

ALZHEIMER'S DISEASE

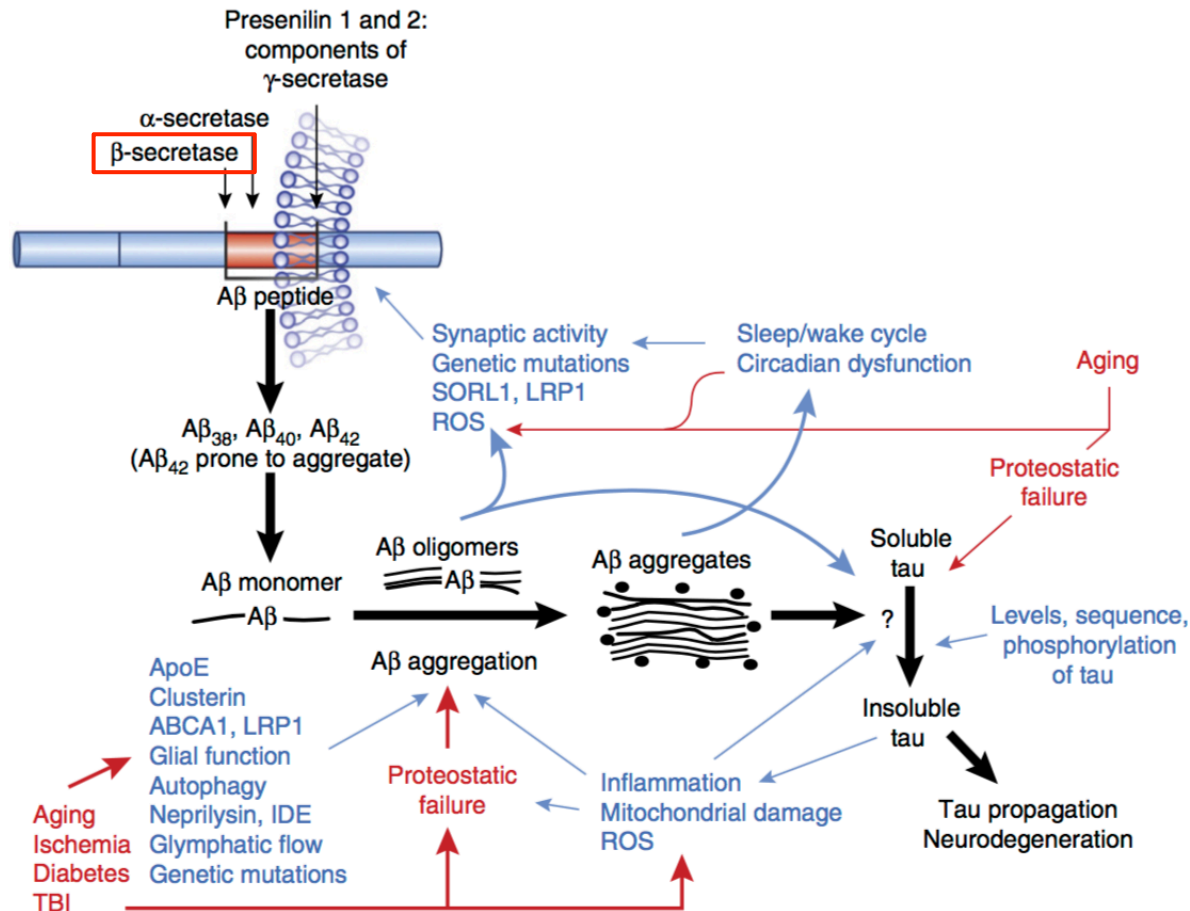
The BACE1 inhibitor verubecestat (MK-8931) reduces CNS β -amyloid in animal models and in Alzheimer's disease patients

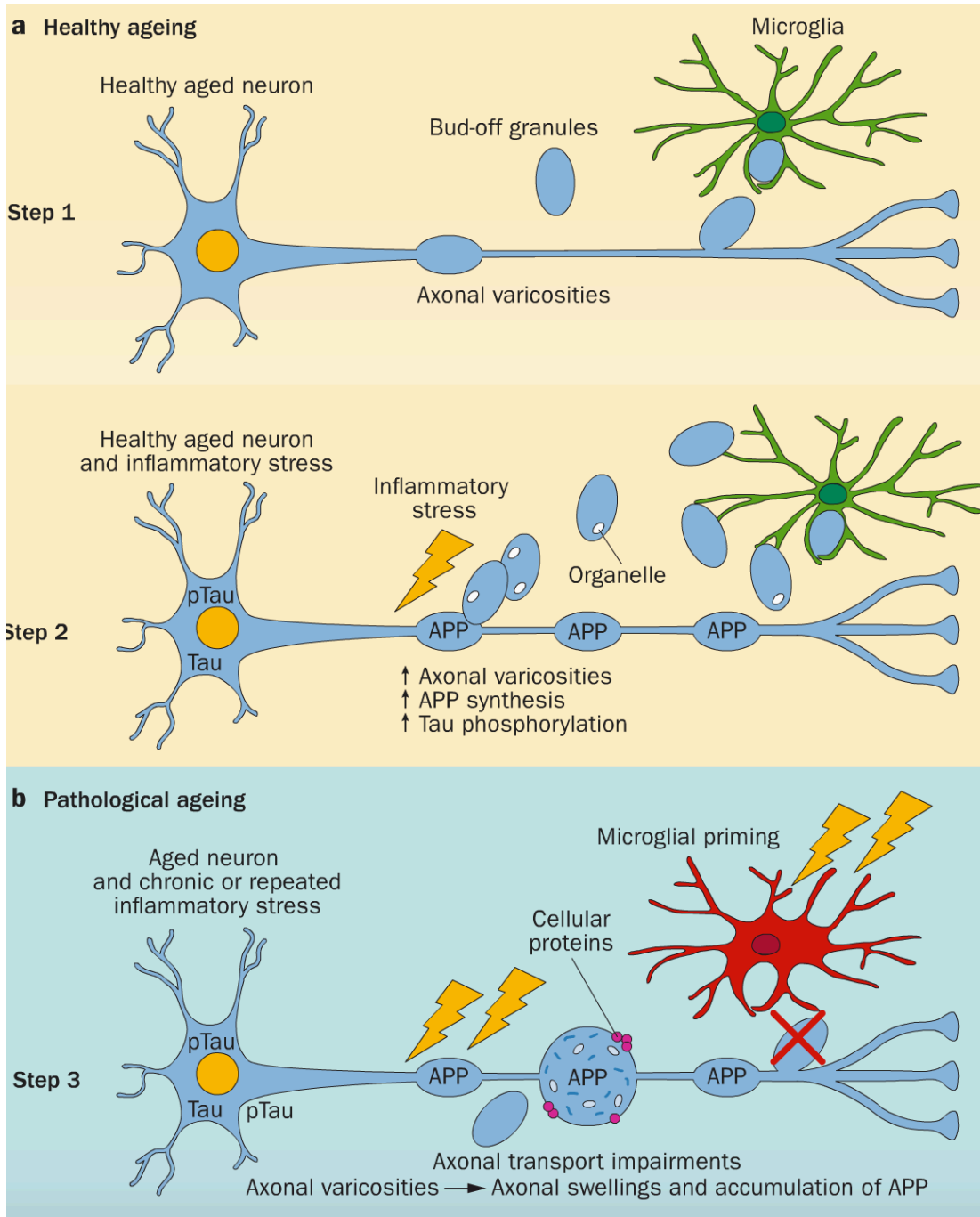
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Sci. Transl. Med. 8, 363ra150 (2016)*

b-Amyloid (Ab) peptides are thought to be critically involved in the etiology of Alzheimer's disease (AD). The aspartyl protease b-site amyloid precursor protein cleaving enzyme 1 (BACE1)(**beta-secretase**) is required for the production of Ab, and BACE1 inhibition is thus an attractive target for the treatment of AD.

BACE1 cleavage of APP produces a secreted N-terminal fragment known as soluble amyloid precursor protein b (sAPPb) and a C-terminal integral membrane protein fragment known as C99. The subsequent heterogeneous processing of C99 by g-secretase produces a family of Ab peptides, some of which (most notably Ab42) are prone to aggregate into toxic species



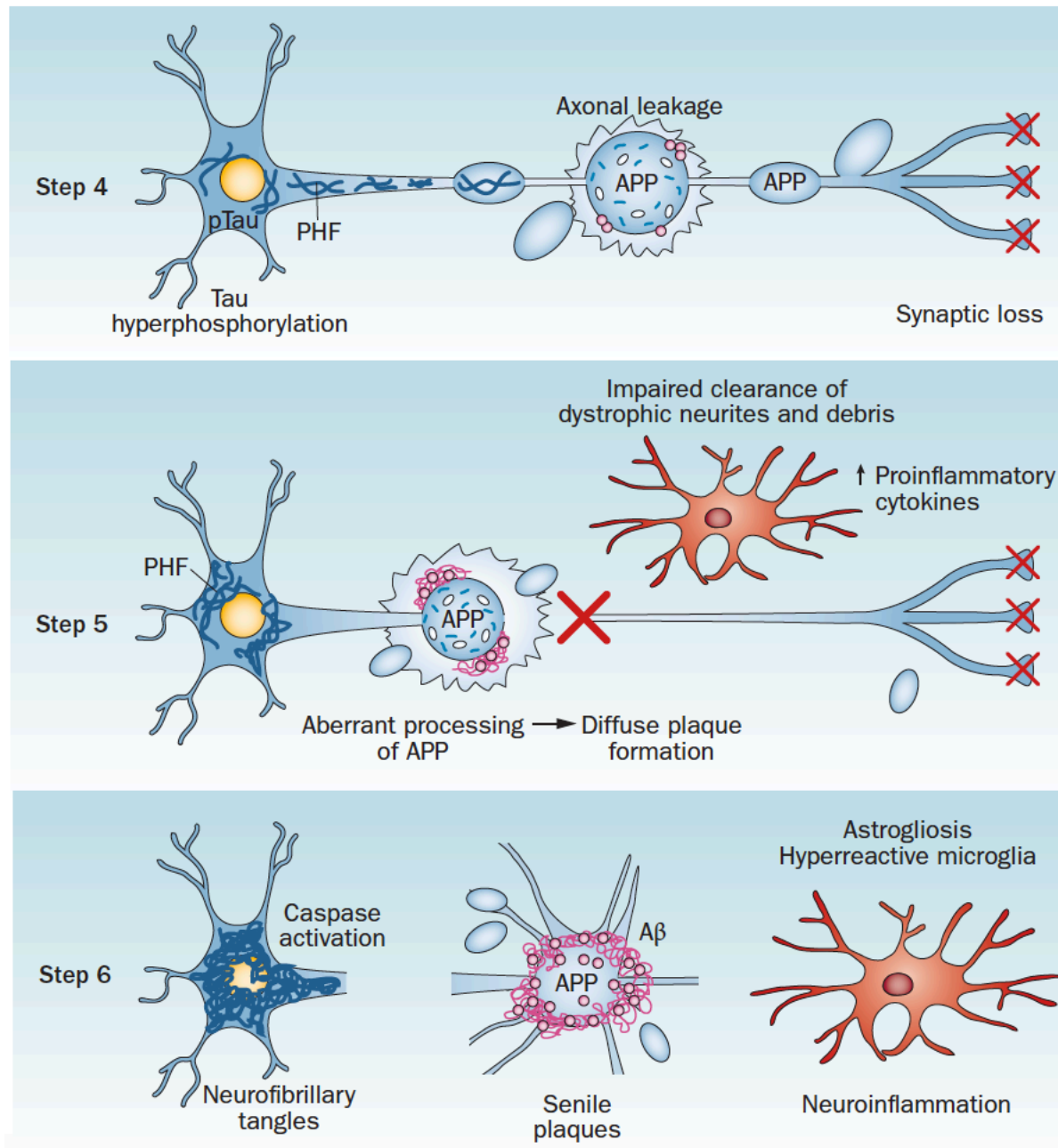


The inflammation hypothesis of late-onset Alzheimer disease.

a | During healthy ageing, a conserved protein extrusion mechanism compensates for ageing- dependent failures in protein clearance and degradation (step 1). Cellular stress to ageing neurons accelerates formation of varicosities and their extrusion into the extracellular matrix, where they are phagocytosed by surrounding glia (step 2). **If aged neurons experience chronic inflammation, tau becomes hyperphosphorylated and is missorted to somatodendritic compartments, which impairs axonal transport (steps 2 and 3).**

b | Consequently, stress-induced APP accumulates in axonal compartments and in larger swellings (step 3). Chronic inflammation also 'primes' microglia to subsequent immune challenges (step 3).

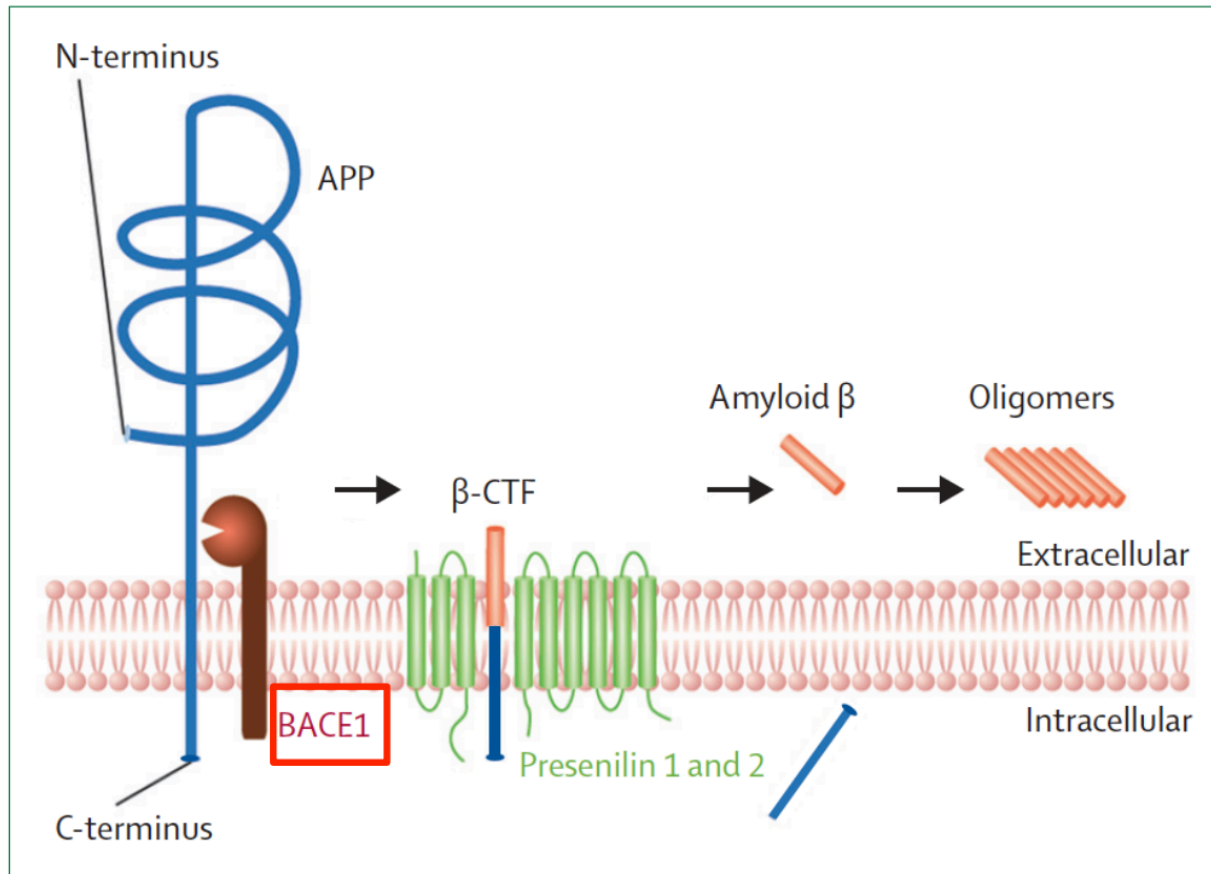
Krstic, D., & Knuesel, I. (2012). Deciphering the mechanism underlying late-onset Alzheimer disease. Nature reviews. Neurology, 9(1), 25–34



Blockade of axonal transport leads to synaptic destabilization or loss, and is accompanied by formation of PHFs in neurites and membrane leakage at axonal swellings (step 4). Axonal leakage exposes cellular proteins to lysosomal proteinases, promoting formation of neurotoxic peptides. Hyperreactive microglia cannot properly remove dystrophic neurites, and create a toxic proinflammatory environment that affects surrounding neurons. **Senile amyloid- β -plaques begin to form** (step 5). In response to neuritic degeneration, caspase activation triggers formation of neurofibrillary tangles (step 6). Imbalances in excitatory– inhibitory neurotransmission and the neurotoxic proinflammatory environment initiate pathology in interconnected brain areas.

Abbreviations: APP, amyloid precursor protein; PHF, paired helical filament.

Krstic, D., & Knuesel, I. (2012). Deciphering the mechanism underlying late-onset Alzheimer disease. *Nature reviews. Neurology*, 9(1), 25–34



Amyloidogenic processing of amyloid precursor protein

Amyloid precursor protein (APP) is a type 1 transmembrane protein that is sequentially cleaved by two aspartate proteases. **β-site APP cleaving enzyme 1 (the β-secretase BACE1)** cleaves the protein to yield a C-terminal fragment (β-CTF) and secreted soluble peptide APPβ. β-CTF is then processed by presenilin 1 and 2 (part of the γ-secretase complex) to release the amyloid β peptide. The process results in differentially truncated C-termini, ranging from amino acid 37 to 42. **The 42-aminoacid form (Aβ1–42) has a particularly strong tendency to form soluble oligomers and fibrils.** These Aβ aggregates bind to cell-surface receptors on microglia, inducing an inflammatory activation that results in the secretion of proinflammatory cytokines, including TNFα and interleukin 1β. In this context, it has been shown that interleukin 1β aggravates plaque formation by modulation of APP expression. Additionally, expression of BACE1 is upregulated by some cytokines, resulting in increased production of Aβ species.

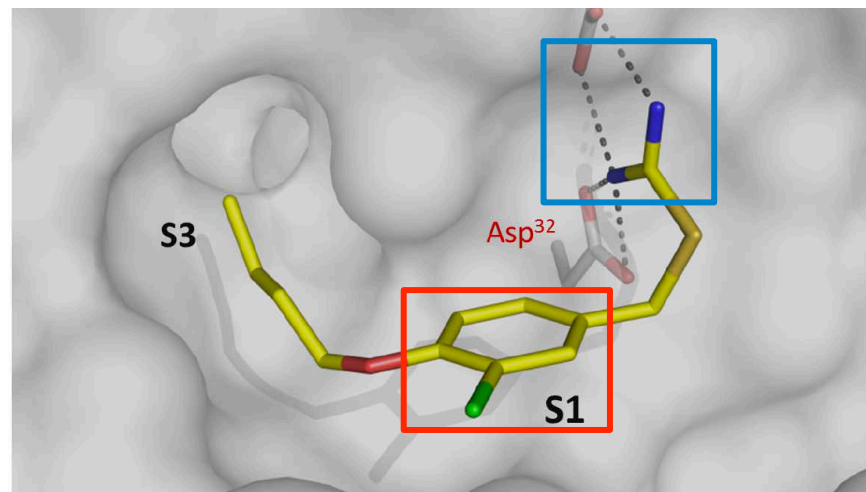
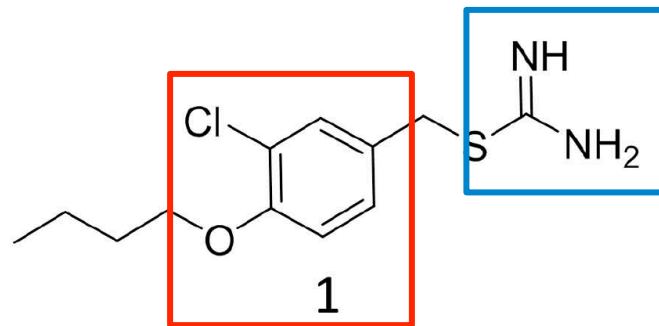
Heneka, M., Carson, M., Khoury, J., Landreth, G., Brosseon, F., Feinstein, D., Jacobs, A., et al. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*, 14(4), 388–405.

Heneka, M., Carson, M., Khoury, J., Landreth, G., Brosseon, F., Feinstein, D., Jacobs, A., et al. (2015). Neuroinflammation in Alzheimer's disease. The Lancet Neurology, 14(4), 388–405.

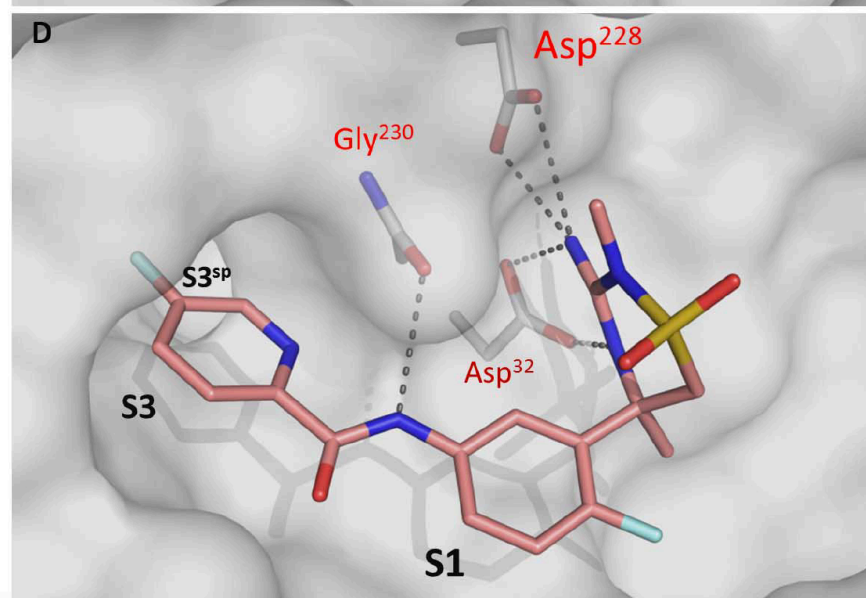
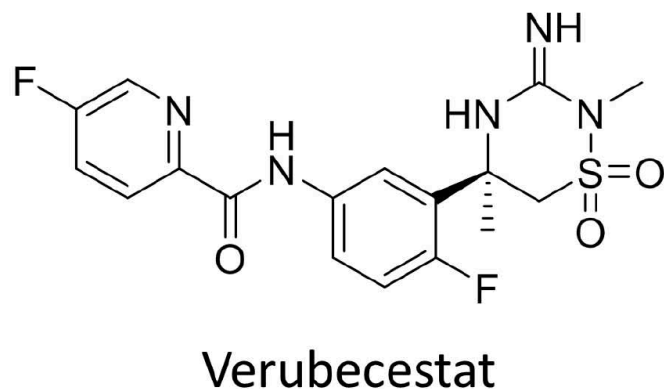
Jonsson, T., Atwal, J., Steinberg, S., Snaedal, J., Jonsson, P., Bjornsson, S., Stefansson, H., et al. (2012). A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature, 488(7409), 96–99.

A coding mutation (A673T) in the APP gene protects against Alzheimer's disease and cognitive decline in the elderly without Alzheimer's disease. **This substitution is adjacent to the aspartyl protease b-site in APP, and results in an approximately 40% reduction in the formation of amyloidogenic peptides in vitro.** The strong protective effect of the A673T substitution against Alzheimer's disease provides proof of principle for the hypothesis that reducing the b-cleavage of APP may protect against the disease. Furthermore, as the A673T allele also protects against cognitive decline in the elderly without Alzheimer's disease, the two may be mediated through the same or similar mechanisms.

Verubecestat (MK-8931) is a potent, selective, structurally unique BACE1 inhibitor that reduced plasma, cerebrospinal fluid (CSF), and brain concentrations of Ab40, Ab42, and sAPPb (a direct product of BACE1 enzymatic activity) after acute and chronic administration to rats and monkeys.



c



Discovery of verubecestat from a weakly active fragment lead using structure-based drug design. X-ray crystallography of the isothiourea fragment lead 1 (A) and verubecestat (C) bound to the human BACE1 soluble enzymatic domain were carried out as described (41). The x-ray cocrystal structure of the fragment lead 1 bound to the active site of human BACE1 determined at 1.8 resolution is shown in (B). Verubecestat bound to the active site of human BACE1 determined at 1.74 resolution is shown in (D). Hydrogen bonds are indicated by the dashed lines. S1, subsite 1; S3, subsite 3; S3sp, subsite 3 subpocket.

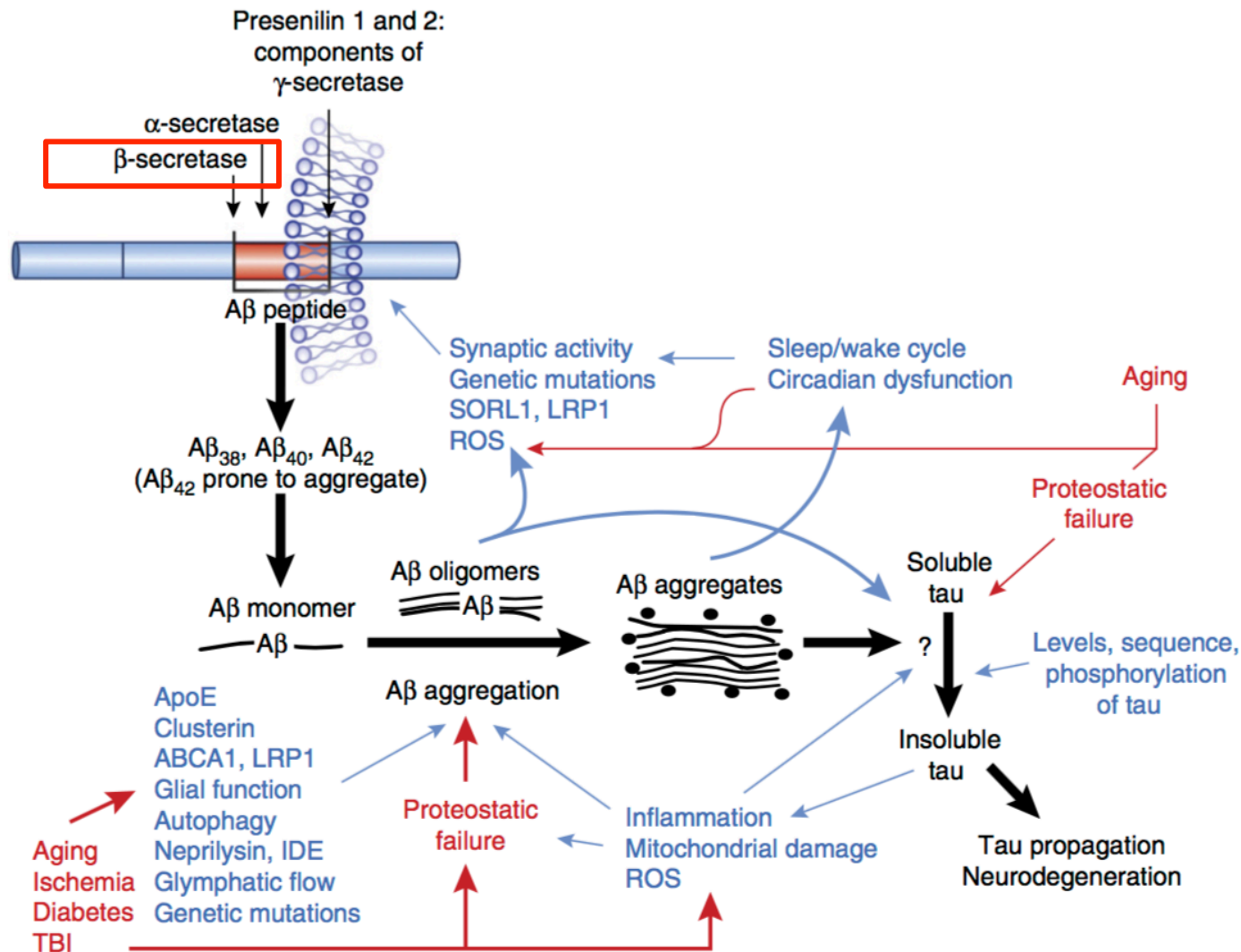
Kennedy et al., Sci. Transl. Med. 8, 363ra150 (2016)

Table 1. Potency of verubecestat to inhibit BACE1 and other human aspartyl proteases. Data are means \pm SD of results from two to five independent experiments performed in duplicate. h, human; m, mouse; HEK293, human embryonic kidney–293.

| Enzyme or cell line | Verubecestat K_i or IC_{50} (nM) |
|---------------------|--------------------------------------|
| hBACE1 | 2.2 \pm 1.4 |
| mBACE1 | 3.4 \pm 0.68 |
| hBACE2 | 0.38 \pm 0.37 |
| hCatD | >>100,000 |
| hCatE | >>100,000 |
| hPepsin | >>100,000 |
| hRenin | 33,800 \pm 12,640 |
| HEK293 A β 40 | 2.1 \pm 1.8 |
| HEK293 A β 42 | 0.7 \pm 0.09 |
| HEK293 sAPP β | 4.4 \pm 1.4 |

Verubecestat is a potent inhibitor of purified human and mouse Bace1 [inhibition constant (K_i) = 2.2 and 3.4 nM, respectively] and also inhibits Ab40, Ab42, and sAPPb production in human cells with similar potency [median inhibitory concentration (IC_{50}) = 2.1, 0.7, and 4.4 nM, respectively; Table 1]. The compound is also a potent inhibitor of purified human BACE2 (K_i = 0.38 nM; Table 1), a structurally related aspartyl protease (3). Verubecestat is essentially inactive in the purified human aspartyl proteases cathepsin D (CatD), cathepsin E (CatE), and pepsin (>45,000-fold selectivity) and is a very weak inhibitor of purified human renin (15,000-fold selectivity).

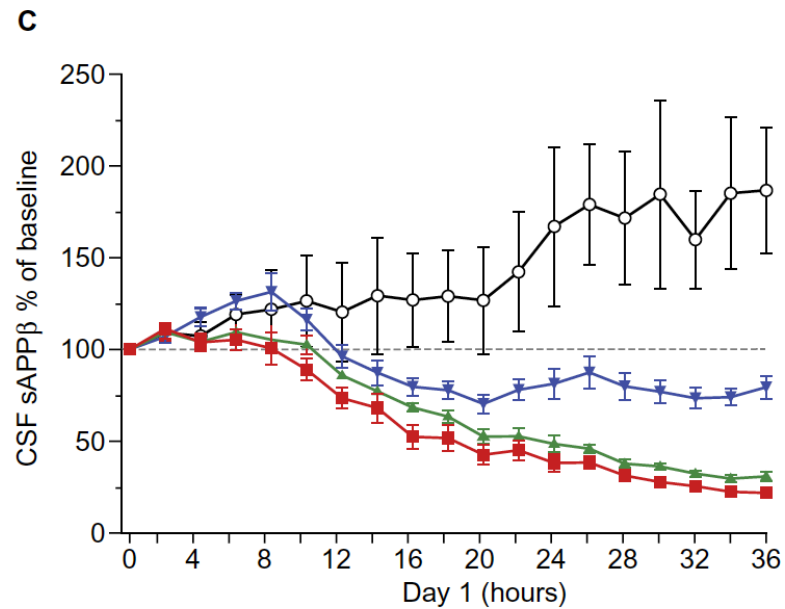
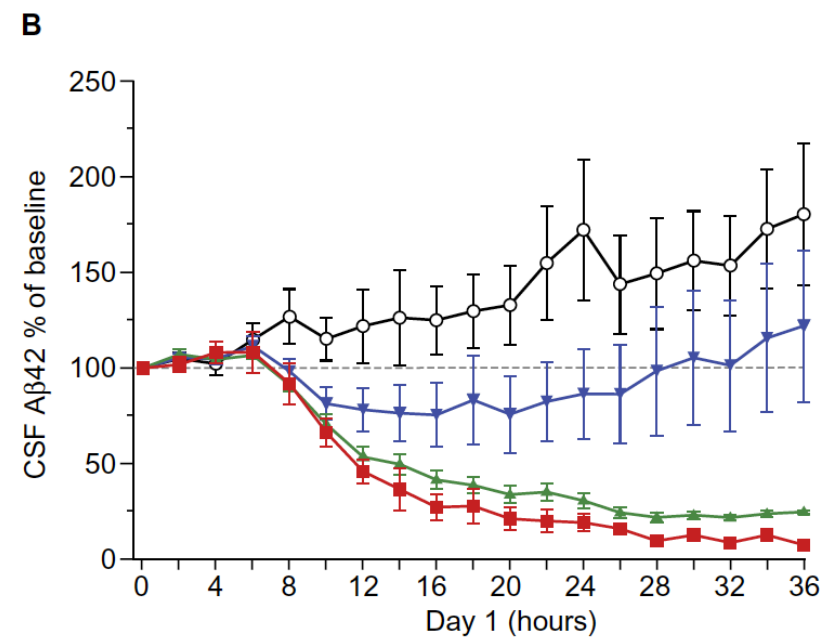
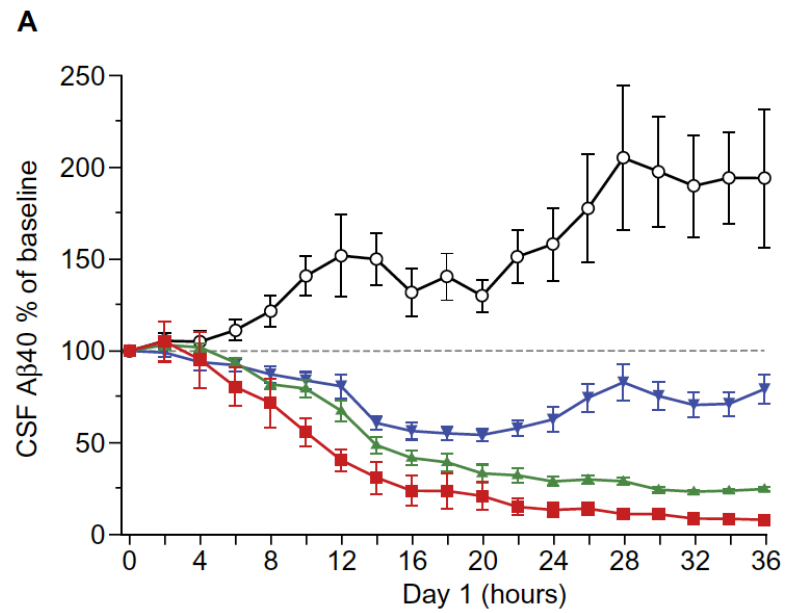
Kennedy et al., Sci. Transl. Med. 8, 363ra150 (2016)



An updated framework of the amyloid hypothesis. The black arrows illustrate the processing of APP by β - and γ -secretases to yield A β species, which subsequently aggregate, ultimately triggering tau aggregation and downstream toxicity. Blue text and arrows illustrate proposed modifiers of the A β cascade, and red text and arrows show the influence of aging and comorbid pathologies. Note that several feedforward cycles are hypothesized, including one involving disturbed sleep promoting A β production (and perhaps A β clearance, although not depicted), whereas A β aggregation in turn disrupts sleep cycles. Multiple factors, from aging to oxidative stress, contribute to proteostatic failure, which in turn promotes aggregation of A β , tau and likely other toxic proteins. Many of the A β -modifying factors interact with each other (such as ApoE modulating inflammation), although this is not depicted. IDE, insulin-degrading enzyme; ROS, reactive oxygen species; TBI, traumatic brain injury.

Musiek, E., & Holtzman, D. (2015). Three dimensions of the amyloid hypothesis: time, space and "wingmen". *Nature neuroscience*, 18(6), 800–6.

Single and multiple doses were generally well tolerated and produced reductions in Ab40, Ab42, and sAPP β in the CSF of both healthy human subjects and AD patients.



Oral administration of verubecestat to healthy nonelderly adults.

Subjects with indwelling lumbar catheters in the intrathecal space were orally administered placebo or single doses of 20, 100, or 550 mg of verubecestat. CSF and blood samples were obtained before dosing and every 2 hours for 36 hours after dosing via lumbar puncture or via indwelling catheters. Single doses of verubecestat reduced CSF A β 40 (A), A β 42 (B), and sAPP β (C).

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Because of its favorable initial safety profile and its ability to markedly reduce CSF Ab and sAPP β concentrations, verubecestat was the first BACE inhibitor to progress to phase 3 clinical trials. The EPOCH phase 2/3 trial (ClinicalTrials.gov identifier:NCT01739348) is testing the impact of 18 months of treatment with 12 and 40 mg of verubecestat on cognitive and functional measures in ~2000 subjects with mild to moderate AD. Because the degree of Ab reduction required to improve cognition and/or slow the progression of AD is unknown and because there may be unforeseen adverse effects of chronic, nearly complete BACE1 inhibition in humans, the 12- and 40-mg doses will test the effects of both partial and near-maximal Ab reduction. Partial reduction of Ab has positive effects on cognition and plaque levels in animal models (7, 8), and human genetic data suggest that modest changes in Ab production significantly modify the risk of AD (5, 9).

5. *E. S. Musiek, D. M. Holtzman, Three dimensions of the amyloid hypothesis: Time, space and 'wingmen'. Nat. Neurosci. 18, 800–806 (2015).*

7. M. Ohno, S. L. Cole, M. Yasvoina, J. Zhao, M. Citron, R. Berry, J. F. Disterhoft, R. Vassar, BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mice. *Neurobiol. Dis.* 26, 134–145 (2007).

8. L. McConlogue, M. Buttini, J. P. Anderson, E. F. Brigham, K. S. Chen, S. B. Freedman, D. Games, K. Johnson- Wood, M. Lee, M. Zeller, W. Liu, R. Motter, S. Sinha, Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP transgenic mice. *J. Biol. Chem.* 282, 26326–26334 (2007).

9. *T. Jonsson, J. K. Atwal, S. Steinberg, J. Snaedal, P. V. Jonsson, S. Bjornsson, H. Stefansson, P. Sulem, D. Gudbjartsson, J. Maloney, K. Hoyte, A. Gustafson, Y. Liu, Y. Lu, T. Bhangale, R. R. Graham, J. Huttenlocher, G. Bjornsdottir, O. A. Andreassen, E. G. Jónsson, A. Palotie, T. W. Behrens, O. T. Magnusson, A. Kong, U. Thorsteinsdottir, R. J. Watts, K. Stefansson, A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature 488, 96–99 (2012).*

Because deposition of amyloid begins several years before AD is diagnosed, it is possible that administration of an anti-amyloid agent will be more effective if given early in the disease process (5). Consequently, the APECS phase 3 trial (ClinicalTrials.gov identifier: NCT01953601) is being conducted to test the effect of 2 years of treatment with 12 and 40 mg of verubecestat on cognitive and functional measures in ~1500 subjects with prodromal AD who have significant amyloid deposition, as measured by amyloid positron emission tomography imaging. Given that the doses being tested in the ongoing phase 3 trials reduce CSF Ab by >80% and assuming that the compound continues to demonstrate an acceptable safety and tolerability profile, these trials will be able to determine whether verubecestat can be a much-needed disease modifying treatment for AD.

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5. E. S. Musiek, D. M. Holtzman, Three dimensions of the amyloid hypothesis: Time, space and 'wingmen'. Nat. Neurosci. 18, 800–806 (2015).

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

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^dNatural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany

^eADx NeuroSciences, Gent, Belgium

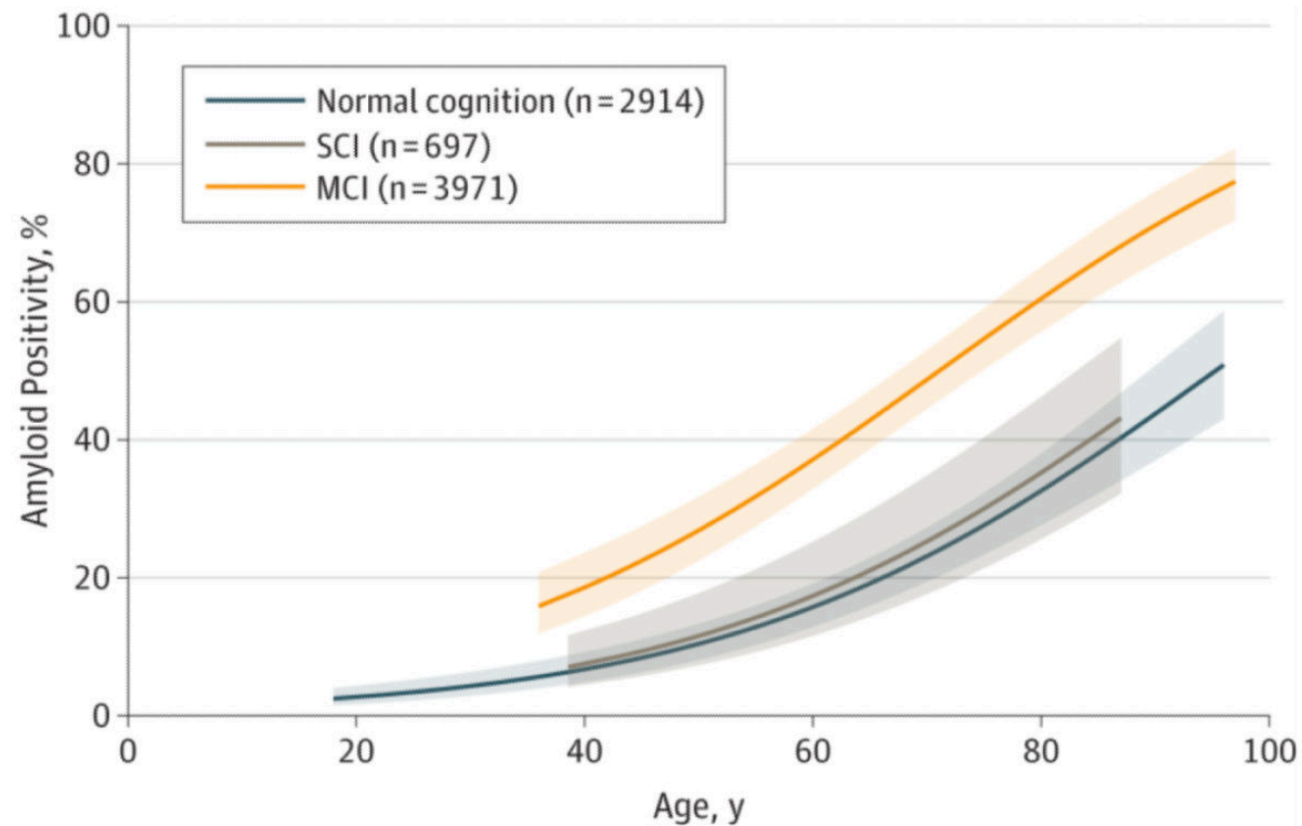
^fDZNE, German Center for Neurodegenerative Diseases, Bonn, Germany

^gNovartis Institutes for BioMedical Research, Neuroscience, Basel, Switzerland

We demonstrate that long-term BACE1 inhibition prevents CSF tau increase both in early depositing APP transgenic mice and APP transgenic mice with moderate Ab pathology. Our results demonstrate that BACE1 inhibition not only reduces Ab generation but also downstream AD pathophysiology.

The tight correlation between Ab aggregation in brain and CSF tau levels renders CSF tau a valuable marker to predict the effectiveness of BACE inhibitors in current clinical trials.

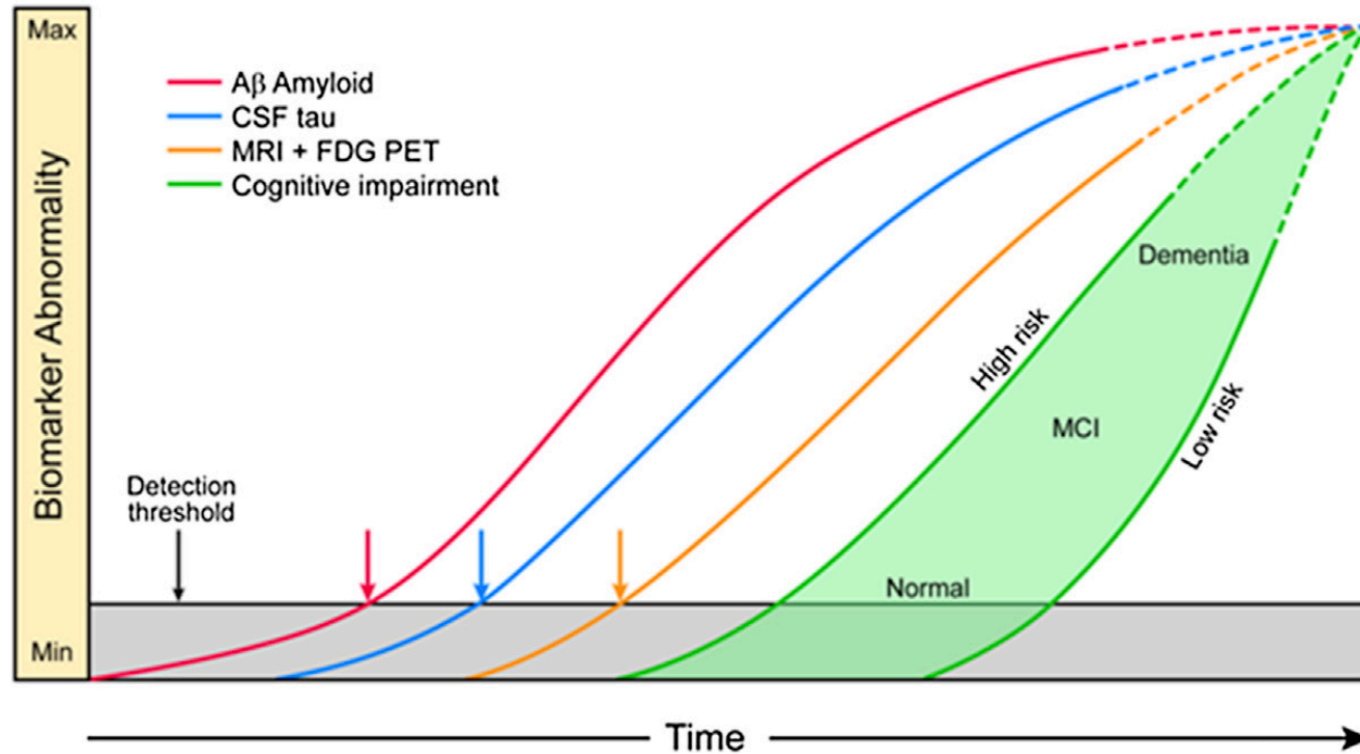
Schelle, J., Häslér, L., Göpfert, J., Joos, T., Vanderstichele, H., Stoops, E., Neumann, U., et al. (2016). Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. *Alzheimer's & Dementia*..1-9 doi:10.1016/j.jalz.2016.09.005



Association of Age With Prevalence Estimates of Amyloid Positivity According to Cognitive Status

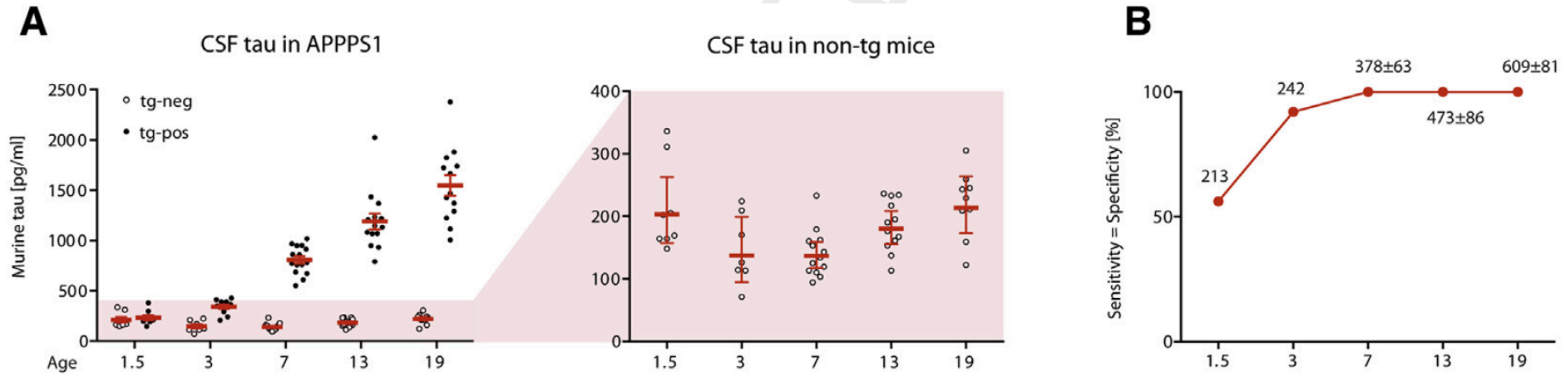
The prevalence estimates were generated from generalized estimating equations. The model included age and cognitive status as predictors. Shading indicates 95% CIs; SCI, subjective cognitive impairment; MCI, mild cognitive impairment.

Jansen, W.; Ossenkoppele, R.; Knol, D.; Tijms, B.; Scheltens, P.; Verhey, F.; Visser, P.; Group, A.; Aalten, P.; Aarsland, D.; et al. Prevalence of Cerebral Amyloid Pathology in Persons Without Dementia: A Meta-Analysis. *Jama* 2015, 313, 1924–1938.



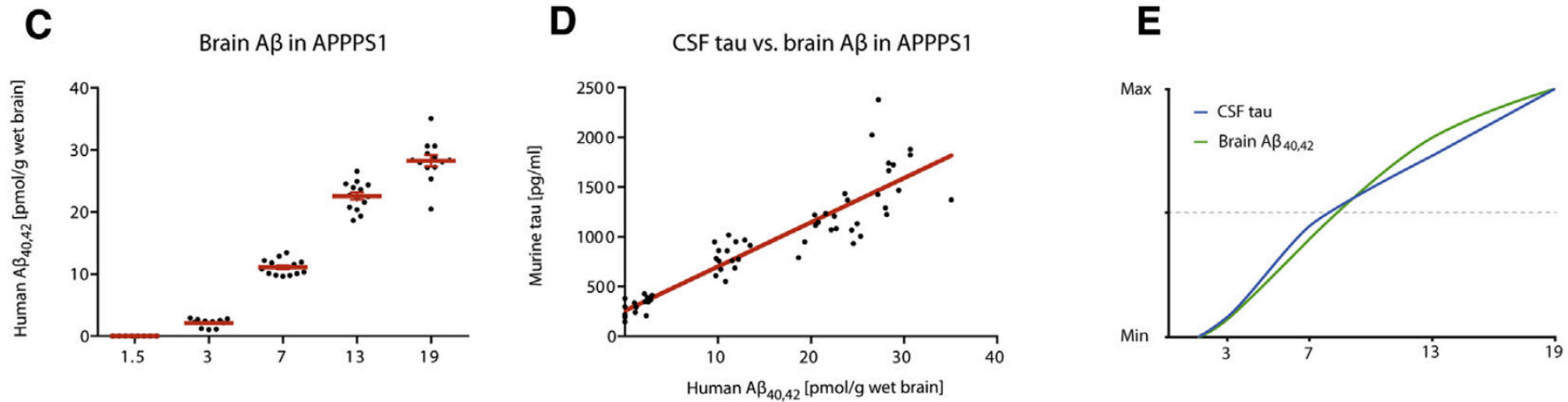
Biomarker model of pure AD. The horizontal axis represents time and the vertical axis severity of biomarker abnormality from completely normal (min) to abnormal (max). The threshold for biomarker detection of pathophysiology is denoted by a horizontal black line. The gray area denotes the zone in which abnormal pathophysiology lies below the biomarker detection threshold. Amyloid biomarkers become abnormal first, followed by CSF tau, followed by FDG PET and MRI. Cognitive impairment (green filled area) is the last event in the progression of the disease. A range of cognitive responses are possible that depend on the individual's risk profile. The cognitive response curve is shifted to the left for those with low cognitive reserve and to the right for those with high cognitive reserve.

Jack, C., & Holtzman, D. (2013). Biomarker modeling of Alzheimer's disease. Neuron, 80(6), 1347–58.



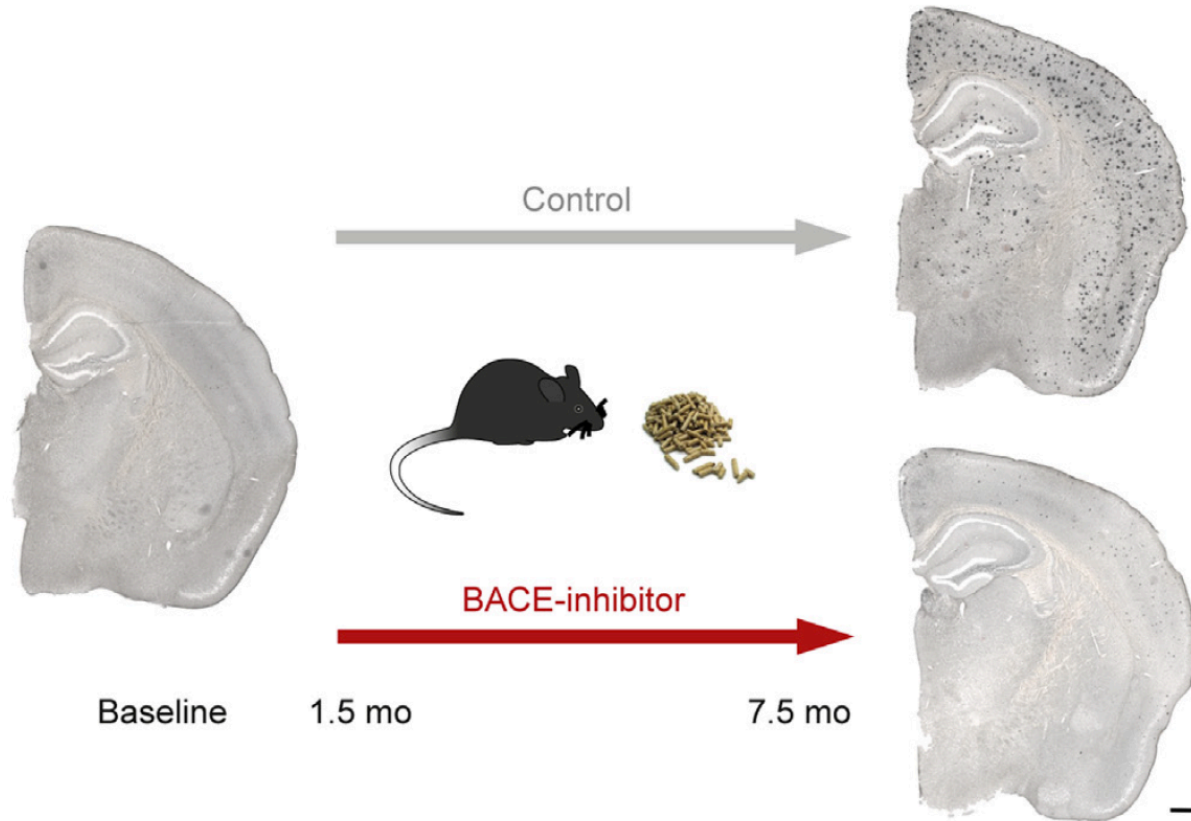
Age-dependent increase of CSF tau and brain Ab in APPPS1 mice. (A) CSF tau was measured in 1.5- to 19-month-old male APPPS1 mice and non- tg littermates (n 5 7–15 per group) using a highly sensitive novel tau assay. Two-way analysis of variance (ANOVA) revealed significant age- genotype interaction [F(4, 100) 5 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.

Schelle, J., Häslér, L., Göpfert, J., Joos, T., Vanderstichele, H., Stoops, E., Neumann, U., et al. (2016). Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. *Alzheimer's & Dementia*..1-9 doi:10.1016/j.jalz.2016.09.005



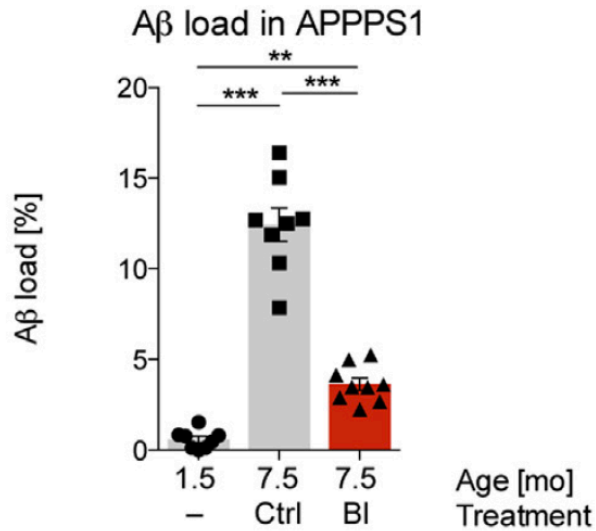
(C) Total brain Ab levels (sum of Abx –40 and Abx –42, i.e., the species ending at amino acid 40 and 42) were measured in the same tg mice and revealed a robust increase with aging [ANOVA: $F(4, 56) = 2101$, $P < .001$]. The Tukey post hoc test indicated a significant difference among all age groups ($P < .001$), except between 13 and 19 months. (D) The relationship between CSF tau (A) and brain Ab concentration (C) is best described by a linear regression ($r^2 = 0.84$, $P < .001$) indicating a strong positive correlation (the Spearman rank correlation test: $r = 0.94$, $P < .001$). (E) The percentage increase of total brain Ab (green) and CSF tau (blue) in APPPS1 mice shows a very similar profile with aging. The curves are based on the mean values of panels (A) and (C).

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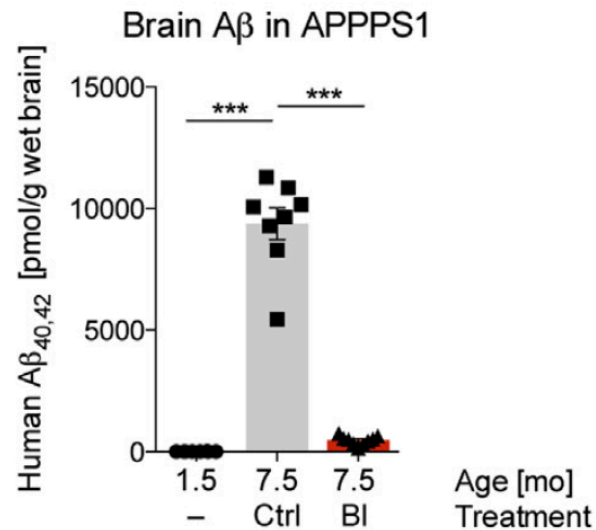
A

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 μ m).

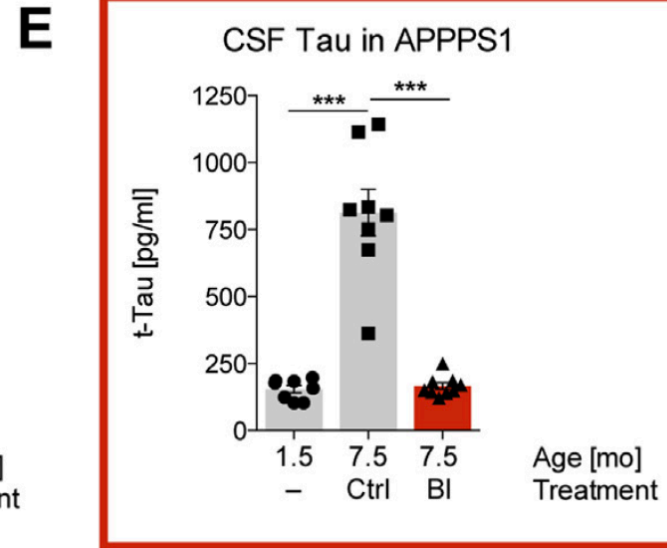
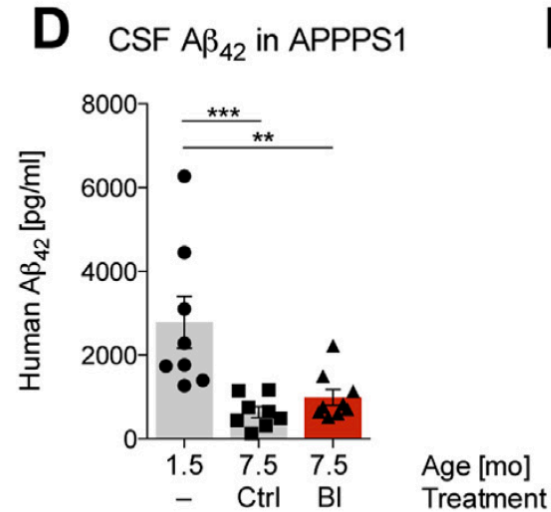
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B

(B,C) Stereological quantification of neocortical Ab immunostaining (Ab load) and measurements of Ab levels in brain exhibited significant increases with age, which could largely be blocked by BACE1 inhibition [analysis of variance (ANOVA) F(2, 22) 5114.6 and 209.5, respectively, Ps , .001; the Tukey post hoc test **P <0.1, ***P <0.01].

C

Schelle, J., Häslér, L., Göpfert, J., Joos, T., Vanderstichele, H., Stoops, E., Neumann, U., et al. (2016). Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. Alzheimer's & Dementia..1-9 doi:10.1016/j.jalz.2016.09.005



(D) CSF A β_{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) 5 13.58, P 5.0001; the Tukey post hoc test **P ,.01, ***P ,.001]. A similar result was found for A β_{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) 557.01, 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 \pm 6 standard errors of mean.

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