of CN3 (1:2000) [2]. According to standard protocols, sections were counterstained with Congo red for amyloid detection.

2.9. *Stereological analysis of total Aβ plaque load*

Aβ plaque load was quantified in the neocortex of a representative section from an Aβ-immunostained set of every 12th systematically sampled, serial, coronal section. A microscope equipped with a motorized *x–y–z* stage coupled to a video microscopy system and the Stereo Investigator software (MicroBrightField, Inc., Williston, VT, USA) was used as previously described [15]. The person who performed the analysis was blinded to the treatment groups. The total Aβ load (percentage) was determined by calculating the areal fraction occupied by CN5-positive staining in two-dimensional sectors (20 × /0.45 objective).

2.10. *Statistical analysis*

JMP (version 11.2) and GraphPad Prism (version 6.0) were used for statistical analyses and graphics. Normal distribution was assessed using the Shapiro-Wilk test. Non–normally distributed variables were logarithmically transformed. In all cases, statistical significance was set at $P < .05$. All values are expressed as means ± standard errors of mean.

3. Results

3.1. *Age-related increase of tau in CSF of APPPS1 mice*

We have previously reported an age-dependent increase of CSF tau in APP tg mouse models, concomitant with the progression of cerebral β-amyloidosis [2]. However, the commercially available assay that we used originally was not sensitive enough to reliably measure murine CSF tau in non-tg and young (pre- or early-depositing) APP tg mice. Thus, we now developed a novel sandwich immunoassay using the Simoa technology, which improved sensitivity about 100-fold compared with other immunoassay platforms (for assay development and validation, see Supplementary Fig. 1).

CSF was collected for tau quantification from cohorts of APPPS1 mice and non-tg littermates between 1.5 and 19 months of age. CSF tau in APPPS1 mice revealed an age-dependent increase of up to sevenfold compared with the early-depositing phase and non-tg control mice (Fig. 1A). Our newly developed assay almost completely discriminated APPPS1 from non-tg control mice already at

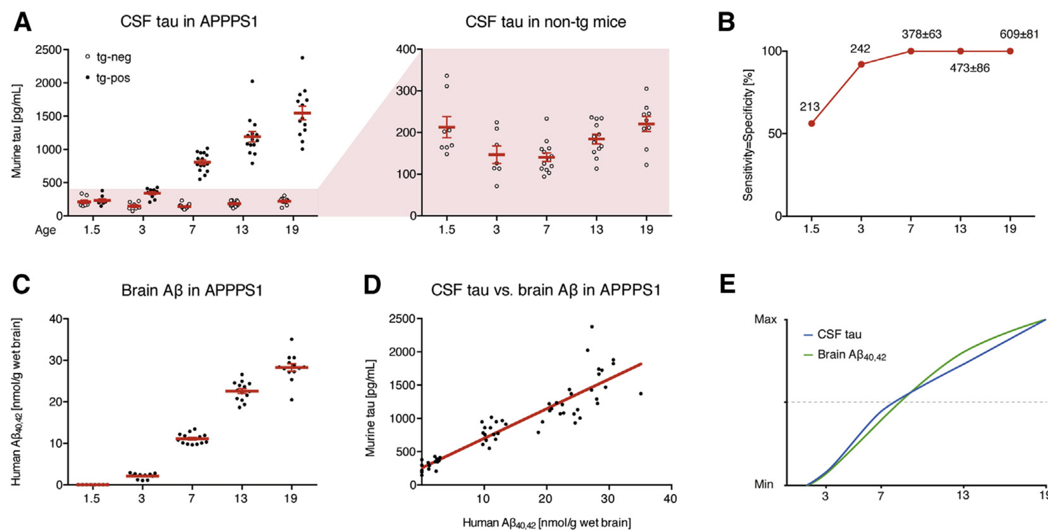


Fig. 1. Age-dependent increase of CSF tau and brain Aβ in APPPS1 mice. (A) CSF tau was measured in 1.5- to 19-month-old male APPPS1 mice and non-tg littermates (n = 7–15 per group) using a highly sensitive novel tau assay (Supplementary Fig. 1). Two-way analysis of variance (ANOVA) revealed significant age × genotype interaction [$F(4, 100) = 45.8$, $P < .001$]. Notably, the Tukey post hoc test revealed that CSF tau was already significantly increased in the 3-month-old APPPS1 mice compared with age-matched non-tg littermates ($P < .001$) and also compared with the 1.5-month-old APPPS1 mice ($P < .05$). Detailed analysis of the CSF tau levels in the non-tg mice (right panel) revealed a transient drop, which became significant at 7 months of age ($P < .05$). (B) Binary classification of APPPS1 and non-tg littermates based on CSF tau levels revealed a sensitivity and specificity of 55% and 92% for 1.5- and 3-month-old mice, respectively, and 100% for all the other age groups, assuming equal sensitivity and specificity. Arbitrary cutoff values (pg tau/mL CSF) were chosen as indicated for each age group to achieve best discrimination between the two genotypes. (C) Total brain Aβ levels (sum of $A\beta_{x-40}$ and $A\beta_{x-42}$, i.e., the species ending at amino acid 40 and 42) were measured in the same tg mice and revealed a robust increase with aging [ANOVA: $F(4, 56) = 2101$, $P < .001$]. The Tukey post hoc test indicated a significant difference among all age groups ($P < .001$), except between 13 and 19 months. (D) The relationship between CSF tau (A) and brain Aβ concentration (C) is best described by a linear regression ($r^2 = 0.84$, $P < .001$) indicating a strong positive correlation (Spearman rank correlation test: $\rho = 0.94$, $P < .001$). (E) The percentage increase of total brain Aβ (green) and CSF tau (blue) in APPPS1 mice shows a very similar profile with aging. The two curves are based on the mean values of panels (A) and (C). Data in (A) and (C) are presented as group means ± standard errors of mean.

3 months of age (with 92% specificity and sensitivity); in the older age groups, CSF tau concentrations allowed an unambiguous segregation of APPPS1 mice and non-tg littermates (Fig. 1B).

As expected, Aβ levels in the brains of APPPS1 mice revealed a robust age-dependent increase (Fig. 1C) closely paralleling the CSF tau increase (Fig. 1E). A significant positive correlation between brain Aβ and CSF tau was apparent, indicating that the CSF tau concentration reflects the Aβ pathology (Fig. 1D).

3.2. Aβ pathology–associated tau increase in CSF is impeded by prolonged BACE1 inhibition

To more directly demonstrate a causal relationship between the stage of Aβ pathology in brain and tau in CSF, we tested if suppression of Aβ pathology would affect CSF tau concentrations. Therefore, we treated 1.5-month-old APPPS1 mice for 6 months with the BACEi NB-360 [6] while littermates were fed with control pellets (Fig. 2A). Aβ immunohistochemistry revealed a 71% lower cortical Aβ plaque load in the brains of NB-360–treated mice compared with controls (Fig. 2B). Consistently, brain Aβ concentrations of NB-360–treated mice revealed a very strong reduction in comparison to mice fed with control food pellets and were similar to those of the 1.5-month-old mice (Fig. 2C). As anticipated from previous work [2], $A\beta_{42}$ significantly decreased with age in CSF of APPPS1 mice. However, NB-360 treatment did not further decrease CSF $A\beta_{42}$ (Fig. 2D).

When CSF tau was analyzed using our newly developed high-sensitivity tau sandwich immunoassay, we found the

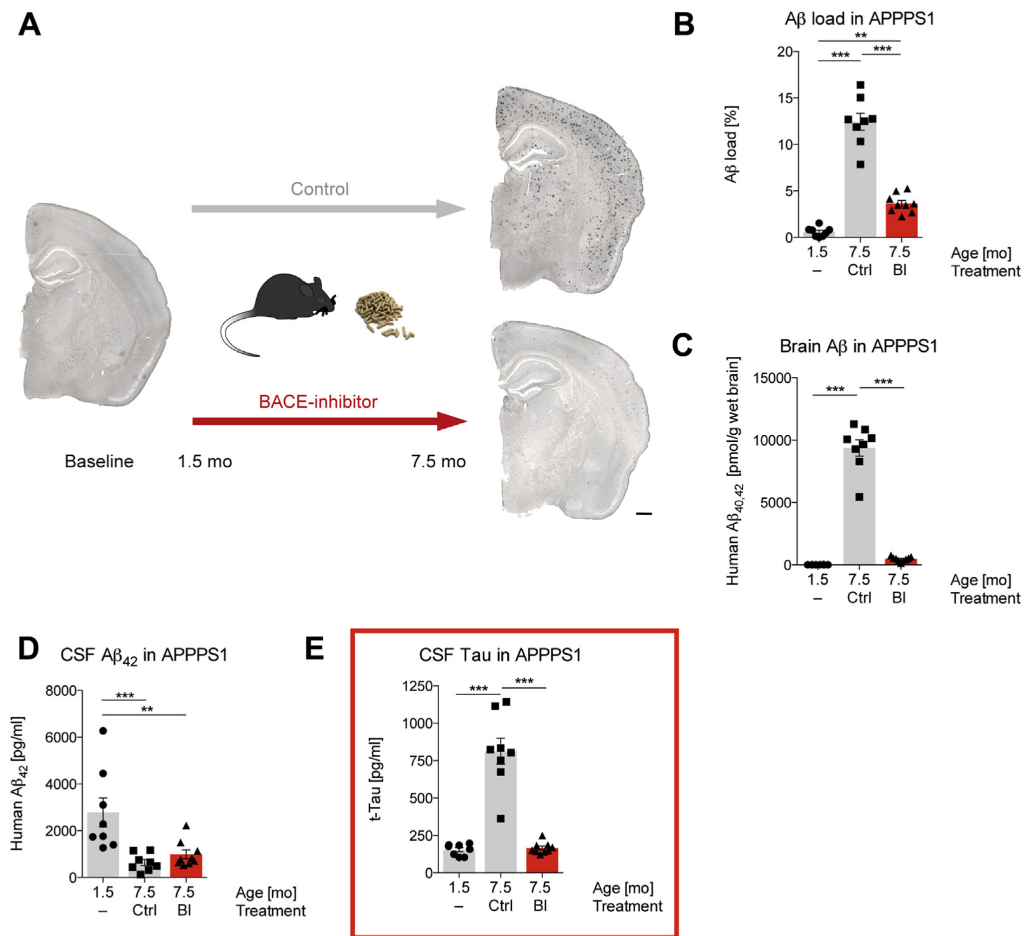


Fig. 2. BACE1 inhibition in APPPS1 mice prevents tau increase in CSF. Male and female APPPS1 mice (1.5–7.5 months of age; 8–9 mice per group, gender equally distributed, i.e., 4–5 males/females per group) were either fed with food pellets containing a BACE1 inhibitor NB-360 (BI) or control pellets (Ctrl) for 6 months (27 weeks). (A) Schematic overview of experimental design. Shown are representative cortical brain sections for each group (scale bar is 500 μm). (B, C) Stereological quantification of neocortical Aβ immunostaining (Aβ load) and measurement of Aβ levels in brain exhibited significant increases with age, which could largely be blocked by BACE1 inhibition [analysis of variance (ANOVA) $F(2, 22) = 114.6$ and 209.5, respectively, $P < .001$; the Tukey post hoc test $**P < .01$, $***P < .001$]. (D) CSF $A\beta_{42}$ in the 7.5-month-old APPPS1 mice was significantly decreased compared with 1.5-month-old mice with no further decrease after BACE1 inhibition [ANOVA: $F(2, 22) = 13.58$, $P = .0001$; the Tukey post hoc test $**P < .01$, $***P < .001$]. A similar result was found for $A\beta_{40}$ (not shown). (E) Tau in CSF revealed a significant age-dependent increase in control mice, which could be prevented by the application of a BACEi [ANOVA: $F(2, 22) = 57.01$, $P < .001$; the Tukey post hoc $***P < .001$]. No gender effect was found in any of the measurements and males and females were combined. All data are represented as group means ± standard errors of mean.

expected increase from 1.5 to 7.5 months of age in the APPPS1 mice fed with the control pellets. In striking contrast, CSF tau concentrations remained at baseline level (levels at the time the treatment was started) in the NB-360–treated APPPS1 mice (Fig. 2E).

To confirm these observations in a second mouse model and to assess the effect of BACE1 inhibition in a situation of preexisting Aβ pathology, 15-month-old APP23 mice were treated with the same BACEi until the age of 21.5 months (Fig. 3A). Similar to APPPS1 mice, the increase in Aβ plaque load and brain Aβ levels could be almost completely impeded (Fig. 3B and 3C). Interestingly, in this mouse line, CSF $A\beta_{42}$ was significantly reduced in NB-360–treated animals compared with baseline and control mice (Fig. 3D), and remarkably, NB-360 fully prevented the increase of CSF tau levels also in APP23 mice (Fig. 3E).

3.3. CSF tau levels stay unaltered after short-term BACE1 inhibition

To test the possibility that the reduction of soluble Aβ or the BACEi itself could directly lead to a decline of CSF tau, that is, in the absence of changes in Aβ deposition, young APPPS1 and APP23 mice were treated with NB-360–containing food pellets for one week. After this acute treatment, CSF $A\beta_{42}$ levels revealed a significant reduction demonstrating the pharmacodynamic effect of the drug (Fig. 4A and 4C). However, CSF tau levels remained unaffected by the short-term treatment in both APPPS1 and APP23 mice (Fig. 4B and 4D).

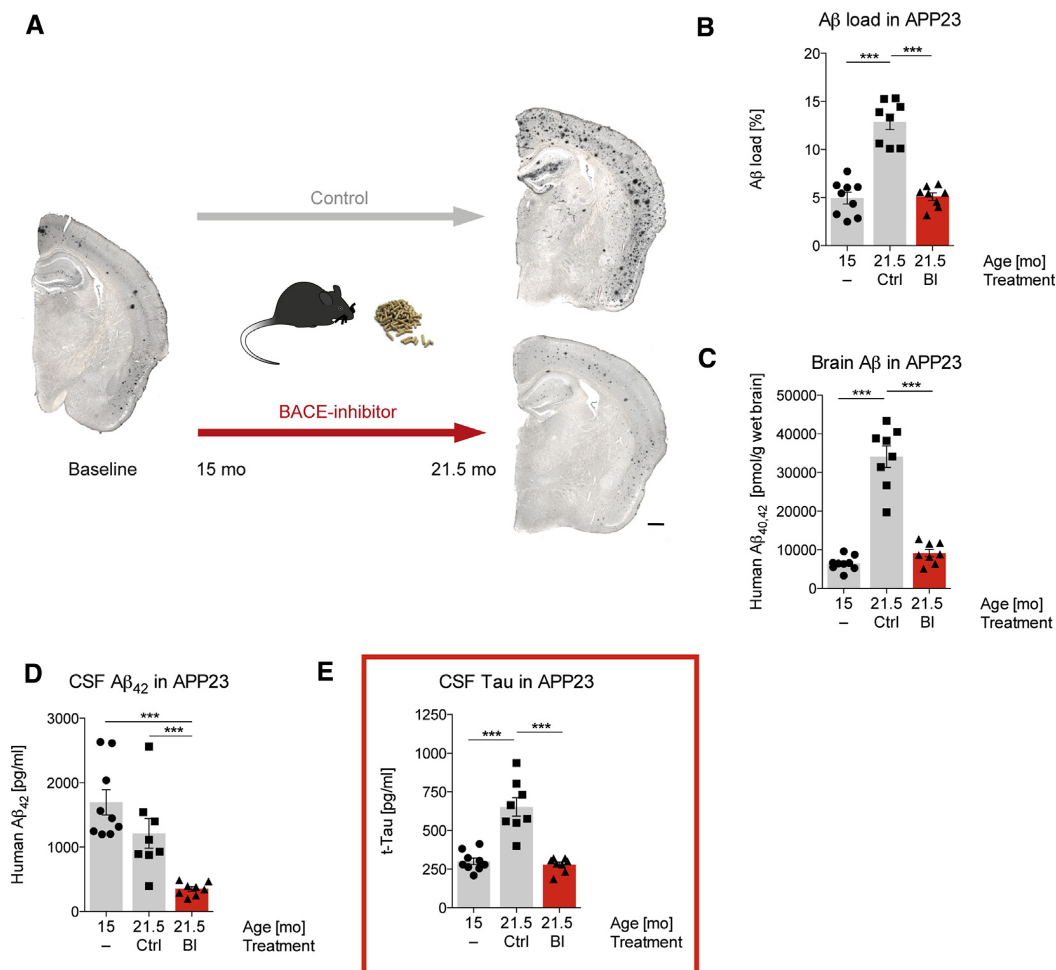


Fig. 3. BACE1 inhibition in APP23 mice prevents tau increase in CSF. Male APP23 mice (15–21.5 months of age; 8–9 mice per group) were either fed with food pellets containing a BACE1 inhibitor NB-360 (BI) or control pellets (Ctrl) for 6.5 months (29 weeks). (A) Schematic overview of experimental design including representative cortical brain sections for each group (scale bar is 500 μm). (B, C) Stereological quantification of cortical Aβ immunostaining (Aβ load) and measurements of Aβ levels in brain exhibited significant age-dependent increases, which could be completely blocked by BACE1 inhibition [ANOVA: $F(2, 22) = 52.61$ and 81.58, $P < .0001$; the Tukey post hoc test ***$P < .001$]. (D) CSF $A\beta_{42}$ in the NB-360–treated mice was significantly decreased compared with 15-month-old mice and age-matched controls [ANOVA: $F(2, 22) = 33.81$, $P < .0001$; the Tukey post hoc test ***$P < .001$]. A similar result was found for $A\beta_{40}$ (not shown). (E) CSF tau exhibited a significant age-dependent increase in control mice, which could be suppressed by the application of BACEi [ANOVA: $F(2, 22) = 31.85$, $P < .0001$; the Tukey post hoc ***$P < .001$]. All data are represented as group means ± standard errors of mean.

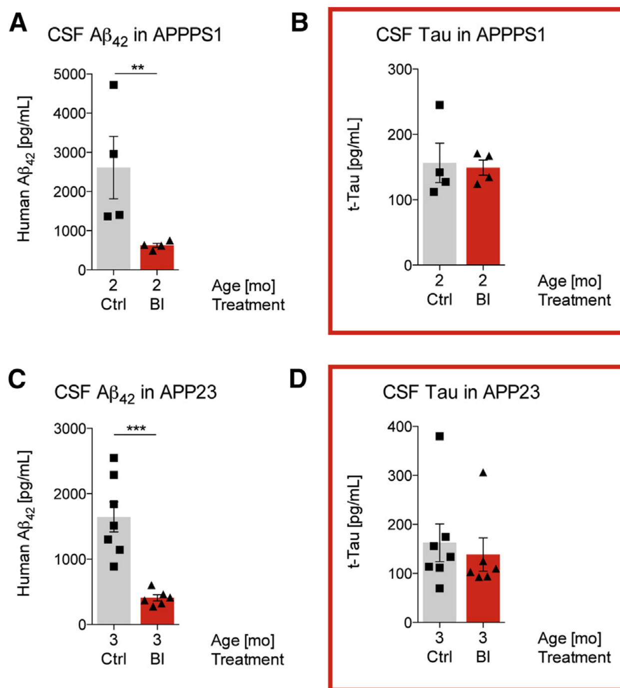


Fig. 4. Short-term BACE1 inhibition does not affect CSF tau levels. Male and female APPPS1 mice (2 months of age; 4 mice per group; gender equally distributed, i.e., 2 males/females per group) and male APP23 mice (3 months of age; 6–7 mice per group) were either fed with food pellets containing a BACE1 inhibitor NB-360 (BI) or control pellets (Ctrl) for one week. (A) In APPPS1 mice, CSF Aβ_{42} was reduced by 76% after NB-360 application (t-test $t_6 = 4.134$, ** $P < .01$). (B) Treatment of APPPS1 mice with a NB-360 for one week did not reveal significant changes in CSF tau levels (t-test $t_6 = 0.051$, $P > .05$). (C) In APP23 mice, CSF Aβ_{42} was also significantly reduced after NB-360 treatment (t-test $t_{11} = 7.300$, *** $P < .001$). (D) Again no significant difference could be detected after BACE1 inhibition for 1 week (t-test $t_{11} = 0.497$, $P > .05$). All data are represented as group means ± standard errors of mean.

4. Discussion

Tau is, besides Aβ, the most thoroughly investigated and validated CSF biomarker for AD [16–20] and hence has already been implemented as a monitoring marker for clinical trials [1]. Our previous observation of CSF biomarker changes in aging APP tg mice suggests that tangle formation and global neuron loss are not required to induce a substantial tau rise in the CSF [2]. Instead, presumed neuronal injury below the threshold of histological detection caused by progressing cerebral Aβ deposition was suggested to trigger an increased release of tau from nerve cells, which ultimately leads to elevated CSF tau levels. However, proving such a mechanistic link between Aβ deposition and CSF tau changes requires a direct interference with Aβ aggregation and deposition.

To this end, we now treated two different APP tg mouse models with a potent BACEi. Indeed, reducing Aβ production over 6 months not only attenuated Aβ deposition but also affected CSF tau levels. In both mouse models, long-term BACE1 inhibition kept CSF tau at baseline levels, whereas control mice revealed the expected age-related increase. Thus, CSF tau levels mirrored the histological plaque load and total brain Aβ levels after BACEi treatment.

In further support of a mechanistic link between CSF tau levels and brain Aβ deposition is the observation that BACE1 inhibition kept CSF tau at baseline but did not reduce further to levels of wild type mice. This observation indicates that the preexisting Aβ plaques, which were obviously not cleared by long-term BACEi treatment, were responsible for the elevated CSF tau levels. This is consistent with the observation that short-term BACE1 inhibition and thus soluble Aβ had no effect on CSF tau levels (Fig. 4). Although NB-360 is known to inhibit both BACE1 and also BACE2 [6] with several substrates known for these secretases [21], it seems unlikely that a substrate other than APP would be responsible for the CSF tau changes given that short-term BACEi treatment had no effect on CSF tau levels.

While our results demonstrate that CSF tau levels reflect brain Aβ deposits, they also suggest that CSF Aβ_{42} may be a misleading readout for brain Aβ deposition in the context of BACE1 inhibition because CSF Aβ levels are affected by two opposite mechanisms. On the one hand, BACE1 inhibition directly reduces the generation of Aβ and thus lowers CSF Aβ levels, which was also evident in the short-term experiments in both mouse models (Fig. 4). On the other hand, long-term BACE1 inhibition attenuates Aβ deposition that in turn leads to reduced Aβ sequestration to plaques and hence maintains CSF Aβ_{42} at higher levels. These antagonistic mechanisms may also explain why in APP23, but not in APPPS1 mice, CSF Aβ_{42} concentrations were considerably lower after BACE1 treatment compared with mice that were fed with the control food pellets (Figs. 2D and 3D). Differences in CSF Aβ between APP23 and APPPS1 mice after BACE1 inhibition may also be related to differences in the Aβ species generated in the two models, which may affect the overall turnover of brain Aβ, its aggregation/sequestration to plaques, and the type of amyloid deposited. Overall, these observations and results highlight that CSF Aβ_{42} may be a misleading readout in the context of BACE1 inhibition as it is confounded by the stage (and type) of amyloid deposition at the start of treatment. While there are no data published regarding CSF tau changes in clinical trials on BACE1 inhibition, it has been reported that immunotherapy directed against Aβ decreased CSF tau levels in AD patients [22,23]. However, as no significant difference in CSF Aβ_{42} was found, the interpretation of decreased tau CSF tau levels remained unclear at the time.

In conclusion, the present study demonstrates a mechanistic link between brain Aβ deposition and CSF tau, and thus, CSF tau may present an important readout of Aβ deposition in mouse models and likely in AD. Furthermore, the effect of BACE1 inhibition is not restricted to Aβ and amyloid plaque deposition but can positively influence other downstream pathologic events underscoring the high potential of this target for the treatment of AD.

Acknowledgments

The authors thank U. Obermüller, M. Lambert, A. Bühler, J. Odenthal, and S. Fritschi for their experimental help. M. Eichner's (Institute for Biometry, University of Tübingen, Germany) statistical assistance is greatly appreciated. They also thank H. Dawson (Durham, NC, USA) for providing tau-deficient mice and A. Jeromin (Lexington, MA, USA) and Dave Holtzman (St. Louis, MI, USA) for expert technical advice.
Authors' contribution: J.S., L.H., J.C.G., and S.A.K. performed the experimental work. J.S. and S.A.K carried out the statistical analysis. T.O.J., H.V., E.S., U.N., D.R.S., E.-M.M., and M.S. provided crucial research reagents. J.S., M.S., M.J., and S.A.K. designed the study and with the help of all other authors prepared the manuscript.
Conflict of interest: U.N., D.R.S., and M.S. are or were employees and shareholders of Novartis Pharma AG, Basel Switzerland. H.V. and E.S. are employees of ADx NeuroSciences. The remaining authors declare no competing interests.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2016.09.005.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using PubMed. While there have been several publications describing CSF tau changes in APP transgenic mice and also direct effects of BACE1 inhibitors such as the reduction of Aβ levels in CSF and brain, there are no data published yet regarding downstream effects of BACE1 inhibition and a causal relationship between CSF tau and β-amyloidosis. The relevant citations are appropriately cited.

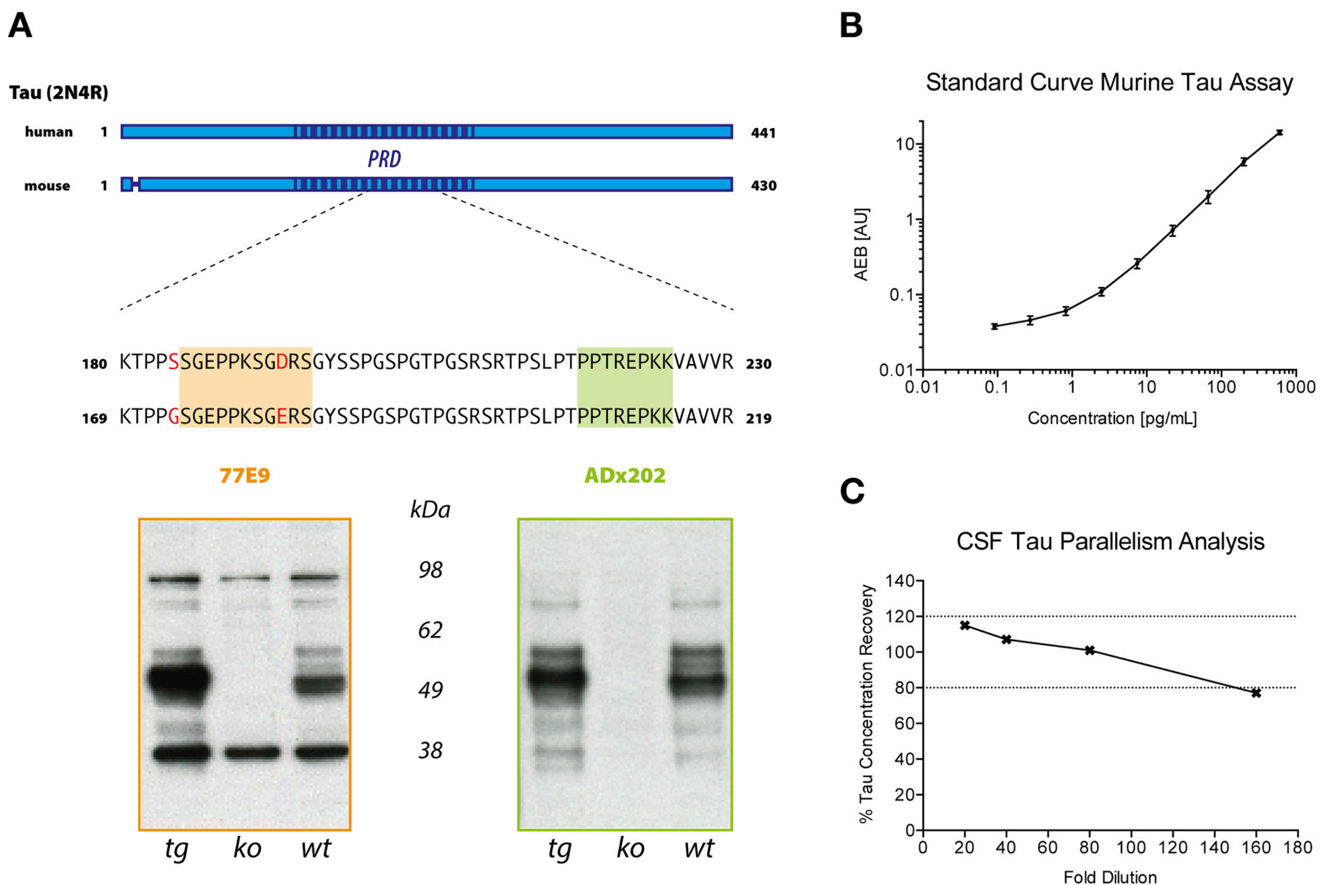
2. Interpretation: Our findings led to a validated hypothesis describing CSF tau changes on BACE1 inhibition. This hypothesis renders CSF tau a valuable marker to predict the effectiveness of BACE1 inhibitors in current clinical trials.

3. Future directions: The manuscript proposes a framework for the generation of new hypotheses and the conduct of additional studies. Examples include further understanding (a) the mechanistic link between CSF tau levels and brain Aβ deposition and (b) potential other downstream pathological events caused by BACE1 inhibition.

References

[1] Jack CR, Holtzman DM. Biomarker modeling of Alzheimer's disease. Neuron 2013;80:1347–58.
[2] Maia LF, Kaeser SA, Reichwald J, Hruscha M, Martus P, Staufenbiel M, et al. Changes in Amyloid-β and Tau in the Cerebrospinal Fluid of Transgenic Mice Overexpressing Amyloid Precursor Protein. Sci Transl Med 2013;5:194re2.
[3] Mullard A. BACE race gains steam. Nat Rev Drug Discov 2016; 15:151.
[4] Fukumoto H, Takahashi H, Tarui N, Matsui J, Tomita T, Hirode M, et al. A noncompetitive BACE1 inhibitor TAK-070 ameliorates Abeta pathology and behavioral deficits in a mouse model of Alzheimer's disease. J Neurosci 2010;30:11157–66.
[5] Jacobsen H, Ozmen L, Caruso A, Narquizian R, Hilpert H, Jacobsen B, et al. Combined treatment with a BACE inhibitor and anti-Aβ antibody gantenerumab enhances amyloid reduction in APPLondon mice. J Neurosci 2014;34:11621–30.
[6] Neumann U, Rueeger H, Machauer R, Veenstra SJ, Lueoend RM, Tintelnot-Blomley M, et al. A novel BACE inhibitor NB-360 shows a superior pharmacological profile and robust reduction of amyloid-β and neuroinflammation in APP transgenic mice. Mol Neurodegener 2015; 10:44.
[7] Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, et al. Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. EMBO Rep 2006; 7:940–6.
[8] Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci U S A 1997;94:13287–92.
[9] Eisele YS, Obermüller U, Heilbronner G, Baumann F, Kaeser SA, Wolburg H, et al. Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. Science 2010;330:980–2.
[10] Shimshek DR, Jacobson LH, Kolly C, Zamurovic N, Balavenkatraman KK, Morawiec L, et al. Pharmacological BACE1 and BACE2 inhibition induces hair depigmentation by inhibiting PMEL17 processing in mice. Sci Rep 2016;6:21917.
[11] Liu L, Herukka SK, Minkeviciene R, van Groen T, Tanila H. Longitudinal observation on CSF Abeta42 levels in young to middle-aged amyloid precursor protein/presenilin-1 doubly transgenic mice. Neurobiol Dis 2004;17:516–23.
[12] Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science 2009;326:1005–7.
[13] Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. J Cell Sci 2001;114:1179–87.
[14] Allen B, Ingram E, Takao M, Smith MJ, Jakes R, Virdee K, et al. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. J Neurosci 2002; 22:9340–51.
[15] Bondolfi L, Calhoun M, Ermini F, Kuhn HG, Wiederhold KH, Walker L, et al. Amyloid-associated neuron loss and gliogenesis in the neocortex of amyloid precursor protein transgenic mice. J Neurosci 2002;22:515–22.
[16] Andreasen N, Vanmechelen E, Van de Voorde A, Davidsson P, Hesse C, Tarvonen S, et al. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow up study. J Neurol Neurosurg Psychiatr 1998;64:298–305.
[17] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 2009; 65:403–13.
[18] Tapiola T, Alafuzoff I, Herukka SK, Parkkinen L, Hartikainen P, Soininen H, et al. Cerebrospinal fluid {beta}-amyloid 42 and tau

proteins as biomarkers of Alzheimer-type pathologic changes in the brain. Arch Neurol 2009;66:382–9.
[19] Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol 2010;9:1118–27.
[20] Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367:795–804.
[21] Vassar R, Kuhn PH, Haass C, Kennedy ME, Rajendran L, Wong PC, et al. Function, therapeutic potential and cell biology of BACE proteases: current status and future prospects. J Neurochem 2014;130:4–28.
[22] Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 2005;64:1553–62.
[23] Blennow K, Zetterberg H, Rinne JO, Salloway S, Wei J, Black R, et al. Effect of immunotherapy with bapineuzumab on cerebrospinal fluid biomarker levels in patients with mild to moderate Alzheimer disease. Arch Neurol 2012;69:1002–10.



Supplemental Fig. 1. Tau assay development and validation. (A) From a set of different antitau antibodies, the most sensitive pair has been chosen for the full development of an assay on the Simoa platform. Both antibodies ADx202 (green) for capturing and 77E9 (orange) for detection bind to the proline-rich domain of tau, recognizing the epitope 218–225 and 185–195 (human sequence), respectively. The human (top) and the mouse (bottom) sequence are shown and differences indicated in red. Western blot analyses (12% Bis-Tris PAGE) using 10% (wt/vol) brain homogenates from a TauP301S (tg), Tau null (ko), and C57BL6/J wild-type (WT) mouse revealed some unspecific bands (at ~98 and ~38 kDa) with 77E9 but not with ADx202 (lower panel). (B) Average calibration curve based on four individual runs using recombinant murine tau on the Simoa technology. Shown are the mean signals ± SD, indicated as average enzymes per bead (AEBs), within a concentration range from 0.091 to 600 pg/mL. (C) Linear dilution analyses of pooled CSF from C57BL/6J wild-type mice showed 115%, 107%, 101%, and 77% recovery of endogenous tau when diluted 20, 40, 80, and 160 times, respectively (the average of all recalculated concentrations was considered as the 100% reference value). Note: even a 160-fold dilution yielded a recovery of 77% (CV = 23.7%) although tau concentration of 0,66 pg/mL was slightly below the lower limit of quantification (0.69 pg/mL).

3.3 Persistence of Aβ seeds in APP null mouse brain

Lan Ye*, Sarah K. Fritschi*, **Juliane Schelle**, Ulrike Obermüller, Karoline Degenhardt, Stephan A. Kaeser, Yvonne S. Eisele, Lary C. Walker, Frank Baumann, Matthias Staufenbiel, and Mathias Jucker *contributed equally

Published in:

Nature neuroscience 2015, 18(11), pp.1559–1561

Persistence of Aβ seeds in APP null mouse brain

Lan Ye[1–3,5], Sarah K Fritschi[1,2,5], Juliane Schelle[1–3], Ulrike Obermüller[1,2], Karoline Degenhardt[1–3], Stephan A Kaeser[1,2], Yvonne S Eisele[1,2], Lary C Walker[4], Frank Baumann[1,2], Matthias Staufenbiel[1,2] & Mathias Jucker[1,2]

Cerebral β-amyloidosis is induced by inoculation of Aβ seeds into APP transgenic mice, but not into *App*$^{-/-}$ (APP null) mice. We found that brain extracts from APP null mice that had been inoculated with Aβ seeds up to 6 months previously still induced β-amyloidosis in APP transgenic hosts following secondary transmission. Thus, Aβ seeds can persist in the brain for months, and they regain propagative and pathogenic activity in the presence of host Aβ.

© 2015 Nature America, Inc. All rights reserved.

In cerebral β-amyloidoses such as Alzheimer's disease (AD), misfolded Aβ proteins can form proteopathic seeds that structurally corrupt similar proteins, which in turn further perpetuate the seeding cascade[1]. Aβ seeds are thus self-propagating agents that are responsible for the initiation, progression and spreading of amyloidosis in the brain. Indeed, when young Aβ-precursor protein (APP) transgenic mice are inoculated with Aβ-rich brain extracts from AD patients or aged APP transgenic mice, cerebral β-amyloidosis is induced 4–8 months post-inoculation[2,3]. The seeding agent in the brain extracts has been identified as Aβ aggregates ranging in size from small, soluble proteinase K (PK)-sensitive Aβ assemblies to larger PK-resistant aggregates[4]. Aβ seeds are resistant to *ex vivo* inactivation by such treatments as boiling, drying and exposure to formaldehyde[2,5,6], and thus share key characteristics with infectious prions[7–9].

To shed light on the *in vivo* stability, durability and propagation of Aβ seeds, we inoculated APP null (*App*$^{-/-}$), wild-type (WT) and APP23 transgenic mice with brain extracts of defined Aβ seeding activity (Online Methods). At 1, 7 and 30 d post unilateral inoculation (dpi) into the hippocampus, the injected and contralateral hippocampi were dissected and assessed for the presence of exogenous human Aβ by immunoblotting and immunoassay (**Fig. 1a**,**b**). At 1 dpi, the injected human Aβ was present in the injected hippocampi of all three murine strains at similar levels. Human Aβ decreased at 7 dpi and was below the level of detection at 30 dpi in *App*$^{-/-}$ and WT mice with apparently similar kinetics. In contrast, human Aβ in APP23 mice increased steadily over the 30-d incubation period (**Fig. 1a**,**b**), indicative of a nascent seeding response.

To assess the seeding activity of hippocampal extracts from *App*$^{-/-}$ and APP23 mice that had been previously inoculated with Aβ seeds, we carried out second passage inoculations into young APP23 host mice (**Fig. 1c**–**e**). After a 4-month incubation period, the 1 dpi hippocampal extracts from both *App*$^{-/-}$ and APP23 donor mice had induced amyloid deposition in APP23 host mice that was similar to the induction by 30-dpi hippocampal extracts from the APP23 mice (**Fig. 1c**,**d**). Notably, amyloid induction was still observed for the *App*$^{-/-}$ 30-dpi hippocampal extracts, even though it was minimal after 4 months (**Fig. 1c**,**d**).

We previously found that the seeding of Aβ lesions is time dependent[2,4]. Sensitivity as measured in seeding units increased tenfold when the incubation period was prolonged from 4–6 months, and again tenfold following prolongation from 6–7 months (F.B., M.S. and M.J., unpublished observations). Indeed, when the incubation time of the second passage inoculation was prolonged to 8 months, substantial amyloid induction also was observed for the *App*$^{-/-}$ 30-dpi hippocampal extracts, suggesting the persistence of some Aβ seeds for 30 d in the absence of host-derived Aβ (**Fig. 1c**,**e**).

Our findings were unexpected given that brain extracts from prion-inoculated *Prnp* null mice no longer reveal infectivity 2 weeks post-inoculation using observation periods of up to 16 months following secondary transmission[10–12]. We therefore repeated the experiment and included extended primary incubation periods in the *App*$^{-/-}$ donor mice of 60 and 180 d, as well as additional control inoculations of WT brain extracts (**Fig. 2**). Consistent with the first experiment, human Aβ levels were below detection in the extract-injected hippocampi of *App*$^{-/-}$ mice 30, 60 and 180 dpi using immunoblotting and electrochemiluminescence-linked (ECL) immunoassay. However, using ultra-sensitive bead-based single-molecule array Simoa technology, we were able to detect residual human Aβ even at 180 dpi (**Supplementary Fig. 1**). Second passage inoculation with an 8-month incubation time again revealed robust induction of β-amyloidosis in APP23 hosts by the *App*$^{-/-}$ 30-dpi hippocampal extracts, whereas no induction was observed with the WT extracts (**Fig. 2a**,**b**). Amyloid induction was also observed with the *App*$^{-/-}$ 60-dpi hippocampal extract, albeit somewhat less than the *App*$^{-/-}$ 30-dpi extracts. Notably, even 180 d after the introduction of Aβ seeds, some amyloid induction was instigated by hippocampal extracts from *App*$^{-/-}$ donor mice (**Fig. 2a**–**c**).

In all cases, the induced lesions were partly Congo red positive. All Congo red–positive amyloid (independent of whether the primary incubation occurred in *App*$^{-/-}$ or APP23 hosts) was surrounded by activated microglia and dystrophic neuritic structures (**Supplementary Fig. 2**), as previously reported in the brains of AD patients and APP transgenic mice[13–16]. Notably, cerebral amyloid angiopathy (CAA) was always induced together with parenchymal amyloid (**Supplementary Fig. 3**) and appeared in non-injected brain regions, most notably in the thalamus, similar to the pattern previously observed after primary inoculations[2]. The induced CAA was often associated with microbleeds (**Supplementary Fig. 3**), supporting the

[1]Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany. [2]German Center for Neurodegenerative Diseases (DZNE), Tübingen, Tübingen, Germany. [3]Graduate School of Cellular and Molecular Neuroscience, University of Tübingen, Tübingen, Germany. [4]Yerkes National Primate Research Center and Department of Neurology, Emory University, Atlanta, Georgia, USA. [5]These authors contributed equally to this work. Correspondence should be addressed to M.J. (mathias.jucker@uni-tuebingen.de).

Received 16 July; accepted 25 August; published online 9 September 2015; doi:10.1038/nn.4117

conclusion that the exogenous Aβ seeds retained their pathogenicity in *App*$^{-/-}$ donor mice, even in the absence of replication.

Our results demonstrate a marked longevity of Aβ seeding activity in the living brain despite generally efficacious Aβ clearance mechanisms. The persistence of Aβ seeding activity for at least 180 d in the brain in the absence of host-derived Aβ (and thus replication) substantially exceeds the reported longevity of bioactive prions, at least with regard to prion infectivity in mice[10–12,17]. In conjunction with previous studies demonstrating the resilience of Aβ seeds[2–6], this observation raises the possibility that a similar phenomenon transpires in AD and other human disorders. AD *per se* has not been shown to be infectious under normal circumstances. However, cerebral Aβ deposition is inducible by injection of exogenous Aβ seeds in a prion-like manner in transgenic mice and nonhuman primates[1,9]. In this regard, it may be prudent

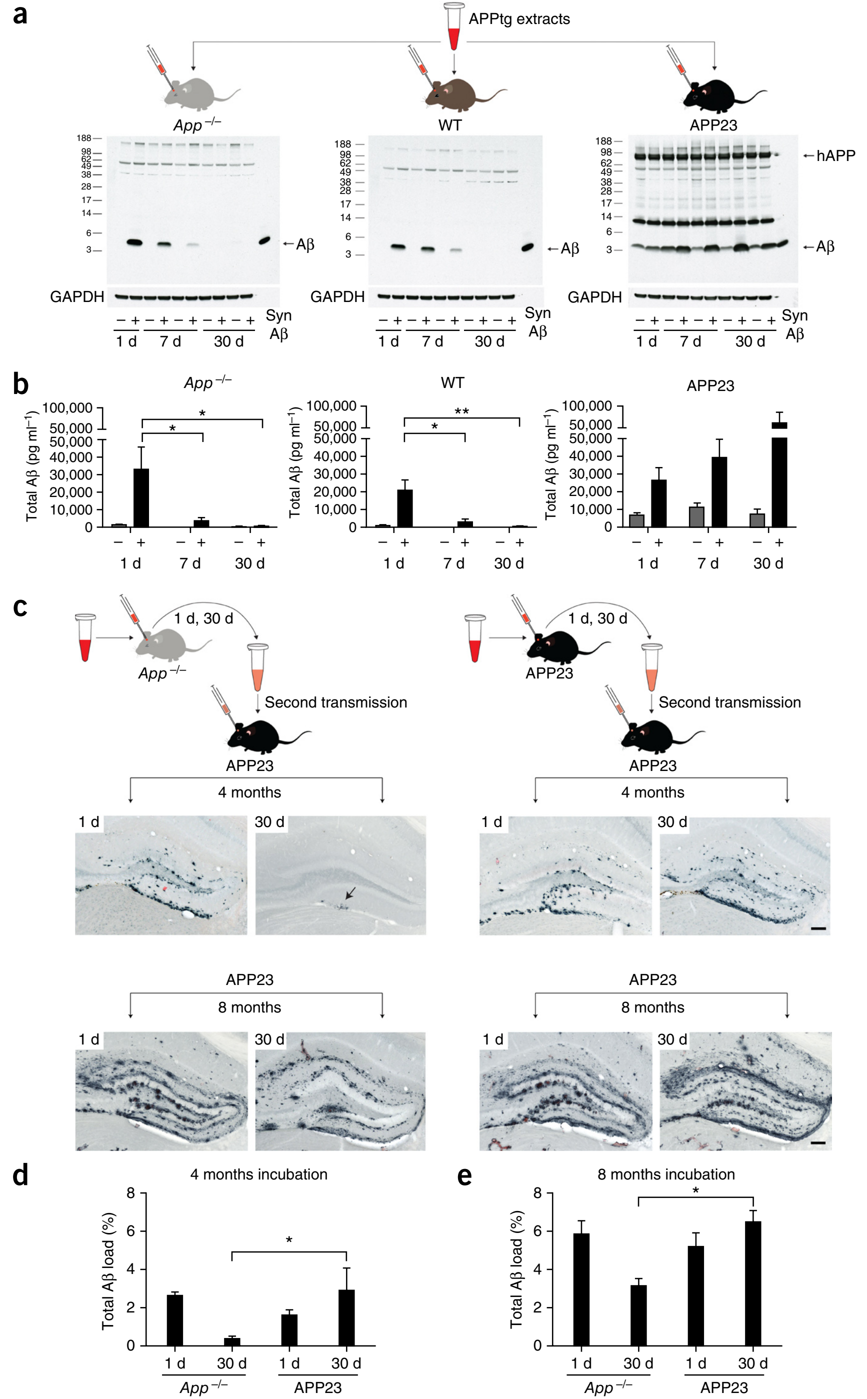


Figure 1 Aβ seed persistence in various murine hosts. Aβ seed–containing APP23 brain extract (APPtg extract) was injected unilaterally into the hippocampus of 3-month-old male and female *App*$^{-/-}$, WT and APP23 mice. Injected and contralateral control hippocampi were biochemically analyzed 1, 7 and 30 dpi. (**a**) Immunoblots with an antibody specific to human Aβ. Although the injected Aβ could be detected 1 dpi in *App*$^{-/-}$, WT and APP23 mice, the injected Aβ was no longer detectable at 30 dpi in *App*$^{-/-}$ or WT mice (left and middle). The injected hippocampus is marked with + and the uninjected side with –. In contrast, human Aβ was still robustly increased compared with the uninjected side in APP23 mice at 30 dpi (right). Shown is one representative mouse for 1 dpi and two mice for 7 and 30 dpi, respectively. Bottom, GAPDH as a loading control. Synthetic (syn) Aβ (0.23 ng) was loaded as a positive control. (**b**) Human Aβ concentration (pg ml^{-1}) in the extracted hippocampus using ECL-linked immunoassay. Although human Aβ in the inoculated hippocampus of *App*$^{-/-}$ and WT mice was significantly decreased to background (–) at 30 dpi (left and middle), APP23 mice did not show such a reduction (right) ($n = 6$, 4–5 and 7–8 mice per time point for *App*$^{-/-}$, WT and APP23 mice, respectively; males and females were equally distributed in each group). One-way ANOVA for the injected hippocampi revealed $F(2,15) = 5.603$, $P = 0.015$ (*App*$^{-/-}$); $F(2,10) = 8.484$, $P = 0.007$ (WT); $F(2,20) = 1.162$, $P = 0.333$ (APP23). Bonferroni *post hoc* test for pairwise comparisons, $^*P < 0.05$, $^{**}P < 0.01$. (**c**) Secondary transmission of injected hippocampal extracts (hippocampi were pooled) from *App*$^{-/-}$ and APP23 mice (1 and 30 dpi) into the hippocampus of young (3 month old) pre-depositing male APP23 mice. Brains were analyzed for Aβ deposition 4 and 8 months later. Shown are combined Aβ-immunostained and Congo red–stained hippocampi. Hippocampal 1-dpi extracts from both *App*$^{-/-}$ (left) and APP23 mice (right) induced Aβ deposition after the 4-month incubation period, and considerably more deposition after 8 months. Consistent with the immunoassays, the 30-dpi APP23 extracts also induced robust seeding activity. Notably, hippocampal extracts from the 30-dpi *App*$^{-/-}$ mice also displayed seeding activity (barely detectable after the 4-month incubation period but robustly present after 8 months). Scale bars represent 100 μm. (**d**,**e**) Stereological quantification of hippocampal Aβ immunostaining (Aβ load). Indicated is the mean ± s.e.m. Kruskal-Wallis test for the 4-month incubation: $P = 0.026$, $n = 3$ mice per group; for the 8-month incubation: $P = 0.032$, $n = 4$–5 mice per group; Mann-Whitney test with Bonferroni correction, $^*P \leq 0.05$.

© 2015 Nature America, Inc. All rights reserved.
npg

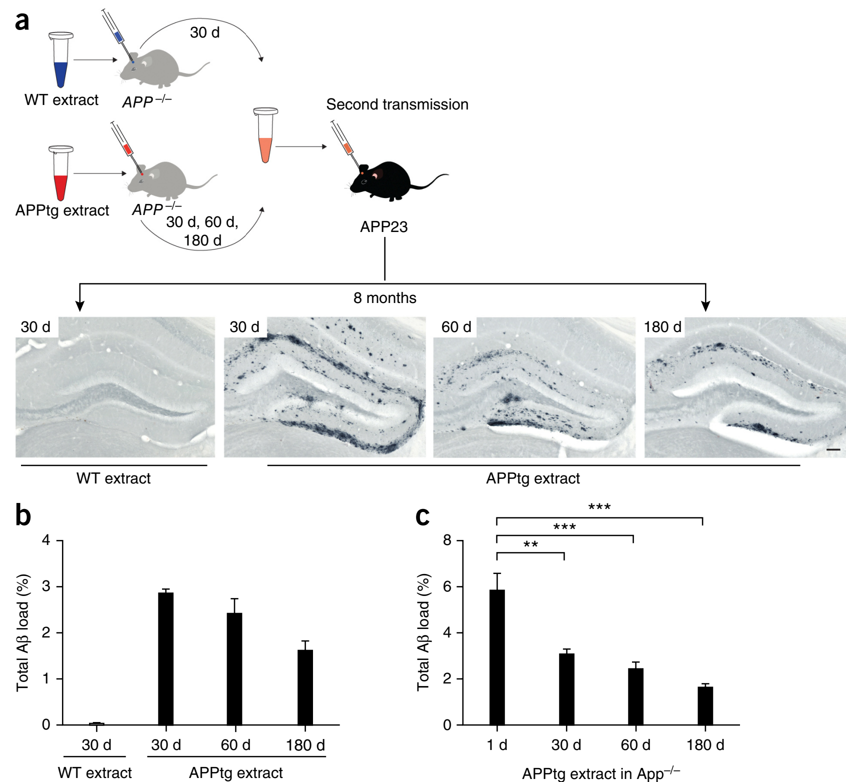


Figure 2 Aβ seed persistence in *App*$^{-/-}$ mice for up to 6 months. (**a**) Aβ seed–containing APP23 brain extract (APPtg extract) or WT mouse brain extract was injected bilaterally into the hippocampus of 3-month-old male *App*$^{-/-}$ mice. The injected hippocampi were isolated 30, 60 or 180 dpi (n = 4–5 mice per group), pooled and used for secondary transmission into the hippocampus of young, 3-month-old APP23 mice. Brains were analyzed for Aβ deposition 8 months later. Shown is the Aβ-immunostained and Congo red–stained hippocampus. Consistent with the first experiment (**Fig. 1**), 30-dpi *App*$^{-/-}$ hippocampal extracts induced robust Aβ deposition, whereas no amyloid deposition was observed with the WT extract. To our surprise, the 60-dpi *App*$^{-/-}$ extract and even the 180-pi *App*$^{-/-}$ extract induced some amyloid deposition. Scale bar represents 100 µm. (**b**) Stereological quantification of hippocampal Aβ immunostaining (Aβ load). Indicated is the mean ± s.e.m. WT and APPtg extract 30 dpi, n = 2 mice each (but see **Fig. 1**); APPtg extract 60 and 180 dpi, n = 4 and n = 3 mice per group, respectively. (**c**) Diminished induction of Aβ deposition by *App*$^{-/-}$ hippocampal extracts with increasing dpi (animals from this experiment are combined in this graph with the 1- and 30-dpi animals from **Fig. 1e**). One-way ANOVA revealed $F(3,14)$ = 15.210, P = 0.0001; Bonferroni *post hoc* test for pairwise comparisons, $^{**}P < 0.01$, $^{***}P < 0.001$.

to consider more stringent measures to mitigate the transmission of Aβ seeds by such means as contaminated surgical instruments or biologics[1]. To this end, the murine Aβ-seeding model is an exquisitely sensitive bioassay for the presence and functionality of Aβ seeds.

The extreme longevity of Aβ seeds *in vivo* also provides new insights into the long, clinically silent period that precedes the onset of dementia in AD[18,19]. Current estimates, based on the assessment of Aβ by positron emission tomography imaging and cerebrospinal fluid measurements, indicate that preclinical AD begins approximately 20 years before the first clear signs of incipient dementia[18,19] and is characterized by the progressive appearance of cerebral Aβ deposits in neocortical areas with subsequent spreading to other parts of the brain[20]. The discovery that Aβ seeds can exist in the brain at levels below routine detection indicates that the initial changes that drive AD pathogenesis may occur even earlier. From a therapeutic standpoint, these findings indicate that the most effective treatments for AD should target the Aβ cascade well before the characteristic deposits of Aβ become detectable by currently available methods. However, our findings in WT mice and *App*$^{-/-}$ mice also indicate that impeding the production of Aβ will inhibit the generation of new seeds and thereby delay or prevent the onset of dementia.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We would like to thank L. Goepfert and T. Joos (NMI, Reutlingen) and M. Hruscha, M. Lambert and L. Haesler for help with the immunoassays, A. Bosch, C. Krüger, J. Odenthal and all of the other members of our department for experimental help, and C. Haass (Munich) for antibody donation. This work was supported by grants from the Competence Network on Degenerative Dementias (BMBF-01GI0705) and the EC Joint Programme on Neurodegenerative Diseases (JPND-NeuTARGETs). L.Y. was supported by a Chinese Scholarship Council grant for PhD scholarship.

AUTHOR CONTRIBUTIONS

L.Y., S.K.F., J.S. and U.O. performed the experimental work. L.Y., S.K.F., J.S., U.O., K.D., S.A.K., F.B. and M.J. carried out the analysis. Experimental design and preparation of the manuscript was done by L.Y., S.K.F., Y.S.E., L.C.W., M.S. and M.J.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Jucker, M. & Walker, L.C. *Nature* **501**, 45–51 (2013).
2. Meyer-Luehmann, M. *et al. Science* **313**, 1781–1784 (2006).
3. Eisele, Y.S. *et al. Science* **330**, 980–982 (2010).
4. Langer, F. *et al. J. Neurosci.* **31**, 14488–14495 (2011).
5. Eisele, Y.S. *et al. Proc. Natl. Acad. Sci. USA* **106**, 12926–12931 (2009).
6. Fritschi, S.K. *et al. Acta Neuropathol.* **128**, 477–484 (2014).
7. Prusiner, S.B. *Annu. Rev. Genet.* **47**, 601–623 (2013).
8. Aguzzi, A. & Falsig, J. *Nat. Neurosci.* **15**, 936–939 (2012).
9. Morales, R., Callegari, K. & Soto, C. *Virus Res.* **207**, 106–112 (2015).
10. Büeler, H. *et al. Cell* **73**, 1339–1347 (1993).
11. Prusiner, S.B., Groth, D., Serban, A., Stahl, N. & Gabizon, R. *Proc. Natl. Acad. Sci. USA* **90**, 2793–2797 (1993).
12. Sailer, A., Bueler, H., Fischer, M., Aguzzi, A. & Weissmann, C. *Cell* **77**, 967–968 (1994).
13. Rupp, N.J., Wegenast-Braun, B.M., Radde, R., Calhoun, M.E. & Jucker, M. *Neurobiol. Aging* **32**, 2324.e1–2324.e6 (2011).
14. Stalder, M. *et al. Am. J. Pathol.* **154**, 1673–1684 (1999).
15. Mackenzie, I.R., Hao, C. & Munoz, D.G. *Neurobiol. Aging* **16**, 797–804 (1995).
16. Probst, A., Basler, V., Bron, B. & Ulrich, J. *Brain Res.* **268**, 249–254 (1983).
17. Safar, J.G. *et al. J. Gen. Virol.* **86**, 2913–2923 (2005).
18. Jack, C.R. Jr. & Holtzman, D.M.B. *Neuron* **80**, 1347–1358 (2013).
19. Jansen, W.J. *et al. J. Am. Med. Assoc.* **313**, 1924–1938 (2015).
20. Thal, D.R., Rub, U., Orantes, M. & Braak, H. *Neurology* **58**, 1791–1800 (2002).

© 2015 Nature America, Inc. All rights reserved.
npg

ONLINE METHODS

Mice. For all inoculations 3–4-month-old C57BL/6J-Tg(Thy1-$APP_{K670N;M671L}$)23 mice (APP23 mice), C57BL/6J (WT), and C57BL/6J-*App* ($App^{-/-}$)[21,22] mice were used. All mice were kept under specific pathogen-free conditions. The experimental procedures were carried out in accordance with the veterinary office regulations of Baden-Württemberg (Germany) and approved by the local Animal Care and Use Committees (Regierungspräsidium Tübingen).

Preparation of brain tissue extracts for intracerebral inoculation. We used extraction protocols as previously described[2,3]. Tissue for extract preparation was derived from aged (24.5–26.0 month old) amyloid-depositing male APP23 transgenic mice containing approximately 400,000 ID_{50} units per ml of seeding agent as estimated by serial dilutions (Ye, L., Kaeser, S.A., Marzeso, A., Obermüller, U., Odenthal, J., Fritschi, S.K., Staufenbiel, M., Baumann, F. & Jucker, M., unpublished observations), and from age-matched non-transgenic WT mice. After removal of the cerebellum and lower brainstem, forebrain samples were immediately fresh-frozen on dry ice and stored at −80 °C until use. Tissue was then homogenized (Ultra Turrax T8, IKA-Werke) at 10% (wt/vol) in sterile phosphate-buffered saline (PBS, Lonza), vortexed, sonicated 3 × 5 s (LabSonic, B. Braun Biotech International GmbH, 0.5 mm diameter sonotrode, cycle 1, amplitude 80%) and centrifuged at 3000*g* for 5 min to remove tissue debris. The supernatant was aliquoted and stored at −80 °C until use. Different extracts were used for the experiments described in **Figures 1** and **2**. The amount of Aβ in the APP23 extracts was ~10 ng μl^{-1}, similar to previous publications[2,3].

Intrahippocampal inoculation. Stereotactic injections were made under a mixture of ketamine/xylazine in PBS (ketamine 118 mg per kg of body weight, xylazine 8 mg per kg). Inoculates were stereotactically placed with a Hamilton syringe into the hippocampus (unilateral: AP −2.5 mm, L −2.0 mm, DV −1.8 mm, bilateral: AP −2.5 mm, L ± 2.0 mm, DV −1.8 mm,). 2.5 μl were injected at the speed of 1.25 μl min^{-1} and the needle was kept at the injection site for an additional 2 min before withdrawal. The surgical area was cleaned with sterile PBS and the incision was sutured. Postoperatively, the mice were maintained under infrared light and monitored until they had recovered from the anesthesia.

Preparation of hippocampal extracts from primary inoculation. Hippocampi from inoculated $App^{-/-}$, WT and APP23 mice were dissected 1, 7, 30, 60 and 180 dpi. Each hippocampus was immediately fresh-frozen on dry ice and stored at −80 °C until use. Tissue was then homogenized at 10% (wt/vol) in PBS using a 1 ml BD Plastikpak syringe (Becton Dickson). Homogenate was then vortexed, sonicated 3 × 5 s and centrifuged at 3000*g* for 5 min. The supernatant was aliquoted and immediately frozen at −80 °C until use. Hippocampal extracts from the primary inoculation in each group were pooled for secondary inoculation.

Quantification of Aβ by immunoassays. Hippocampal extracts were pretreated with formic acid (Sigma-Aldrich, final concentration: 70%, vol/vol), sonicated for 30 s on ice and centrifuged at 25,000*g* for 1 h at 4 °C. Supernatants were equilibrated in neutralization buffer (1 M Tris base, 0.5 M Na_2HPO_4, 0.05% NaN_3 (wt/vol)). Human Aβ was measured either by an electrochemiluminescence (ECL)-linked immunoassay (Meso Scale Discovery, MSD), as previously described[4,23], or by an ultra-sensitive bead-based single molecule array (Simoa, Quanterix). All samples and calibrators were run in duplicates. For the MSD platform commercial Human (6E10) Abeta Triplex Assay was used according to the manufacturer's instructions. In brief, 96-well plates pre-spotted with C-terminal capture antibodies against Aβx-38, Aβx-40 and Aβx-42 were blocked for 1 h with 1% bovine serum albumin (BSA in Tris buffer, wt/vol) and washed three times with Tris buffer. Formic acid–treated samples were co-incubated with the SULFO-TAG 6E10 detection antibody solution on the plate for 2 h. After washing, MSD Read Buffer T was added and the plate was measured immediately on the Sector Imager 6000. Data analysis used MSD DISCOVERY WORKBENCH software 2.0. Internal QC samples were used for quality control of the assay performance and inter-plate variability. Total Aβ was the sum of Aβx-38, Aβx-40 and Aβx-42.

Samples with very low Aβ levels were also measured using the commercial Simoa Human Aβ40 and Human Aβ42 kits from Quanterix. Depending on the expected Aβ concentration formic acid treated samples were diluted (at least 1:2) in sample diluent before being subjected to the fully automated analysis. Aβ was captured by antibody-coated beads and co-incubated with biotinylated detector antibodies against Aβ40 (or Aβ42) to form a complex. After a wash step, a streptavidin-β-galactosidase (SBG) conjugate was added and after further washing, resuspension in a substrate solution led to a fluorescence signal in the presence of SBG. Following transfer to the array disc, beads were individually sealed in microwells and quantified by the optical system of the Simoa HD-1 Analyzer. Each labeled Aβ molecule yielded a measurable signal. For the calibration, the software uses a Cubic ($1/Y^2$ with zero point custom weight of 0.1) or a 4 Parameter Logistic ($1/Y^2$ weighted) curve fit data reduction method for the human Aβ40 and Aβ42 assays, respectively. For internal quality control, 3 in-house QC samples (at high, intermediate, and low Aβ concentration) were measured in each individual run. Total Aβ was the sum of Aβ40 and Aβ42.

Immunohistochemistry and histology. Mice were deeply anesthetized with ketamine (300 mg per kg)/xylazine (20 mg per kg), transcardially perfused with ice-cold PBS (8 min) and decapitated. Brains were removed, immersion-fixed for 48 h in 4% paraformaldehyde in PBS (wt/vol), cryoprotected in 30% sucrose (wt/vol) in PBS for an additional 48 h, and then frozen in 2-methylbutane. Fixed-frozen brains were cut into 25 μm-thick coronal sections and collected in cryoprotectant buffer (35% ethylene glycol, 25% glycerol in PBS, vol/vol). Sections were stored at −20 °C until use. Immunohistochemistry was performed according to standard protocols with the Vectastain Elite ABC Kit (Vector Laboratories). As primary antibody, polyclonal rabbit Aβ-antibody NT12 and polyclonal rabbit anti-ionized calcium binding adaptor molecule 1 (Iba1, Wako Chemicals) were used as previously described[2]. In addition rabbit polyclonal antibody 5313 to the ectodomain of APP (generous gift of C. Haass, Munich) was used. Staining with Congo red for amyloid or Perl's Prussian blue for ferric iron was conducted according to standard protocols.

Quantification of total Aβ load. Aβ load was quantified on Aβ-immunostained sets of every 12th systematically sampled, serial, coronal section throughout the entire hippocampus. Researchers who performed the analysis were blinded to the inoculation groups. Stereological analysis was performed using a microscope equipped with a motorized *x-y-z* stage coupled to a video-microscopy system and the Stereo Investigator software (MicroBrightField) as previously described[2]. Total Aβ load was determined by calculating the % areal fraction occupied by Aβ-positive staining in two-dimensional sectors at a single focal plane (20×, 0.45 NA objective).

SDS-PAGE and immunoblot analysis. Hippocampal extracts were analyzed on NuPage Bis-Tris mini gels using NuPage LDS sample buffer and MES running buffer (Invitrogen). Proteins were transferred onto a nitrocellulose membrane, probed with monoclonal antibody 6E10 specific to human Aβ (Covance) and visualized using Amersham ECL Plus (GE Healthcare).

Statistics. Data in figures are expressed as mean ± s.e.m. Statistical analysis was performed using the GraphPad Prism software, version 6.0. The Kolmogorov-Smirnov test was used to assess the normality of the data distribution. Depending on the outcome of the Kolmogorov-Smirnov test, one-way ANOVA followed by the Bonferroni *post hoc* test or the Kruskal-Wallis test followed by the Mann-Whitney test with Bonferroni correction were used.

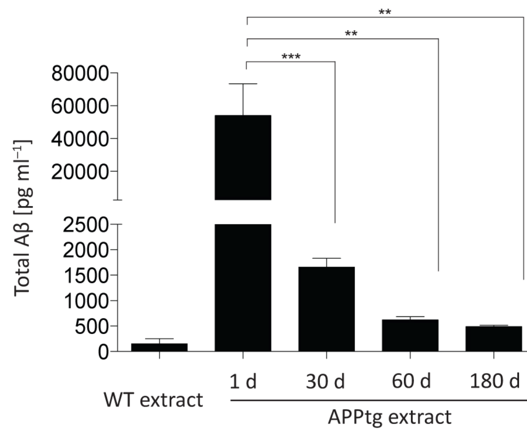
A **Supplementary Methods Checklist** is available.

21. Calhoun, M.E. *et al. Proc. Natl. Acad. Sci. USA* **96**, 14088–14093 (1999).
22. Eisele, Y.S. *et al. J. Neurosci.* **34**, 10264–10273 (2014).
23. Maia, L.F. *et al. Sci. Transl. Med.* **5**, 194re192 (2013).

© 2015 Nature America, Inc. All rights reserved.

npg

doi:10.1038/nn.4117

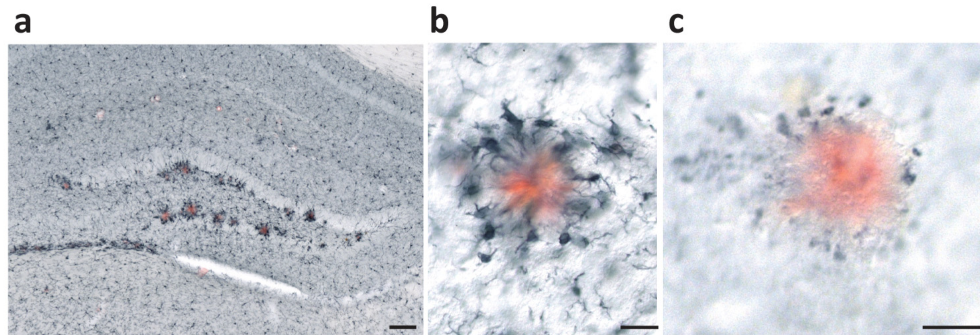


Supplementary Figure 1

Ultra-sensitive bead-based Simoa technology detects residual human Aβ in hippocampal extracts of inoculated *App*$^{-/-}$ mice.

Aβ seed-containing APP23 brain extract or seed-negative WT mouse brain extract was injected into the hippocampus of *App*$^{-/-}$ mice. The injected hippocampi were isolated 1, 30, 60, or 180 dpi (see Figures 1 and 2). While ECL-linked immunoassay no longer detected any Aβ at and beyond 30 dpi (Figure 1), the bead-based Simoa technology detected human Aβ up to 180 dpi. Note that the majority of Aβ was cleared within the first 30d with much slower clearance of residual Aβ thereafter. (The same samples were analysed as in Figures 1 and 2, thus n=6, 10, 5, and 5 for 1d, 30d, 60d, and 180d, respectively). One-way ANOVA for the APP transgenic extracts revealed $F(3,22)=8.932$, $P=0.0007$. Bonferroni post hoc test for pairwise comparisons, $^{**}P<0.01$, $^{***}P<0.001$. WT-injected mice (n=4; 30 or 60 dpi).

Nature Neuroscience: doi:10.1038/nn.4117

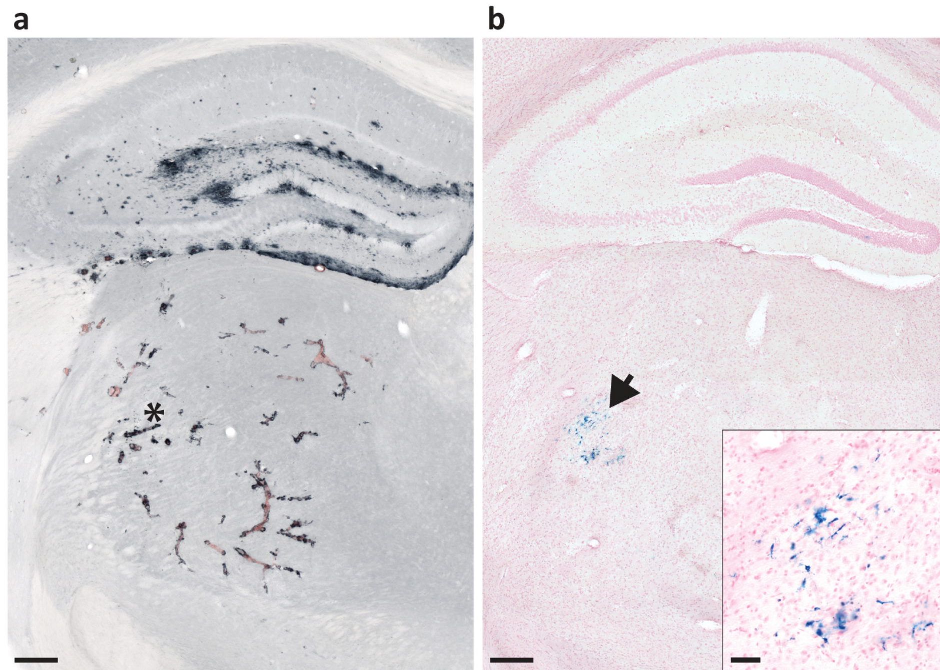


Supplementary Figure 2

Induced amyloid lesions are partly congophilic and surrounded by activated microglia and dystrophic boutons.

(**a**) Congo red-positive amyloid deposits induced in the dentate gyrus were surrounded by Iba1-positive microglia (black). (**b)** Congo red-positive plaque with surrounding hypertrophic microglial cell bodies and processes at higher magnification. (**c)** APP-immunoreactive dystrophic processes and boutons (black) in close proximity to a congophilic amyloid plaque. Images are from a 1 or 30 dpi *App*$^{-/-}$ hippocampal extract-inoculated APP23 mouse. Scale bar: 100µm (a), 20 µm (b,c).

Nature Neuroscience: doi:10.1038/nn.4117



Supplementary Figure 3

Cerebral amyloid angiopathy (CAA) was always induced in addition to parenchymal amyloid.

(**a**) Shown are the Aβ-immunostained and Congo red-stained hippocampus and thalamus of an APP23 mouse that had been inoculated with a 30 dpi *App*$^{-/-}$ hippocampal extract 8 months earlier. Note the prominent CAA in the thalamus (asterisk). Scale bar: 200 µm. (**b**) Perl´s Prussian blue staining of an adjacent section reveals multiple microhemorrhages in the CAA-laden thalamic region (arrow). Insert shows the microhemorrhages at higher magnification. Scale bars: 200 µm and (inset) 50 µm.

Nature Neuroscience: doi:10.1038/nn.4117

3.4 Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds

Juliane Schelle, Sarah K. Fritschi, Ulrike Obermüller, Lisa M. Häsler, Marius Lambert, Stephan A. Kaeser, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker

Manuscript in preparation

Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds

Juliane Schelle[1,2,3], Sarah K. Fritschi[1,2,3], Ulrike Obermüller[1,2], Lisa M. Häsler[1,2,4], Marius Lambert[1,2], Stephan A. Kaeser[1,2], Ulf Neumann[5], Derya R. Shimshek[5], Matthias Staufenbiel[1], Mathias Jucker[1,2]

[1]Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany; [2]DZNE, German Center for Neurodegenerative Diseases, 72076 Tübingen, Germany; [3]Graduate School of Cellular and Molecular Neuroscience, University of Tübingen, 72074 Tübingen, Germany; [4]Natural and Medical Sciences Institute at the University of Tübingen, 72770 Reutlingen, Germany; [5]Novartis Institutes for BioMedical Research, Neuroscience, 4056 Basel, Switzerland

Number of words: 216 (abstract); 6500 (total, including all)
Figures: 6

Correspondence: Mathias Jucker, mathias.jucker@uni-tuebingen.de.

Key words: Alzheimer's disease, Aβ seeds, preventive therapy, BACE inhibitor, anti-Aβ immunization

ABSTRACT

The insight that Alzheimer's disease (AD) pathogenesis precedes dementia symptoms by 10-20 years suggests that therapeutic compounds are most effective when applied at a pre-clinical disease stage before neurodegeneration has become apparent. As the aggregation of the β-amyloid (Aβ) peptide is assumed to initiate the pathological process of the disease, therapeutical approaches for AD have been focused on anti-Aβ strategies. Amyloid deposition as an early event in AD pathogenesis together with the nucleated-dependent Aβ aggregation seen *in vitro* suggest a slow pre-amyloid lag phase in which Aβ seeds are presumably formed. Therefore a preventive therapy based on the interference with Aβ seed formation has the potential to show clinical efficacy in AD. Hence, in this study we treated two different APP transgenic mouse models at the beginning of plaque deposition with an Aβ specific antibody targeting Aβ seeds and a potent BACE inhibitor to lower Aβ production. We demonstrate that the short but early combination approach not only reduced brain Aβ levels acutely but also significantly decreased amyloid deposition even after discontinuation of the treatment for several months. Thus, targeting initial Aβ seeds at an early pre-amyloid stage by anti-Aβ immunization and BACE inhibition might be an effective and promising paradigm in order to delay or even prevent the development of cerebral β-amyloidosis and eventually AD.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide and the leading cause of late-life dementia. However, so far no treatments are available to significantly slow down or stop AD. Recent studies have shown that AD pathogenesis starts 10-20 years before the onset of first clinical symptoms (Villemagne et al. 2013; Bateman et al. 2012; Buchhave et al. 2012; Jack et al. 2010). Hence, the failure of previous clinical trials for AD can be explained by the fact that they were initiated in symptomatic patients, in which massive amyloid deposition and neurodegeneration have already begun and progressed for many years inducing a clinical phenotype (Selkoe 2012; Golde et al. 2011). Eventually the efficacy and safety of therapeutic compounds can be increased when given at a pre-clinical

disease stage before regular brain function is affected and irreversible damage has occurred (Bateman et al. 2012; Golde et al. 2011).

According to the amyloid hypothesis and multiple genetic and biochemical evidences aggregation of the amyloid β (Aβ) peptide is suggested to be the first and initial lesion in AD brain (Selkoe & Hardy 2016; Holtzman et al. 2011; De Strooper 2010; Hardy & Selkoe 2002). Given that Aβ accumulation is a key trigger of the disease, targeting Aβ seems to be a very reasonable therapeutical approach (Golde et al. 2010).

From *in vitro* studies it is known that Aβ can form amyloid fibrils by a highly concentration-dependent nucleated polymerization process. After a certain lag phase and a series of intermediate states an initial nucleus (Aβ seed) is formed. This so called slow nucleation phase is subsequently followed by a phase of exponential increase of Aβ aggregation and fibril formation due to addition of soluble Aβ to Aβ seeds by conformational conversion (Jucker & Walker 2013; Eisenberg & Jucker 2012; Chiti & Dobson 2006; Harper & Lansbury 1997). With increasing size the growing fibrils break and form new self-propagating fragments and Aβ seeds (Jucker & Walker 2013). The lag time of amyloid formation *in vitro* can be shortened by the addition of small amounts of Aβ seeds (Harper & Lansbury 1997).

These *in vitro* observations of Aβ amyloid formation have recently been translated *in vivo* by the demonstration that Aβ aggregation can be induced and accelerated in young, pre-depositing APP transgenic (tg) mice by intracerebral injections of brain extract containing Aβ seeds (Meyer-Luehmann et al. 2006; Kane et al. 2000). Similar to i*n vitro* assays the *in vivo* induction of Aβ deposition is dependent on the concentration of the Aβ seeds as well as the availability of soluble Aβ to be incorporated into the growing Aβ aggregates (Jucker & Walker 2013; Hamaguchi et al. 2012; Jucker & Walker 2011; Meyer-Luehmann et al. 2006).

Aβ specific antibodies applied at early stages of plaque formation are intended to bind and inactivate already existing microplaques and therefore promote clearance of Aβ seeds. As the β-site of APP cleaving enzyme (BACE) initiates the production of the Aβ peptide it seems to be another attractive therapeutic target (Golde et al. 2011; Citron 2010; De Strooper et al. 2010). Therefore an early intervention using a combinational treatment based on Aβ vaccination to remove pre-existing Aβ aggregates and a BACE inhibitor to block the processing of APP and thereby reduce the production of soluble Aβ may effectively delay or prevent cerebral β-amyloidosis.

In this study such a combined therapy based on an anti-Aβ specific immunization strategy together with a BACE inhibitor was used during a defined pre-depositing stage targeting first endogenous Aβ seeds in two different APP tg mouse models. This short and early intervention of initial Aβ aggregation delayed the onset of Aβ plaque pathology even after discontinuation of the treatment. Our findings suggest that targeting amyloid seeds at a pre-amyloid stage and thereby interfering with the initial seeding phase of amyloid formation by immunotherapy and BACE inhibition is an effective therapeutic strategy to prevent cerebral β-amyloidosis and eventually AD.

METHODS

APP23 mice

Male 6-month-old heterozygous APP23 mice (Sturchler-Pierrat et al. 1997) were all bred at the Hertie-Institute for Clinical Brain Research. APP23 mice express the K670M/N671L mutated human APP (Swedish double mutation) under control of the neuron-specific Thy1 promoter element at about 7-fold over endogenous (murine) APP. The mice were generated on a B6D2 background but have since been bred with C57BL/6J mice over more than 20 generations. In APP23 mice plaque formation starts at 6–8 months of age with a faster plaque development in females than in males (Eisele et al. 2010; Sturchler-Pierrat et al. 1997). Therefore only male animals were used for this study. While single male mice enjoyed housing enrichment, cages with several male mice were not provided with additional enrichment material due to possessive behavior. Mice were kept under specific pathogen-free conditions. All experimental procedures were conducted in accordance with veterinary office regulations of Baden-Württemberg (Germany) and approved by the local Animal Care and Use Committees.

APP51 mice

Male and female 12-month-old heterozygous APP51 mice (Bodendorf et al. 2002) were bred at the Hertie-Institute for Clinical Brain Research. APP51 mice express the human wild-type APP under control of the neuron-specific Thy1 promoter element at about 7-fold over endogenous (murine) APP and were bred on a C57BL/6J background. APP51 mice develop the first plaques between 13 and 15 months of age. Aβ plaque development is faster in females than in males. Therefore experiments were always conducted in mice of the same sex

(Figure 5 females, Figure 6 males). While female mice and single male mice enjoyed housing enrichment, cages with several male mice were not provided with additional enrichment material due to possessive behavior. Mice were kept under specific pathogen-free conditions. All experimental procedures were carried out in compliance with veterinary office regulations of Baden-Württemberg (Germany) and approved by the local Animal Care and Use Committees.

Stereotactic injection of brain extract

For intracerebral injections, 10% brain homogenates (without cerebellum) in sterile phosphate-buffered saline (PBS, Lonza) derived from β-amyloid-depositing APP23 mice (25-26.0 month-old) were vortexed, sonicated 3 x 5 seconds (LabSonic, B. Braun Biotech International GmbH, 0.5 mm diameter sonotrode, cycle 1, amplitude 80%), and centrifuged at 3,000 x g for 5 min. The supernatant (brain extract) was collected and stored at −80 °C until use. Host mice were 3- to 4-month-old female APP23 mice (n=3-6 per group). The mice were anaesthetized with a mixture of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) in saline. 2.5 µl brain extract were bilaterally injected into the hippocampus (AP -2.5 mm, L +/-2.0 mm, DV -1.8 mm) with a Hamilton syringe. Injections were performed at 1.25 µl/minutes, and the needle was kept in place for additional 2 minutes before being slowly withdrawn. The surgical area was cleaned with sterile PBS, the incision was sutured, and the mice were maintained under infrared light and monitored until recovery from anesthesia.

Passive Aβ immunization

APP23 mice inoculated with Aβ seeds containing brain extract, 6-months-old male APP23, and 12-months-old female APP51 mice were immunized intraperitoneally (i.p.) with 0.5 mg mouse monoclonal immunoglobulin IgG2a antibody Beta-1 (β1) or a control IgG2a antibody (anti-wheat germ agglutinin) once daily for five consecutive days. 12-months-old male APP51 mice were injected i.p. with 2 mg β1 or control antibody five times once a week. The β1 antibody recognizes amino acids 3-6 of human Aβ and has been shown to be effective *in vivo* in a passive immunization paradigm (Meyer-Luehmann et al. 2006; Pfeifer et al. 2002).

BACE inhibitor treatment

6-month-old male APP23 mice (Figure 3 and 4) as well as 12-month-old female (Figure 5) and male (Figure 6) APP51 mice were fed with food pellets containing the BACE inhibitor NB-360 (0,5 g inhibitor / kg food pellets; Novartis, Basel, Switzerland (Schelle et al., 2017; Shimshek et al. 2016; Bacioglu et al. 2016; Neumann et al. 2015) for six weeks, respectively. Age-matched control mice received control pellets without the drug for the same time period. For the short-term experiments (Figure 3 and 5), NB-360-containing food pellets were replaced by control pellets three days before preparation except for groups 3 and 4 in Figure 3 that received NB-360-containing food pellets until preparation. Food pellets were available *ad libitum* until the day of preparation.

Brain tissue harvesting

Mice were anesthetized with ketamine (100 mg/kg body weight) and xylazine (10 mg/ml) and sacrificed by transcardial perfusion with ice-cold PBS. Brains were removed and one hemisphere was snap-frozen on dry ice and stored at -80 ºC until use. The other hemisphere was immersion-fixed in 4% paraformaldehyde in PBS, pH 7.6, at 4 ºC for 48 hours, cryoprotected in 30% sucrose in PBS at 4 ºC for additional 48 hours, frozen in 2-methylbutane, and stored at -80 ºC until use.

Brain tissue homogenization and extraction

Forebrains (hemispheres without cerebellum) from APP23 and APP51 mice were homogenized at 10% (w/v) in PBS or homogenization buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA and protease/phosphatase inhibitor cocktail from Thermo Scientific) twice at 5500 rpm and 4 °C for 10 sec using 7 ml lysing tubes with 2.8 mm ceramic beads and a tissue homogenizer (Precellys Bertin, Montigny-le-Bretonneux, France). The homogenized brain tissue was aliquoted and stored at -80 ºC until use.

For Aβ measurements the brain homogenates were either extracted using formic acid (FA) or measured directly after centrifugation. For the FA-extraction aliquots were thawed on ice, mixed 1:3.2 with cold FA (min. 96% purity, Sigma, St. Louis, MO, USA), sonicated on ice for 35 seconds, and spun at 25,000 x g at 4 °C for one hour. The supernatant was collected as the “FA-soluble fraction” and equilibrated (1:20) in neutralization buffer (1M Tris base, 0.5M Na_2HPO_4, 0.05% NaN_3). For direct measurement (grey bars in Figure 2) aliquots were thawed on ice and spun at 25,000 x g at 4 °C for one hour. Supernatant was pipetted into a new tube and used for the measurement.

Ultrasensitive sandwich immunoassay for *Aβ* measurements in brain extracts of pre-depositing mice

Aβ40 and Aβ42 concentrations in brain extracts from 1-8 month-old APP23 mice (Figure 2) were determined with an ultrasensitive bead based immunoassay using the human Aβ40 and human Aβ42 assay on the Simoa platform (Quanterix, Lexington, MA, USA). FA-soluble brain extracts were diluted 1:16 in sample diluent (Quanterix) for measurement of Aβ40 and 1:4 for Aβ42 measurements. 25.000 x g supernatants of total brain extracts were diluted 1:64 for direct measurements of Aβ40 and 1:32 for direct measurements of Aβ42 in the same buffer. FA-soluble total brain extracts from APP knockout and wildtype mice served as samples for testing specificity and were diluted 1:2 in Aβ40 and Aβ42 sample diluent for measurement of Aβ40 and Aβ42, respectively. Samples were measured on a Simoa HD-1 Analyzer (Quanterix) using the provided assay protocol for human Aβ40 and human Aβ42 assay. Data analysis was done using the Simoa software (Quanterix). Aβ40 and Aβ42 control samples in the low, mid, and high assay range served as a control and were measured in every Aβ40 and Aβ42 assay respectively.

Aβ measurement in brain extracts of depositing mice

Aβ40 ($A\beta_{x-40}$) and Aβ42 ($A\beta_{x-42}$) concentrations in brain extracts from APP tg mice were determined with an electrochemiluminescence-linked immunoassay using the V-PLEX Aβ Peptide Panel 1 (4G8) Kit (Meso Scale Discovery, Gaithersburg, MD, USA). FA-soluble total brain extracts were diluted up to 1:10 in buffer (Diluent 35, Meso Scale Discovery) before measurement. Data analysis used MSD® DISCOVERY WORKBENCH® software 2.0. Internal reference samples were used as a control in every plate.

Aβ immunohistochemical analysis

After freezing, fixed brains were cut into serial, 25 µm-thick coronal sections using a freezing-sliding microtome. The sections were collected in tissue cryoprotection solution (35% ethylene glycol, 25% glycerol in PBS) and stained immunohistochemically with a customized polyclonal anti-Aβ-antibody and the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) as previously described (Eisele et al. 2010). Sections were counterstained with Congo red for amyloid according to standard protocols. Representative images are shown in Figure 1 for the two different treatment groups.

Quantification of total Aβ plaque load

In mice inoculated with Aβ seeds Aβ plaque load was stereologically quantified in the neocortex of representative sections of an Aβ-immunostained set of every 12th systematically sampled, serial, coronal section. A microscope equipped with a motorized *x–y–z* stage coupled to a video-microscopy system and the Stereo Investigator software (MicroBrightField, Inc., Williston, VT, USA) was used as previously described (Bondolfi et al. 2002). The person who performed the analysis was blind to the treatment groups. The total Aβ load (percentage) was determined by calculating the areal fraction occupied by Aβ-positive staining in two-dimensional sectors (20×/0.45 objective).

For the rest of the mice the number of Aβ positive stainings was counted manually in representative sections of an Aβ-immunostained set of every 12th systematically sampled, serial, coronal section using a 10x objective (0.30 numerical aperture) and a Zeiss Axioskop 2 microscope (Zeiss, Oberkochen, Germany).

Statistical Analysis

GraphPadPrism (version 6.0) was used for statistical analyses and graphics. Normal distribution was assessed using the Shapiro-Wilk test. Non-normal-distributed variables were logarithmic-transformed. In all cases statistical significance was set at $P < 0.05$. All values are expressed as means ± SEM.

RESULTS

Passive immunization targeting early exogenously applied Aβ seeds decreases cerebral β-amyloidosis

We have previously reported that the Aβ specific antibody β1 mixed with an APP23 brain extract can effectively attenuate amyloid induction (Meyer-Luehmann et al. 2006). To investigate whether immunotherapy using the β1 antibody to remove Aβ seeds at early stages prevents the induction of cerebral β-amyloidosis we immunized young pre-depositing APP23 mice with the β1 antibody after intrahippocampal inoculation with amyloid-laden brain extracts of aged APP23 animals. Once daily for the first five consecutive days starting three hours post Aβ seed inoculation mice were intraperitoneally injected with either the β1 or a control antibody (Figure 1 A). Four months later immunohistochemical analyses revealed

less induced Aβ deposition in APP23 host mice that were injected with the β1 antibody compared to control animals (Figure 1 B, C). Stereological quantification revealed a significant reduction by 77% (Figure 1 D) indicating that an early inactivation of Aβ seeds by anti-Aβ immunotherapy might be an effective paradigm to prevent amyloid formation.

BACE inhibition combined with Aβ immunization target initial endogenous Aβ seeds in APP23 mice

In order to translate our findings in immunized mice injected with exogenously applied Aβ seeds to targeting strategies of endogenous Aβ seeds we used young pre-depositing APP23 mice. We first set out to define the pre-depositing phase of APP23 animals to narrow down the time of putative first Aβ seed generation. Brain Aβ levels of 1-8 month-old APP23 mice were measured with and without formic acid (FA) extraction to reveal the age when first FA-soluble Aβ seeds become apparent. While the omission of FA did not reveal any difference in cerebral Aβ40,42 levels of 1-8 month-old APP23 mouse brain extracts, the FA-soluble fraction of the same samples showed a significant increase in brain Aβ concentration with age (Figure 2). After seven months of age first significant increases in FA soluble brain Aβ or Aβ seeds could be measured compared to younger animals.

Based on those results we treated pre-depositing APP23 mice with the β1 antibody, a potent BACE inhibitor NB-360 (0,5 g inhibitor / kg food pellets; Novartis, Basel, Switzerland (Shimshek et al. 2016; Bacioglu et al. 2016; Neumann et al. 2015), or a combination of both for a short but defined time starting at six months of age before significant Aβ seeds became apparent (Figure 3 A). The β1 antibody was intraperitoneally injected once daily for the first five consecutive days in accordance to the previous study. The BACE inhibitor was applied in food pellets either for 45 days until preparation (group 3 and 4) or for 42 days until replacement by control pellets three days before preparation (group 5 and 6). Already 24 hours after administration NB-360 is not detectable anymore in blood and brain and therefore soluble Aβ levels are in a physiological range again (Neumann et al. 2015). A control group of mice received a control antibody as well as control food pellets. After 45 days all the mice were prepared. Mice that received until preparation the BACE inhibitor alone or in combination with β1 showed significantly less brain Aβ40,42 levels compared to control littermates or mice only treated with β1 (Figure 3 B). Similar results were obtained in NB-360-treated and NB-360 and β1-treated mice that were fed with control food pellets three days before preparation. The slight tendency towards less brain Aβ40,42 in mice that

received the BACE inhibitor until preparation compared to those animals whose food pellets were changed to control ones after 42 days can be explained by acutely decreased soluble Aβ levels due to the presence of NB-360. Soluble Aβ levels are expected to normalize after three days of NB-360 absence in the administered food pellets. Aβ immunohistochemistry revealed more than 75% less positive Aβ staining in NB-360-treated mice with β1 immunization (group 4 and 6) or without β1 treatment (group 5) in comparison to mice treated with only the β1 or a control antibody (Figure 3 C). Because of the small number of animals and one biological outlier the number of Aβ positive staining was only reduced by 60% in mice treated with β1 in addition to NB-360 for 45 days (group 3). Control and only β1-treated groups did not differ. Therefore a combinational treatment based on NB-360 application and β1 immunization seems to be the best strategy to prevent the development of first detectable Aβ seeds.

Interference with initial Aβ seeds delays plaque deposition in APP23 mice

To evaluate the long-term effect of our combination treatment we provided 6 month-old APP23 mice with the NB-360 pellets for 45 days and injected the β1 antibody once daily for the first five consecutive days (Figure 4 A). Afterwards the mice were incubated for another 4.5 months without any treatment. Control littermates received control food pellets as well as a control antibody. Control mice sacrificed already after 10.5 months of age were used as a baseline group. In NB-360 and β1 treated animals brain Aβ40,42 was kept on baseline level (Figure 4 B). Furthermore the age-dependent increase in amyloid plaque formation was completely prevented after combination treatment at early stages of deposition (Figure 4 C).

BACE inhibition combined with Aβ immunization target initial endogenous Aβ seeds in APP51 mice

APP51 mice do not harbor the Swedish mutation which causes higher affinity of BACE1 for APP and therefore less potency of a BACE inhibitor (Citron 2002; Bodendorf et al. 2002; Vassar et al. 1999) but express human wildtype APP. Hence, similar to our experiments in APP23 mice pre-depositing APP51 mice were treated with NB-360 containing food pellets for 42 days, the β1 antibody for the first five consecutive days, or a combination of both (Figure 5 A). To diminish the acute BACE inhibitor effect NB-360-containing food pellets were exchanged by control pellets three days before preparation. Control pellets and control antibody were applied to littermates. Biochemical analyzes revealed significantly reduced brain Aβ40,42 concentrations (Figure 5 B) as well as Aβ positive staining (Figure 5 C) in

groups of mice that received NB-360 either alone or in addition to the β1 antibody when compared to control mice. Immunization with the β1 antibody alone did not differ from control littermates.

Interference with initial Aβ seeds delays plaque deposition in APP51 mice

In order to establish whether our combination treatment in APP51 mice at the beginning of seed formation effects the further development of amyloid deposition we again fed mice the NB-360 pellets for 45 days and immunized the mice using the β1 antibody (Figure 6 A). However, in this experiment 2 mg β1 antibody were applied once weekly to increase the probability to target arising Aβ seeds rather than residual soluble Aβ. Control mice, fed with control food pellets and injected with a control antibody, were either sacrificed after 18 or 16.5 months of age as baseline group. Aβ40,42 concentrations in the brains of 18 month-old control mice were elevated compared to 1.5 months younger animals. Early NB-360 and β1 treatment completely blocked this age-dependent increase in brain Aβ (Figure 6 B). Similar results were obtained with regards to Aβ immunohistochemistry. Early combination treatment prevented the age-dependent increase in plaque formation (Figure 6 C).

DISCUSSION

To date estimated 40 million patients suffer from AD worldwide. So far treatments for AD induce relatively rapid but only temporary amelioration of the symptoms without slowing the decline of cognitive functions over years. Approved symptomatic drugs include acetyl cholinesterase inhibitors and a glutamate antagonist, memantine, and have become the standard of care for AD patients (Ising et al. 2015). However, disease-modifying compounds are highly needed in order to slow disease progression without major side effects. Recent therapeutic approaches for AD have been focused on anti-amyloid agents targeting Aβ as the key trigger of the disease. Genetics of the human disease including inherited missense mutations of APP (Hardy & Selkoe 2002) and presenilin 1 or 2 causing familial forms of AD (De Strooper et al. 1998), Aβ seed inoculation experiments in APP tg mice inducing cerebral β-amyloidosis (Meyer-Luehmann et al. 2006; Walker et al. 2002; Kane et al. 2000), and several fluid and neuroimaging biomarker studies (Villemagne et al. 2013; Bateman et al. 2012; Buchhave et al. 2012; Jack et al. 2010) support the amyloid cascade hypothesis proposing a key role for Aβ dyshomeostasis in initiating AD (Selkoe & Hardy 2016; Hardy

& Selkoe 2002). Specific reasons for the failures of therapeutic agents targeting Aβ in recent clinical trials are mainly based on insufficient safety standards, relative imprecision of a clinical diagnosis of AD, and the mild to moderate clinical stage of AD patients (Selkoe 2013). Recent biomarker analyses of a familial AD cohort suggest that Aβ42 levels in CSF begin to decline 25 years before the onset of the expected abnormalities on standard cognitive tests for dementia (Bateman et al. 2012). This is followed by the appearance of fibrillar amyloid deposits in the brain and increased CSF tau levels. Given that pathological alterations occur long before diagnosis of dementia, anti-amyloid approaches seem to be clinically feasible when applied before the onset of significant neurodegeneration. In this study we used the Aβ specific antibody β1 (Meyer-Luehmann et al. 2006; Pfeifer et al. 2002) to bind and clear exogenously applied Aβ seeds and therefore ameliorate the induction and propagation of Aβ aggregation in APP tg mice after several months. We then transferred this passive Aβ immunization approach to targeting strategies of early endogenous Aβ seeds and could not detect significant Aβ reductions in brain of young APP tg mice. These results suggest that endogenous Aβ seeds are different from exogenously applied Aβ seeds. Reasons may be that the exogenously applied seeds end up in a cellular department more accessible to antibodies, or that initial endogenous seeds have different conformational features compared to the seeds extracted from an aged amyloid-laden brain. Support for the latter interpretation comes from recent findings that the most seeding active Aβ aggregates occur at early stages of the amyloid formation (Ye et al., under review). Another explanation for the ineffectiveness of the β1 antibody in targeting endogenous Aβ seeds might be that the ratio of Aβ seeds and soluble Aβ species is much higher in mice inoculated with APP tg brain material compared to young pre-depositing APP tg mice. Therefore the β1 antibody was combined with a BACE inhibitor, which is supposed to block the amyloidogenic pathway of APP processing and therefore lowers the production of soluble Aβ monomers. In two different APP tg mouse models we show that a short combinational treatment applied at a defined pre-depositing stage significantly decreased both Aβ seeds acutely and amyloid deposition even after discontinuation of the treatment for several months. Although APP harboring the Swedish mutation is known to increase BACE1 cleavage of APP (Citron 2002; Bodendorf et al. 2002; Vassar et al. 1999) and should therefore lower the potency of a drug targeting this enzyme our results revealed similar reductions in amyloid pathology independent of the presence of the Swedish mutation. While chronic BACE inhibition or Aβ immunotherapy can evoke adverse neurochemical effects our short therapeutic intervention seems to be a promising therapeutic approach in order to safely delay the onset of cerebral β-

amyloidosis. Thus, interference with the early phase of amyloid formation by targeting initial Aβ seeds might be an effective paradigm to prevent cerebral β-amyloidosis and eventually AD. The preventive effect of the combined anti-amyloid agents in mice expressing human APP harboring the Swedish double mutation as well as in human wildtype APP tg mice suggests the potential efficacy of the short and early treatment in not only familial AD patients but also sporadic AD cases.

ACKNOWLEDGEMENTS

The authors would like to thank Lan Ye, Jörg Odenthal, and Ruth Dröge for their experimental help. Authors' contribution: J.S., S.K.F., U.O., L.H., M.L., and S.A.K. performed the experimental work. J.S. carried out the statistical analysis. U.N., D.R.S., and M.S. provided crucial research reagents. J.S., S.K.F., S.A.K., M.S., and M.J. designed the study and with the help of all other authors prepared the manuscript. Conflict of interest: U.N., D.R.S., and M.S. are or were employees and shareholders of Novartis Pharma AG, Basel Switzerland. The remaining authors declare no competing interests.

REFERENCES

Bacioglu, M. et al., 2016. Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. *Neuron*, 91(1), pp.56–66.

Bateman, R.J. et al., 2012. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *The New England journal of medicine*, 367(9), pp.795–804.

Bodendorf, U. et al., 2002. Expression of human beta-secretase in the mouse brain increases the steady-state level of beta-amyloid. *Journal of neurochemistry*, 80(5), pp.799–806.

Bondolfi, L. et al., 2002. Amyloid-associated neuron loss and gliogenesis in the neocortex of amyloid precursor protein transgenic mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(2), pp.515–522.

Buchhave, P. et al., 2012. Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Archives of general psychiatry*, 69(1), pp.98–106.

Chiti, F. & Dobson, C.M., 2006. Protein misfolding, functional amyloid, and human disease.

Annual review of biochemistry, 75, pp.333–366.

Citron, M., 2010. Alzheimer's disease: strategies for disease modification. *Nature reviews. Drug discovery*, 9(5), pp.387–398.

Citron, M., 2002. Emerging Alzheimer's disease therapies: inhibition of beta-secretase. *Neurobiology of aging*, 23(6), pp.1017–1022.

De Strooper, B., 2010. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. *Physiological reviews*, 90(2), pp.465–494.

De Strooper, B. et al., 1998. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*, 391(6665), pp.387–390.

De Strooper, B., Vassar, R. & Golde, T., 2010. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nature reviews. Neurology*, 6(2), pp.99–107.

Eisele, Y.S. et al., 2010. Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. *Science (New York, N.Y.)*, 330(6006), pp.980–982.

Eisenberg, D. & Jucker, M., 2012. The amyloid state of proteins in human diseases. *Cell*, 148(6), pp.1188–1203.

Golde, T.E., Petrucelli, L. & Lewis, J., 2010. Targeting Abeta and tau in Alzheimer's disease, an early interim report. *Experimental neurology*, 223(2), pp.252–266.

Golde, T.E., Schneider, L.S. & Koo, E.H., 2011. Anti-aβ therapeutics in Alzheimer's disease: the need for a paradigm shift. *Neuron*, 69(2), pp.203–213.

Hamaguchi, T. et al., 2012. The presence of Aβ seeds, and not age per se, is critical to the initiation of Aβ deposition in the brain. *Acta neuropathologica*, 123(1), pp.31–37.

Hardy, J. & Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)*, 297(5580), pp.353–356.

Harper, J.D. & Lansbury, P.T., 1997. Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annual review of biochemistry*, 66, pp.385–407.

Holtzman, D.M., Morris, J.C. & Goate, A.M., 2011. Alzheimer's disease: the challenge of the second century. *Science translational medicine*, 3(77), p.77sr1.

Ising, C., Stanley, M. & Holtzman, D.M., 2015. Current thinking on the mechanistic basis of Alzheimer's and implications for drug development. *Clinical pharmacology and therapeutics*, 98(5), pp.469–471.

Jack, C.R. et al., 2010. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. *Brain : a journal of neurology*, 133(11), pp.3336–3348.

Jucker, M. & Walker, L.C., 2011. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Annals of neurology*, 70(4), pp.532–540.

Jucker, M. & Walker, L.C., 2013. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465), pp.45–51.

Kane, M.D. et al., 2000. Evidence for seeding of beta -amyloid by intracerebral infusion of Alzheimer brain extracts in beta -amyloid precursor protein-transgenic mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(10), pp.3606–3611.

Meyer-Luehmann, M. et al., 2006. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science (New York, N.Y.)*, 313(5794), pp.1781–1784.

Neumann, U. et al., 2015. A novel BACE inhibitor NB-360 shows a superior pharmacological profile and robust reduction of amyloid-β and neuroinflammation in APP transgenic mice. *Molecular neurodegeneration*, 10(1), p.44.

Pfeifer, M. et al., 2002. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science (New York, N.Y.)*, 298(5597), p.1379.

Schelle, J. et al., 2017. Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. *Alzheimer's & dementia: the journal of the Alzheimer's Association*, 13(6), pp.701–709.

Selkoe, D.J., 2012. Preventing Alzheimer's disease. *Science (New York, N.Y.)*, 337(6101), pp.1488–1492.

Selkoe, D.J., 2013. The therapeutics of Alzheimer's disease: where we stand and where we are heading. *Annals of neurology*, 74(3), pp.328–336.

Selkoe, D.J. & Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO molecular medicine*, 8(6), pp.595–608.

Shimshek, D.R. et al., 2016. Pharmacological BACE1 and BACE2 inhibition induces hair depigmentation by inhibiting PMEL17 processing in mice. *Scientific reports*, 6, p.21917.

Sturchler-Pierrat, C. et al., 1997. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proceedings of the National Academy of Sciences of the United States of America*, 94(24), pp.13287–13292.

Vassar, R. et al., 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science (New York, N.Y.)*, 286(5440), pp.735–741.

Villemagne, V.L. et al., 2013. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet neurology*, 12(4), pp.357–367.

Walker, L.C. et al., 2002. Exogenous induction of cerebral beta-amyloidosis in betaAPP-transgenic mice. *Peptides*, 23(7), pp.1241–1247.

Ye, L. et al., 2017. Aβ seeding potency peaks in the early stages of cerebral β-amyloidosis. Accepted in *EMBO Reports*.

FIGURES

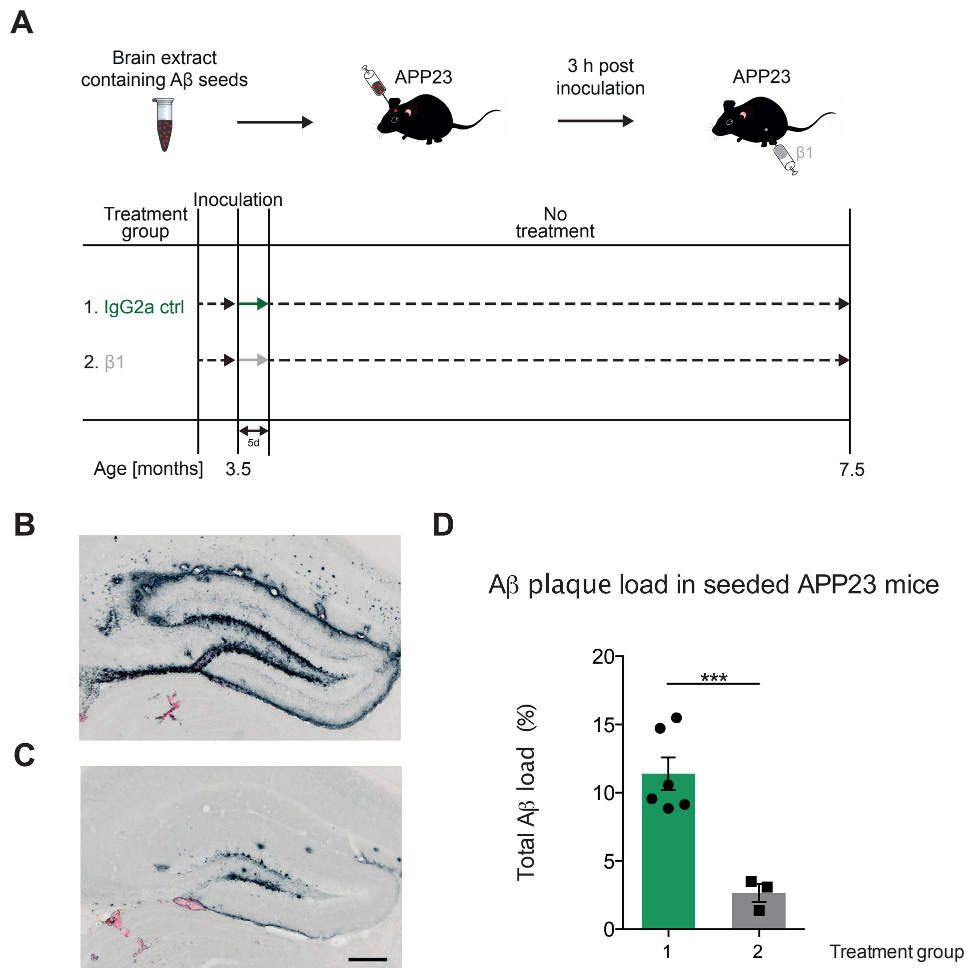


Figure 1: *Short and early anti-Aβ immunization attenuates induced cerebral β-amyloidosis.* Female APP23 mice (3–4 months of age; 3–6 mice per group) were intracerebrally injected with amyloid-laden brain extract of aged APP23 mice and intraperitoneally immunized once daily for five consecutive days starting three hours post inoculation. Aβ seed inoculated mice were either injected with 0.5 mg of the Aβ specific antibody Beta-1 (β1) or 0.5 mg of an IgG2a control antibody (IgG2a ctrl), respectively. Brains were analyzed immunohistochemically four months after intracerebral injection. **(A)** Schematic overview of experimental design. (B, C) Shown are representative sections of the dentate gyrus of the hippocampus for IgG2a ctrl **(B)** or β1-injected mice **(C)** (scale bar is 200 µm). **(D)** Stereological quantification of hippocampal Aβ immunostaining (Aβ load) exhibited significantly less induced Aβ deposition after β1 treatment compared to control mice (t-test $t_7 = 6.207$, ***$P < 0.001$). All data are represented as group means ± SEM.

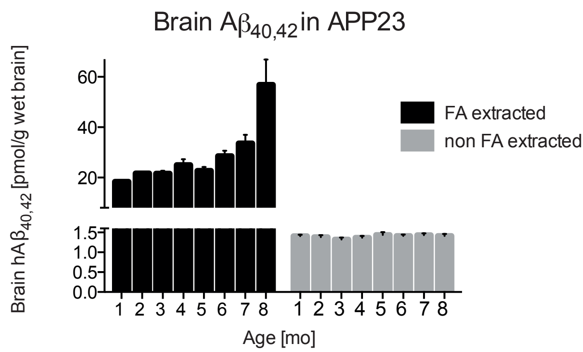


Figure 2: *FA-soluble Aβ seeds increase in APP23 mice at 7 months of age.* 1–8 month-old male APP23 mice (6–7 mice per group) were analyzed regarding their brain Aβ40,42 levels with and without formic acid (FA) extraction. Two-way ANOVA revealed significant age x extraction interaction ($F_{7,45} = 14.1$, $P < 0.001$). Notably, Tukey's *post hoc* test revealed that non-FA extracted brain homogenates did not differ, but FA-extracted brain samples of seven and eight month-old mice showed significantly increased brain Aβ40,42 levels compared to younger animals ($P < 0.001$). All data are represented as group means ± SEM.

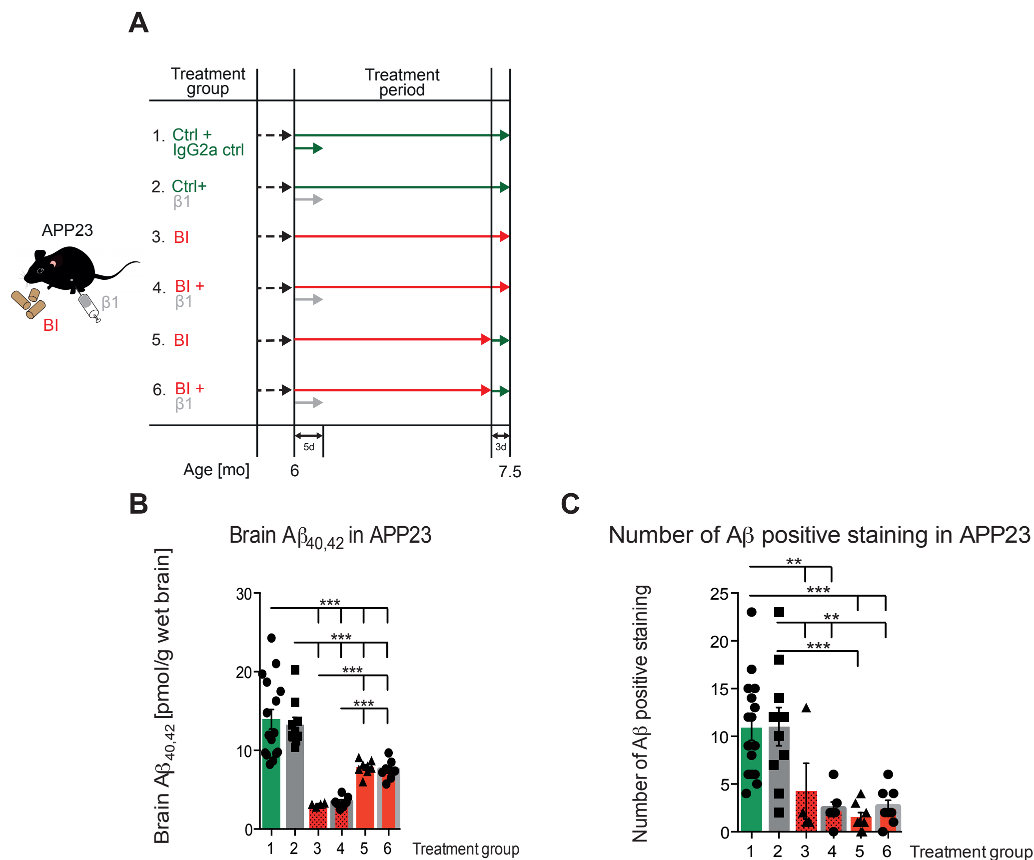


Figure 3: *Passive immunization combined with BACE inhibition reduces brain Aβ levels and plaque deposition in APP23 mice.* Six month-old male APP23 mice (6–8 mice per group) were either intraperitoneally injected with the Aβ specific antibody Beta-1 (β1) once daily for five consecutive days (group 2), fed with BACE inhibitor NB-360-containing pellets (BI) for 45 (group 3) or 42 days (group 5), or received a combination of 45 day NB-360 and five days β1 (group 4) or 42 days NB-360 and five days β1 (group 6). Mice of group 5 and 6 were supplied with control food pellets three days before preparation. Control mice were injected with a control IgG2a antibody (IgG2a ctrl) and fed with control food pellets (Ctrl) (group 1). All mice were sacrificed at 7.5 months of age. **(A)** Schematic overview of experimental design. **(B)** Measurements of Aβ levels in brain exhibited a significant decrease in mice treated with only NB-360 or a combination of β1 and NB-360 independent of the duration of NB-360 administration compared to control or only β1-treated animals (ANOVA $F_{5, 47}$ = 51.36, P < 0.0001; Tukey's *post hoc* test ***P < 0.001). **(C)** Quantification of cerebral Aβ immunostaining revealed a similar result (ANOVA $F_{5, 43}$ = 12.36, P < 0.0001; Tukey's *post hoc* test **P < 0.01, ***P < 0.001). All data are represented as group means ± SEM.

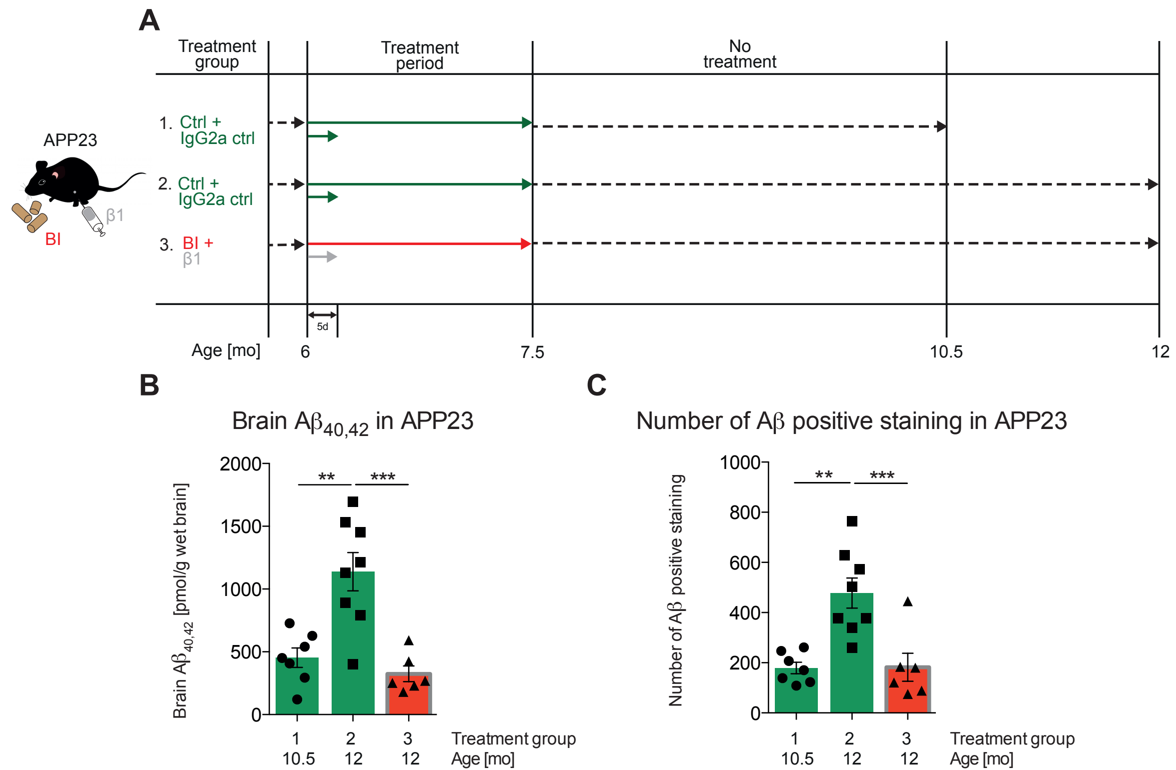


Figure 4: *Targeting early Aβ seeds delays cerebral β-amyloidosis in APP23 mice.* 6 month-old male APP23 mice (6–8 mice per group) were intraperitoneally injected with the Aβ specific antibody Beta-1 (β1) once daily for five consecutive days and were additionally fed with BACE inhibitor NB-360-containing pellets (BI) for 45 days. Littermates were immunized with control IgG2a antibody (IgG2a ctrl) and fed with control food pellets (Ctrl). After 45 days until preparation standard food pellets were supplied. NB-360 and β1-treated animals as well as one control group was prepared after 12 months of age. Other control mice served as baseline group and were prepared with 10.5 months of age. **(A)** Schematic overview of experimental design. **(B)** Brain Aβ level measurements revealed an age-dependent increase in 12 month-old compared to 10.5 month-old animals, which could be completely blocked by NB-360 and β1 treatment (ANOVA $F_{2, 18} = 12.01$, $P < 0.001$; Tukey's *post hoc* test $^{**}P < 0.01$, $^{***}P < 0.001$). **(C)** Immunohistochemistry confirmed those results. NB-360 and β1-treated animals showed similar amounts of Aβ positive staining in the brain than 1.5 months younger controls (ANOVA $F_{2, 18} = 13.01$, $P < 0.001$; Tukey's *post hoc* test $^{**}P < 0.01$, $^{***}P < 0.001$). All data are represented as group means ± SEM.

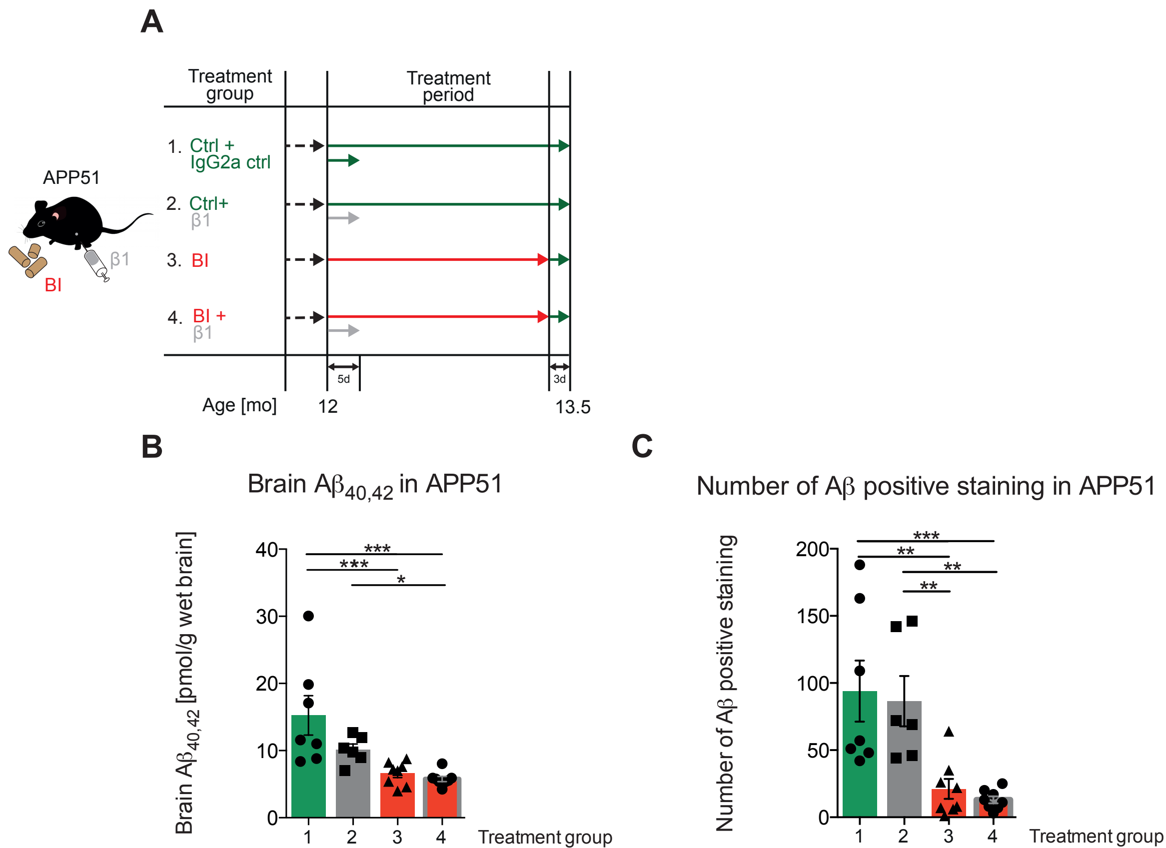


Figure 5: *Passive immunization combined with BACE inhibition reduces brain Aβ levels and plaque deposition in APP51 mice.* 12 month-old female APP51 mice (6–8 mice per group) were either intraperitoneally injected with the Aβ specific antibody Beta-1 (β1) once daily for five consecutive days, fed with BACE inhibitor NB-360-containing pellets (BI) for 42 days, or received a combination of both treatments. Control mice were injected with a control IgG2a antibody (IgG2a ctrl) and fed with control food pellets (Ctrl). All mice were sacrificed with 13.5 months of age and supplied with control food pellets three days before preparation. **(A)** Schematic overview of experimental design. **(B)** Measurements of Aβ levels in brain exhibited a significant decrease in mice treated with only NB-360 or a combination of the β1 antibody and NB-360 compared to control animals (ANOVA $F_{3,\ 25} = 13.15$, $P < 0.001$; Tukey's *post hoc* test $*P < 0.05$, $***P < 0.001$). **(D)** Quantification of cerebral Aβ immunostaining revealed that control mice had 78% and 86% more positive Aβ staining than NB-360 or NB-360 and β1-treated animals, respectively (ANOVA $F_{3,25} = 11.64$, $P < 0.001$; Tukey's *post hoc* test $**P < 0.01$, $***P < 0.001$). All data are represented as group means ± SEM.

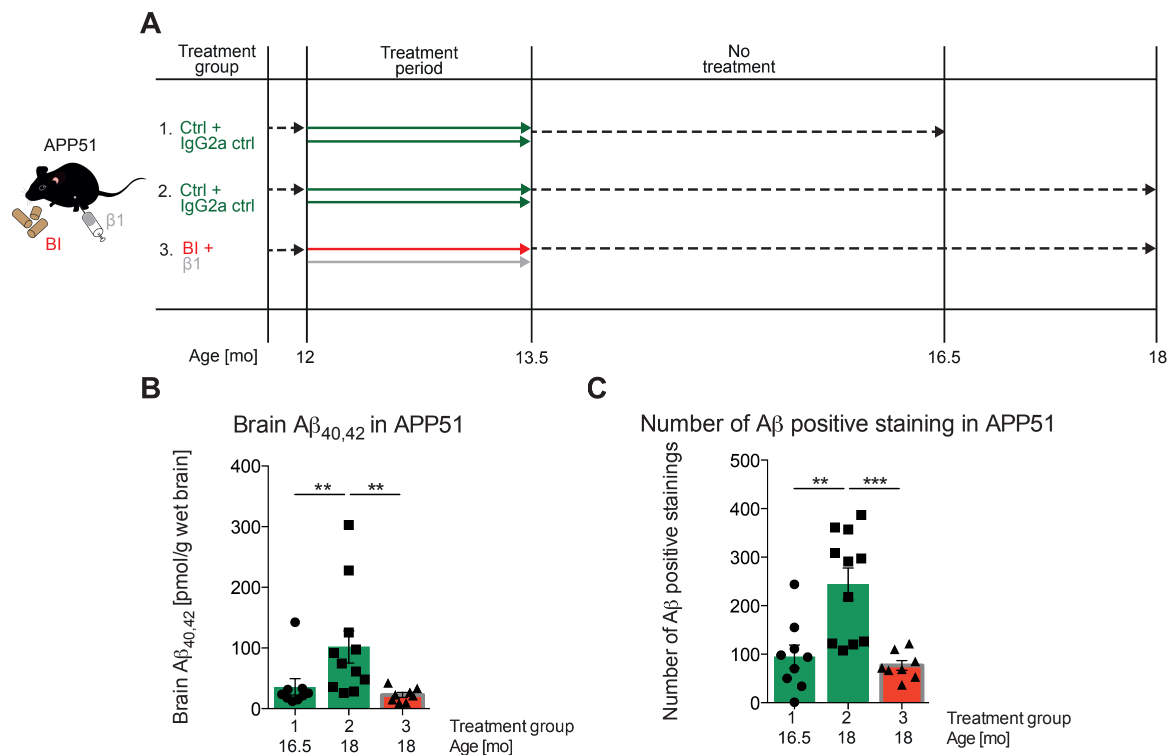


Figure 6: *Targeting early Aβ seeds delays cerebral β-amyloidosis in APP51 mice.* 12 month-old male APP51 mice (8–11 mice per group) were intraperitoneally injected with the Aβ specific antibody Beta-1 (β1) once weekly for five times and were additionally fed with BACE inhibitor NB-360-containing pellets (BI) for 45 days. Littermates were immunized with control IgG2a antibody (IgG2a ctrl) and fed with control food pellets (Ctrl). After 45 days until preparation standard food pellets were supplied. NB-360 and β1-treated animals as well as one control group was prepared after 18 months of age. Other control mice served as baseline group and were prepared with 16.5 months of age. **(A)** Schematic overview of experimental design. **(B)** Brain Aβ level measurements revealed an age-dependent increase in 18 month-old compared to 16.5 month-old animals, which could be completely blocked by NB-360 and β1 treatment (ANOVA $F_{2, 25} = 9.468$, $P < 0.001$; Tukey's *post hoc* test $**P < 0.01$). **(C)** Immunohistochemistry confirmed those results. NB-360 and β1-treated animals showed similar amounts of Aβ positive staining in the brain than 1.5 months younger controls (ANOVA $F_{2, 25} = 12.70$, $P < 0.001$; Tukey's *post hoc* test $**P < 0.01$, $***P < 0.001$). All data are represented as group means ± SEM.

4. Appendix

4.1 Abbreviations

A4	Anti-Amyloid Treatment in Asymptomatic Alzheimer’s Disease trial
ABCA 7	ATP-binding cassette sub-family A member 7
ACh	Acetylcholine
AD	Alzheimer’s disease
ADAD	Autosomal-dominant AD
ADAM	A disintegrin and metalloprotease
AICD	APP intracellular domain
ALS	Amyotrophic lateral sclerosis
Aph-1	Anterior pharynx-defective phenotype 1
API	Alzheimer’s Prevention Initiative
APOE	Apolipoprotein E
APOE4	ε4-allele of the APOE
APP	β-amyloid precursor protein
APP_{695}, APP_{751}, APP_{770}	APP isoforms 695, 751, 770 amino acids in length
APPSwedish	K670M/N671L-mutated human APP
Aβ	Amyloid-β peptide
$A\beta_{40}$, $A\beta_{42}$	Aβ ending at amino acid 40, 42
Aη	Amyloid-η peptide
β1	Beta-1 antibody
BACE1, BACE2	β-site APP cleaving enzyme 1, 2
BBB	Blood-brain-barrier
BIN1	Bridging integrator protein 1
BSE	Bovine spongiform encephalopathy
C-terminal	Carboxyterminal
CD33	Member of the sialic-acid-binding immunoglobulin-like lectins family
CJD	Creutzfeldt-Jakob disease

CNS	Central nervous system
CR1	Complement receptor 1
CSF	Cerebrospinal fluid
CTF	C-terminal fragment
CTFα/C83	CTF generated by α-secretase cleavage of APP (83 amino acids in length)
CTFβ/C99	CTF generated by β-secretase cleavage of APP (99 amino acids in length)
CTFη	CTF generated by η-secretase cleavage of APP (30 amino acids in length)
CWD	Chronic wasting disease
DIAN	Dominantly Inherited Alzheimer Network
DIAN-TU	DIAN Trials Unit
dpi	Days post inoculation
DSM-V	Diagnostic and Statistical Manual of Mental Disorders
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early onset Alzheimer's disease
FA	Formic acid
FAD	Familial Alzheimer's disease
FDA	Food and Drug Administration
FDG	^{18}F-fluorodeoxyglucose
FFI	Fatal familial insomnia
GSS	Gerstmann-Sträussler-Scheinker disease
GWAS	Genome-wide association studies
HIV	Human immunodeficiency virus
i.p.	Intraperitoneal
LOAD	Late onset Alzheimer's disease
MAPT	Microtubule associated protein tau
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
NCT	Nicastrin

NFTs	Neurofibrillary tangles
NMDA	N-methyl-D-aspartate
NINCDS/ ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
p3	Product of the APP processing
PiB	Pittsburgh compound B
PBS	Phosphate-buffered saline
PD	Parkinson's disease
Pen-2	Presenilin enhancer 2
PET	Positron emission tomography
PICALM	Phosphatidylinositol-binding clathrin assembly protein
PK	Proteinase K
Prion	Proteinaceous infectious particle
PrP	Prion protein
PS1/2	Presenilin 1/2
PSEN1, PSEN2	Gene encoding presenilin 1, 2
p-tau	Phosphorylated tau
sAPP	Soluble amyloid precursor protein
sAPPα	sAPP generated by α-secretase
sAPPβ	sAPP generated by β-secretase
SEM	Standard error of the mean
SIMOA	Single molecule array
SORL1	Sortilin-related receptor 1
tg	Transgenic
Thy1	Thymocyte differentiation antigen 1
TOMM40	Translocase of outer mitochondrial membrane 40 homolog
TREM2	Triggering receptor expressed on myeloid cells 2
t-tau	Total tau
wt	Wild type

4.2 Curriculum Vitae

Juliane Schelle	Stöcklestr. 10
* 08.09.1988	72070 Tübingen, Germany
Place of birth: Berlin	E-mail: julischelle@gmail.com
Nationality: German	Phone: +49 176 56526255

Education

12/2013 – now	**PhD position** at Hertie-Institute for Clinical Brain Research, University Tübingen; German Center for Neurodegenerative Diseases (DZNE), Germany PhD thesis: "Initial Aβ seeds as therapeutic target for Alzheimer's disease"
09/2011 – 09/2013	**Master of Science (1.3)** at Eberhard-Karls University Tübingen, Graduate School of Cellular and Molecular Neuroscience, Germany Field of study: Cellular and Molecular Neuroscience
08/2012 – 01/2013	**Semester abroad (A)** at San Diego State University, USA
10/2008 – 07/2011	**Bachelor of Science (1.5)** at Philipps University Marburg, Germany Field of study: Biomedical Science / Human biology with focus on neurobiology

Additional information

09/2015 – now	Student supervision during lab rotation, master thesis, and PhD
12/2013 – 03/2016	PhD student representative of Graduate School for Cellular and Molecular Neuroscience
WT 2015/16, 16/17	Organizer, lecturer, and supervisor for block course "Neurohistology/-pathology and Neuromorphology" for Master students
2014 – now	Organization and supervision of laboratory visits

4.3 Bibliography

Peer-reviewed manuscripts

1. Lan Ye, Jay Rasmussen, Stephan A. Kaeser, Anne-Marie Marzesco, Ulrike Obermüller, Jasmin Mahler, **Juliane Schelle**, Jörg Odenthal, Christian Krüger, Sarah K. Fritschi, Lary C. Walker, Matthias Staufenbiel, Frank Baumann, and Mathias Jucker (2017). Aβ seeding potency peaks in the early stages of cerebral β-amyloidosis. Accepted in *EMBO Reports*.
2. **Juliane Schelle**, Lisa Häsler, Jens C. Göpfert, Thomas O. Joos, Hugo Vanderstichele, Erik Stoops, Eva-Maria Mandelkow, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker, Stephan A. Kaeser (2017). Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. *Alzheimer's & dementia: the journal of the Alzheimer's Association,* 13(6), pp.701–709.
3. Mehtap Bacioglu, Luis F. Maia, Oliver Preische, **Juliane Schelle**, Anja Apel, Stephan A. Keaser, Manuel Schweighauser, Timo Eninger, Marius Lambert, Andrea Pilotto, Derya R. Shimshek, Ulf Neumann, Philipp J. Kahle, Matthias Staufenbiel, Manuela Neumann, Walter Maetzler, Jens Kuhle, and Mathias Jucker (2016). Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. *Neuron*, 91(1), pp.56–66.
4. Derya R. Shimshek, Laura H. Jacobson, Carine Kolly, Natasa Zamurovic, Kamal Kumar Balavenkatraman, Laurent Morawiec, Robert Kreutzer, **Juliane Schelle**, Mathias Jucker, Barbara Bertschi, Diethilde Theil, Annabelle Heier, Karine Bigot, Karen Beltz, Rainer Machauer, Irena Brzak, Ludovic Perrot, and Ulf Neumann (2016). Pharmacological BACE1 and BACE2 inhibition induces hair depigmentation by inhibiting PMEL17 processing in mice. *Scientific reports*, 6, p.21917.
5. Lan Ye*, Sarah K. Fritschi*, **Juliane Schelle**, Ulrike Obermüller, Karoline Degenhardt, Stephan A. Kaeser, Yvonne S. Eisele, Lary C. Walker, Frank Baumann, Matthias Staufenbiel, and Mathias Jucker (2015). Persistence of Aβ seeds in APP null mouse brain. *Nature neuroscience*, 18(11), pp.1559-1561. **equal contribution*
6. Luis F. Maia, Stephan A. Kaeser, Julia Reichwald, Marius Lambert, Ulrike Obermüller, **Juliane Schelle**, Jörg Odenthal, Peter Martus, Matthias Staufbiel, and Mathias Jucker (2015). Increased CSF Aβ during the very early phase of cerebral Aβ deposition in mouse models. *EMBO molecular medicine*, 7(7), pp.895-903.

Book chapter

1. Lary C. Walker, **Juliane Schelle**, and Mathias Jucker (2016). The Prion-Like Properties of Amyloid-β Assemblies: Implications for Alzheimer's Disease. *Cold Spring Harbor perspectives in medicine*, 6(7).

Oral presentations and conference proceedings

1. **Juliane Schelle**, Bettina M. Wegenast-Braun, Ulrike Obermüller, Lisa Häsler, Nina Jährling, Hans-Ulrich Dodt, Stephan A. Kaeser, Sarah K. Fritschi, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker. Prevention of cerebral amyloid angiopathy of the β-amyloid type in mice (2017). *Alzheimer's Association International Conference, London, England (poster presentation).*
2. **Juliane Schelle**, Lisa Häsler, Jens C. Göpfert, Thomas O. Joos, Hugo Vanderstichele, Erik Stoops, Eva-Maria Mandelkow, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker, Stephan A. Kaeser. Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition (2016). *46th annual meeting of the Society for Neuroscience, San Diego, USA (poster presentation).*
3. **Juliane Schelle**, Lisa Häsler, Jens C. Göpfert, Thomas O. Joos, Hugo Vanderstichele, Erik Stoops, Eva-Maria Mandelkow, Ulf Neumann, Derya R. Shimshek, Matthias Staufenbiel, Mathias Jucker, Stephan A. Kaeser. Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition (2016). *2nd Kloster Seeon Meeting on BACE Proteases in Health and Disease, Seeon-Seebruck, Germany (poster presentation).*
4. **Juliane Schelle** (2016). Aβ seeds as therapeutic targets. *EU Joint Programme – Neurodegenerative Disease Research meeting, Paris, France (oral presentation).*
5. **Juliane Schelle** (2016). Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds. *Scientific retreat DZNE Tübingen, Überlingen, Germany (oral presentation).*
6. **Juliane Schelle**, Sarah K. Fritschi, Lan Ye, Ulrike Obermueller, Yvonne S. Eisele, Stephan A. Kaeser, Matthias Staufenbiel, Mathias Jucker. Prevention of cerebral β-amyloidosis by targeting initial Aβ seeds (2015). *EMBO/EMBL symposia mechanisms of neurodegeneration, Heidelberg, Germany (poster presentation).*
7. *International conference on Alzheimer's and Parkinson's Diseases, Nice, France (2015).*