The case for low-level BACE1 inhibition for the prevention of Alzheimer's disease

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Summary

Alzheimer's disease (AD) is the most common cause of dementia in the elderly with a long presymptomatic phase. Disease-modifying or preventive therapies for AD are not yet available. Small molecule inhibitors of the protease β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1, β-secretase), which reduce the production of amyloid-β peptide, are among the most advanced drug candidates against AD. Yet, the phase 2 and 3 clinical trials were concluded without benefit or discontinued due to futility or occurrence of side effects, including

mild, early, non-progressive and reversible but consistent cognitive impairment. This has raised questions regarding the suitability of BACE1 as a drug target for AD. This commentary discusses the status of BACE1 inhibitors, suggests how the recent and upcoming trial results can inform future development of preventive clinical trials, proposes experiments that should be performed for go-no-go decisions, and considers the possibility that low levels of BACE1 inhibition (<50%) may avoid side effects yet still achieve efficacy for AD prevention.

Introduction

A large body of genetic, molecular, cellular, animal model, and human data strongly suggest that the cerebral accumulation of the β-amyloid peptide (Aβ) plays a central, early role in the pathogenesis of AD. This evidence forms the foundation of the amyloid hypothesis and has been a major focus of AD drug development[1,2]. Besides Aβ, additional key proteins and cellular processes contribute to AD pathogenesis, particularly in later phases[2]. The discovery of BACE1 as the β-secretase that initiates Aβ production spurred intense efforts to develop small molecule drugs that inhibit this enzyme[3-6] (Box 1). Consequently, several pharmaceutical companies advanced BACE1 inhibitors into non-clinical and clinical studies[7]. Disappointingly, BACE inhibitor trials in the clinical stages of AD failed to slow AD-associated cognitive decline[8,9]. In fact, both completed and discontinued trials in the symptomatic and presymptomatic stages of AD were even associated with mild, early, non-progressive cognitive worsening and several trials were associated with early, non-progressive reductions in brain volume. These findings have caused the field to reassess the conditions, if any, under which BACE1 inhibitors should be considered in future trials. One conclusion of the trial results is that BACE1 and Aβ are the wrong therapeutic targets for AD[10], particularly at the symptomatic stages, and this is particularly relevant if the recent adverse effects identified in clinical trials is specific to Aβ. Another possibility is that BACE1 is such an important physiologic enzyme that inhibition cannot be achieved in a safe way. Similar conclusions led to the termination of γ-secretase inhibitors[11,12] several years ago, but this discontinuation is now seen by some as having occurred prematurely[13] and recent work on γ-secretase modulators has suggested

opportunities remain in targeting this enzyme (Rynearson 2021). However, before drawing similar conclusions on BACE1, it would be wise to re-evaluate the basic assumptions on which the previous trials were built, to analyze and interpret the outcomes of the BACE1 inhibitor clinical trials as objectively as possible and to study possible ways to overcome the problems before discarding a major drug target for this disease. This is particularly relevant because a) no other suitable drugs for lowering Aβ are available currently for long-term prevention (see Box 2 for alternative Aβ-lowering strategies, their current state of development and their challenges), b) small molecule drugs like BACE1 inhibitors that are orally administered may be less expensive than biologicals, at least when dosed chronically for years, c) the adverse cognitive effects in presymptomatic BACE1 inhibitor trials have now been demonstrated as being almost completely reversible in 3 months' time (REF) and d) it is unknown how long the development of drugs for other targets (REF), such as tau and neuroinflammation may take.

In this article, we will discuss findings from clinical and non-clinical studies of BACE inhibitors and suggest priorities for future research on BACE1 inhibitors to evaluate whether or not they remain a viable option for AD drug development, particularly for prevention trials in populations at high risk of developing AD. Finally, we propose the conditions that may be needed to evaluate BACE inhibitors in trials for at-risk persons who have evidence of no to minimal Aβ plaque deposition at trial start.

Looking back

BACE1 inhibitors efficiently lower soluble Aβ

During the past several years, six different BACE1 inhibitors were tested in Phase 2 or Phase 3 clinical trials at doses that achieved a reduction of Aβ levels in human CSF by up to 90% (**Table 1**), exquisitely similar to results achieved in non-clinical studies (PMID: 27807285, Umibecestat: PMID: 30224383, Lanabecestat: PMID: 26890753). Key lessons learned from

the trials were the robust dose-response relationship between drug dose and Aβ reduction and that a stable continuous level of Aβ reduction can be achieved precisely in participants with AD (e.g., stable 60% Aβ lowering). These points are especially important when considering the potential use of a consistent low-level BACE1 inhibition for long-term prevention in individuals rather than treatment of symptomatic AD. Positron emission tomography (PET) demonstrated that BACE1 inhibitors also lowered insoluble (plaque) Aβ levels in human brains by an average of 5-10% per year, as published for verubecestat[8,14] and lanabecestat[15]. Taken together, BACE1 inhibitors achieved in humans what they were designed for, namely effective target engagement and dose-dependent lowering of Aβ in the CNS. Importantly, at the time these therapies were developed, a high level of BACE1 inhibition to achieve maximal Aβ lowering (i.e. typically greater than 60% lowering) was the goal. As a result, the effect of lower BACE1 inhibition on clinical outcomes is not known as trials with lower doses have not been performed. However, the modest effects that BACE1 inhibitors have on removing established Aβ plaques indicate that the optimal therapeutic window may be prior to the onset of appreciable Aβ plaque deposition, **Figure 1,** as a preventative therapy. Moreover, the mounting evidence that the removal of established Aβ plaques in symptomatic AD results in a modest clinical benefit (REF) supports therapies that could target the accumulation of Aβ peptides prior to significant accumulation significant amyloid pathology for the prevention of cognitive impairment.

BACE1 inhibitors need to be safe for long-term treatment.

As a preventative therapy, BACE1 inhibitors would need to be taken for long periods of time, similar to antihypertensive and cholesterol-lowering medications for the prevention of cardiovascular disease related events. Thus, BACE1 inhibitors need to have an acceptable side effect profile. The dosing paradigms used in recent trials are clearly not acceptable for long term dosing. During BACE1 inhibitor development the side effects observed in non-clinical studies and later clinical trials generally fall into three classes: a) off-target, b) mediated by the BACE1 homolog, BACE2, and c) mechanism-based/BACE1-mediated.

Regarding off-target effects, some early BACE1 inhibitors partially inhibited cathepsin D and showed retinal toxicity[16,17]. Other drugs showed hepatotoxicity [9,18], which was interpreted as an off-target effect, given that most clinically tested BACE1 inhibitors and *Bace1* gene deletion in mice did not lead to hepatotoxicity. The off-target effects resulted in the discontinuation of these drugs.

All six BACE1 inhibitors that were in recent clinical trials not only block BACE1 but also inhibit BACE2, a close homolog of BACE1. Four of the recently tested BACE inhibitors block BACE1 and BACE2 with similar potency, while two of them inhibit BACE1 approximately three-fold more potently than BACE2 in *in vitro* assays, **Table 1**[19]. While BACE1 is highly expressed in brain, BACE2 appears to be mostly expressed outside of the brain, at least under non-inflammatory conditions[20]. In contrast to BACE1, BACE2 does not contribute to Aβ generation, but may even partially prevent Aβ generation[21,22]. While not seen with the more selective BACE1 inhibitors,[20,23-28] one side effect, hair discoloration, was seen in animals[23,29-31] and in less than 10% of the individuals treated with the non-selective BACE inhibitors. In isolation this may be seen as an acceptable side effect. It remains to be seen whether the cognitive worsening reported for most BACE inhibitors is partly due to BACE2 inhibition (discussed below) and whether inhibitors that are more specific to BACE1 over BACE2 would avoid such side effects.

The last class of side effects of BACE1 inhibitors observed in non-clinical studies are mechanism-based, i.e. arising from inhibition of the diverse physiological functions and substrates of BACE1[26-28] (add REF PMID: 32954517). The major side effect of concern identified in the recent trials was cognitive decline. Other important side effects, such as anxiety, weight loss, falls, suicidal ideation and sleep disturbances, were also increased in BACE1 inhibitor clinical trial participants, but not consistently across compounds and only to a mild extent[32]. For example, suicidal ideation rates increased from 6.4% on placebo to 6.8% and 9.3% on a lower and a higher dose of verubecestat in a prodromal AD population, and rate of falls and injuries increased from 20.7% on placebo to 25.4% and 25.7% on verubecestat[8]. Roughly analogous drug-placebo differences were seen with verubecestat in

patients with mild AD dementia[14] , but this adverse event profile was not seen in two large trials of lanabecestat[15]. It is not clear whether aberrant processing of single or multiple BACE1 substrates underlies these symptoms, but the latter appears quite possible, as BACE1 has dozens of substrates with important functions in the nervous system (Box 3).

Taken together, it is possible, but not yet proven that strong inhibition of cleavage of BACE1 substrates may contribute to the occurrence of the cognitive and psychiatric side effects (anxiety, suicidal ideation and sleep disturbances) in BACE1 inhibitor-treated humans[8,14].

BACE1 inhibitors need to stop, slow, or delay the onset of cognitive decline

To date, long-term trials with high-dose BACE1 inhibitors were performed in people with clinical AD, prodromal AD, or in individuals with a high genetic risk of developing AD[33]. The main clinical purpose of BACE1 inhibitors is to stop or slow disease progression or delay the onset of the cognitive decline in AD. In all instances, BACE1 inhibitors have either been stopped for toxicity or futility[14]. The degree of the clinical and cognitive worsening reported was 0.5 points for the high dose in the CDR-sum of boxes (range 0-18; Cohen's d of -0.32 for cognitive decline on the Repeatable Battery of Neuropsychological Status (RBANS))[8],[9,34] and had not been seen in the prior Phase 1 trials, presumably because they were not powered or long enough to reveal a mild effect on cognition. Additionally, a notable reduction of hippocampal volumes, as measured by magnetic resonance imaging (MRI), has been reported for most BACE1 inhibitor trials with sufficient longitudinal data[8,14,15]. Both the cognitive and MRI effects were observed within three months after the treatment was started and did not appear to worsen over time, raising the possibility that they are not related to worsening of the underlying disease itself.

Both the exacerbated cognitive worsening and hippocampal volume loss are seen as severe side effects that need to be resolved if BACE1 inhibitors are to be further tested in clinical trials. The cognitive worsening caused by exposure to umibecestat is almost completely reversible (Cohen's d changed from -0.32 to -0.06) within 3 months after taking trial participants off the drug (since the trials included safety follow up after drug discontinuation, blinded to prior treatment condition), and has been suggested the same for atabecestat[34-36]. This is an

important finding because it suggests that the cognitive side effects do not result from an acceleration of neurodegeneration. This observation leaves open the possibility that lower BACE1 inhibitor doses may avoid this side effect altogether, which, however, still needs to be shown. Likewise, the presence of MRI volumetric changes upon treatment with BACE inhibitors that has been consistently identified also appears to improve with dose discontinuation, as suggested for umibecestat in a preliminary analysis[34]. More follow-up analyses are required, ideally also for some of the other BACE inhibitors tested clinically. At a minimum, these observations offer the opportunity to propose rational trial designs that could protect trial participants from long-term cognitive harm which could help regulatory agencies in reconsidering BACE1 for clinical testing.

While we await additional data on the most recently discontinued BACE1 inhibitor trials to further compare results across all compounds, in the following sections we look forward and discuss if and under what conditions BACE1 inhibition may remain a viable option for AD drug development.

Looking forward

Would dose-lowering prevent side effects of BACE1 inhibitors but retain sufficient Aβ lowering?

For a BACE1 inhibition therapeutic strategy optimized for prevention, a low dose of BACE1 inhibitor administered over years may be fully sufficient to halt or delay the onset of AD pathology and/ or symptoms and decrease the chance of side effects. In support of this claim, heterozygous *Bace1* gene deleted mice, which correspond to 50% BACE1 inhibition[37], showed less of an axon targeting defect in the olfactory bulb compared to mice completely deficient in BACE1[38] and in a separate study resulted in a dramatic reduction in Aβ pathology[37]. Importantly, heterozygous BACE1-deficient mice had normal performance in multiple

behavioral assays, including memory tests[39,40]. Moreover, mice with a genetic reduction of BACE1 by about 50% had normal myelination[41], normal hippocampal structure, and only a mild reduction in LTP[42], suggesting that chronic BACE1 inhibition, but less than 50%, may reduce or prevent the occurrence of side effects such as cognitive worsening. Moreover, there is good evidence of a therapeutic window at lower concentrations of BACE1 inhibitor: at 3 nM concentration APP processing can be inhibited by ~40%, while significant inhibition of BACE dependent neuronal growth cone collapse requires above 300 nM[43]. A second, and perhaps the strongest, line in support of this claim is the Icelandic *APP* gene mutation (A673T) which leads to an approximately 30% life-long reduction of BACE1 cleavage of APP and provides protection against AD[44,45]. However, this mutation also reduces aggregation of the Aβ peptide, which might provide another protection against amyloid pathology[46]. Nevertheless, the Icelandic mutation offers a strong rationale for what level of BACE1 inhibition to target for modest Aβ lowering over long periods of time to prevent AD. Taken together, the heterozygous mouse and Icelandic APP mutation studies strongly suggest that chronic BACE1 inhibitor doses below 50% inhibition, and likely closer to 30%, may be sufficient to delay or even prevent the onset of clinical AD symptoms, if started before Aβ pathology has reached saturation, while the in vitro experiment demonstrates that there is a therapeutic window versus biologically relevant other substrates. However, in the absence of clear data to suggest lower levels of inhibition (e.g. 10%) have an effect on preventing or reversing Aβ plaques, using doses this low would significantly risk the ability to have the necessary target engagement of Aβ needed for long term prevention. Should other Aβ therapies like the passive immunotherapies continue to demonstrate the ability to more rapidly clear Aβ plaques and have clear benefit, it is reasonable to consider that a BACE1 inhibitor in a combination paradigm could be used in the long term to maintain lower Aβ plaque loads following removal by immunotherapies.

Avoidance of side effects may not only be achieved with the chronic, low dose BACE1 inhibition described above, but potentially also with an intermittent dosing paradigm, even at the higher doses used previously. While this is a conceptually different approach, intermittent dosing is frequently used for cancer treatment, where a high dose chemotherapy is given for a short

period of time followed by a recovery phase without dosing before the next therapeutic cycle starts. This treatment strategy is accepted by patients with a tumor diagnosis. However, the risk-benefit evaluation is different for a potential AD prevention, where individuals are still cognitively unimpaired and may be less willing to accept the risk of cognitive worsening even if it was only temporary. Thus, an intermittent dosing strategy at the previously used higher doses would require a treatment time that is shorter than the time of occurrence of cognitive worsening, which still needs to be determined. However, there remain significant challenges in implementing this approach in a population beyond the dominantly inherited AD as it requires a precise determination of when an individual is at a point of very early Aβ plaque development (below current detection thresholds) where the inhibition of BACE1 would be enough to result in the long-term suppression of future growth, as has been demonstrated in preclinical models of AD-transgenic models with gamma secretase inhibitors (Das et al).

Timing of BACE1 inhibition: prevention versus treatment of AD. While there is a large body of evidence demonstrating a key role for Aβ in AD pathogenesis, it appears likely that Aβ deposition has a more prominent role early prior to substantial neurodegeneration, therefore removing Aβ plaques over a prolonged period of time late in the disease process is unlikely to substantially reverse all AD pathogenesis (Figure 1). Additionally, work in transgenic mice has indicated that the secretase inhibitors are more effective at preventing the formation of Aβ plaques[47] rather than reversing them once they are formed[41], suggesting that lowering Aβ production with BACE1 inhibitors at late stages of Aβ accumulation is unlikely to have a significant disease modifying effect as it would likely take many years to substantially lower established Aβ plaques. Likewise, given the availability of therapies that have demonstrated the ability to remove established Aβ plaques (REF) much more efficiently than BACE1 inhibitors, it makes sense that this class of therapies would be used in an Aβ plaque prevention approach.

No trials to date have been completed in participants when Aβ accumulation is low or absent[48-50]. However, the discontinued Alzheimer Prevention Initiative (API) Generation Studies 1 and

2 have recently provided information about the early cognitive and imaging biomarker effects in more than 1,600 unimpaired 60-75-year-old individuals who were APOE4 homozygotes and heterozygotes, including more than 250 Aβ-negative homozygote individuals[34] exposed to umibecestat or placebo. Because a large number of participants had early cognitive testing (13 weeks) and had visits after the discontinuation of the trial, the Generation results have addressed important uncertainties highlighted in other trials, specifically: adverse cognitive effects can be identified as early as 13 weeks, remain stable and are nearly fully reversible within three months of stopping drug, as described above; cognitive effects varied by APOE4 and Aβ PET level with higher Aβ levels being associated with greater cognitive decline; a drop-out rate of three percent of umibecestat to one percent for placebo suggesting the cognitive worsening did not have a clear effect on discontinuation; evidence that the hippocampal atrophy may reverse with drug discontinuation and was not clearly linked to cognition. Together these findings provide important guidance in developing safer trials (see below) and informing potential participants with a more accurate estimate of the risks of enrolling in BACE1 inhibitor trials. A primary prevention test of the amyloid hypothesis (i.e. before the onset of substantial amyloid induced pathological changes) could be started in populations at greatest risk for developing AD including in Aβ-negative APOE4 homozygotes in the 6th and 7th decades as well as in dominantly inherited AD mutation carriers one to two decades before symptom onset, where the majority of mutations are associated with an alteration in the enzymatic processing of APP. Importantly, the development of secretase inhibitors as a potential therapy for the treatment of AD was based on transgenic mouse models using mutations identified in dominantly inherited AD, and have primarily been tested in these models at a stage of amyloid pathology most consistent with primary prevention in humans. Therefore, the greatest potential for translating the information learned in preclinical studies may be in those with dominantly inherited AD mutations first (Mills et al; Reiman et al). A major advantage of primary prevention is that a BACE1 inhibitor may not need to be dosed chronically, but only for a limited time, which – at least in mice – resulted in sustained reduction of amyloid pathology in late life (PMID:

22892055, PMID: 19369565, PMID: 33199898). While conceptually attractive, feasibility and success of such an approach still needs to be tested in humans.

Secondary prevention strategies are also possible, which would start after onset of Aβ deposition in the brain, but before clinical symptoms manifest. The at-risk population could then be broadened to include Aβ-positive APOE4 heterozygotes. In this scenario, suppression of Aβ generation with BACE1 inhibitors may decrease the risk or delay onset of clinical symptoms.

In summary, BACE1 inhibitors are likely to be most effective at early preclinical stages of AD to prevent the formation, rather than the growth, of Aβ plaques. Because this prevention strategy has not yet been fully tested in a clinical trial with any BACE1 inhibitor to date, it is premature to conclude that BACE1 inhibition for AD is a failure, despite the recent clinical trial discontinuations. A major practical constraint is that true prevention studies in AD will likely require thousands of participants and/or very long trials. However, a clear advantage of BACE1 inhibitors over Aβ immunotherapies is the ease of administration and cost for using them. Should any therapies currently in late stage AD trials (e.g. anti-tau therapies) demonstrate clear efficacy and an advantageous safety profile in presymptomatic AD ongoing trials for BACE1 inhibitors might then be discontinued for ethical reasons. However, this is not a scenario unique to BACE inhibitors and will be similar for all AD prevention studies underway and should not preclude moving forward at this time.

Next steps – priorities for BACE1 research

To decide whether or not to continue with the clinical development of BACE inhibitors, we suggest a number of important studies, both preclinical and clinical (Table 2). With the increased understanding of the clinical data highlighted in the preceding sections, we propose that further mechanistic and clinical studies should be conducted in parallel. The most important topic for BACE1 research is the mechanistic understanding of side effects of BACE1 inhibitors in order to develop strategies to avoid them. This may be achieved by a more

comprehensive analysis of the body fluid samples and data obtained in the clinical studies, which is, in fact, currently ongoing. One approach is to identify responsible substrate(s), which may be obtained from CSF and blood samples collected in the recent BACE1 inhibitor trials. The samples should be used to correlate known BACE1 and BACE2-cleaved substrate fragments with the adverse neuropsychiatric and brain imaging effects. This could provide informative biomarkers, along with Aβ (and whether the degree of Aβ lowering is associated with neuropsychiatric or MRI based side effects), for tailoring future therapeutic dosing to avoid unwanted effects. If important substrates related to the neuropsychiatric side effects are identified, these could be used to make go/no-go decisions for lower doses by analyzing them from CSF early in trials. Additionally, the overall safety profiles from the available BACE1 inhibitors would be assessed in order to identify which would be best to bring forward for additional studies.

Other important points are to understand whether the hippocampal atrophy observed in MRI studies is related to the cognitive worsening in the patients and whether the atrophy and the cognitive worsening are reversible upon termination of inhibitor dosing. Preliminary results with umibecestat suggest no relationship between brain volume reduction and cognitive decline, and that both are reversible, at least partially[34] within a three-month period of time after drug discontinuation. Likewise, a recent publication from the verubecestat trial found that hippocampal and whole brain volume changes could be detected within 13 weeks of treatment but did not continue to worsen, were greatest in areas of highest baseline Aβ plaque, were not correlated with a plasma biomarker of neurodegeneration (neurofilament light chain) and were only weakly correlated with cognitive decline (Sur et al). Together, this suggests that the volume changes seen with BACE inhibition are unlikely a reflection of accelerating neurodegeneration and should be relatively easy to monitor in future studies.

It will also be important that the pharma companies release all data from the clinical trials. This will allow a cross-comparison of the inhibitors, e.g. with regard to their effect on Aβ lowering and cognitive worsening and may allow defining a threshold of BACE1 inhibition that should not be exceeded. As an example, as the BACE inhibitor trials were identifying cognitive

adverse effects the Alzheimer's Association convened a committee of academic leaders, pharmaceutical industry representatives (including those with ongoing trials), philanthropic supporters and patient advocacy groups to determine what were the key areas to examine for concluded trials and those that were still underway. This paper reflects the ideas proposed by this group but we acknowledge there remain important steps which we have outlined here. Specifically, we strongly recommend that biofluid samples that were collected during these trials (including all phases) should be released to the research groups that could carry out the analyses highlighted here. Related to this, future studies in AD should include clear language in the consent forms that would allow for the use of samples collected for these types of post-trial collaborations so that there is no need to try to contact participants after.

A complementary approach is to continue preclinical studies with BACE1-deficient and BACE1 inhibitor-treated mice to obtain more detailed mechanistic information about different BACE1 substrates, their physiological functions and their possible contribution to the cognitive worsening in humans exposed to BACE1 inhibition (Table 2).

Taken together the proposed preclinical and clinical studies will provide the data required for informed go or no-go decisions on the further clinical development of BACE inhibitors and the design of future clinical trials. For example, short-term human studies that are powered to detect cognitive decline may be undertaken to find a safe yet effective BACE1 inhibitor dose below 50% inhibition for prevention (ranges of ~30% to 50%). Following the aforementioned studies, including the detailed analysis of the recently terminated clinical trials, a first new trial may need to last for only three to six months, which is the time point when cognitive worsening appears to begin, and test multiple doses either in parallel or in a dose ascending paradigm. If an inhibitor at lower dose does not negatively affect cognition at the three-month time point, it may be carried forward into Phase 3 trials of longer duration with appropriate cognitive assessments, monitoring of brain volume changes by MRI and measurement of BACE1 substrate-based biomarkers. Moreover, it will be necessary to start with populations that have the greatest long-term risk of developing AD (e.g. APOE4 homozygotes and dominantly inherited AD mutation carriers) and would thus have the most to gain should a lower dose

work, while also ensuring that they are provided clear information of the actual risks. Specifically, research participants would be provided with information that puts the known risks in context: 1) all adverse cognitive effects identified thus far have only been detected on sensitive cognitive testing rather than study participant reports; 2) the adverse cognitive effects identified appear to be reversible in within a short period of time when the therapies are discontinued.

In these trials, sensitive cognitive tests for assessing subtle changes would be administered more frequently in order to better detect cognitive impairment, but also changes in everyday functions, thus providing reassurance to participants that any evidence of cognitive decline would result in immediate discontinuation of therapy. Different sensitive cognitive tests are available (REF Mortamais PMID: **27702618)** and one study recently reported differential effects with BACE1 inhibitors on episodic memory versus other measures, such as verbal fluency **(and recent BACE trial data: PMID: 33049114)**, suggesting that cognitive impairment may indeed be identified with high specificity and sensitivity. There are a number of drugs prescribed that have mild cognitive or systemic side effects yet are still frequently used, provided the underlying condition being treated justifies this adverse event (e.g., statin therapies for primary and secondary prevention of cardiovascular events (myositis); antiepileptic medications for prevention of migraine (cognitive effects); anti-hypertensives limit cardiovascular performance, while protecting future events over many years to decades)). This prevention treatment with mild side-effects (especially if reversible) may appropriately balance the risk:beneft for trial participants, and for the patient population in the future if found to be effective. In many ways, this would be very consistent with phase II studies that are conducted all of the time in order to identify the optimal dose for phase III studies. However, in contrast to most phase II studies, at this point there is a large body of data to anticipate the degree of cognitive decline which would be communicated clearly to participants in order for them to make a more informed decision on their own level of risk-benefit tolerance. If a BACE1 inhibitor is able to prevent or significantly delay the onset of AD and has minimal impact on day to day function, reversible, mild, cognitive side effects that are identified on objective tests may be

acceptable in those at high risk. However, before proceeding with these studies, the full data from the most recently discontinued BACE1 inhibitor trials is necessary to better investigate the reversibility of the cognitive changes leading to their discontinuation and to understand whether asymptomatic participants from the atabecestat and umibecestat trials were aware of any adverse cognitive effects. Again, there is no evidence that BACE1 inhibitors led to a continued acceleration of cognitive decline over studies up to 18 months duration. A historical example from another field may be helpful to put in context what is at stake. When statins were being developed for lowering cholesterol based on the 'cholesterol hypothesis', there was a major concern in the field for risk and toxicity of lowering cholesterol and findings of potential carcinogenicity and toxicity halted all programs in development[51]. One program continued slowly and carefully, and eventually demonstrated clinical benefit. Had it not been for that program continuing, it's likely statins beneficial effects would not have been discovered for many years, if at all.

Another important aspect for a potential future prevention trial with BACE1 inhibitors, is the use of suitable biomarkers. AD was originally defined as a cognitive disorder, but the emerging consensus is that AD is in fact a brain disease in which memory deficits and dementia are late-stage phenomena. This has been recently articulated in the NIA-AA Research Framework in which AD is defined as a biological continuum beginning with the presence of amyloid pathology (A+/T-/N-) that progresses to tau pathology (A+/T+/N-) and eventually ending with neurodegeneration (A+/T+/N+) (Jack reference). In this scheme, low-dose BACE1 inhibition may be most effective during early stages of pathology (A-/T-/N- or A+/T-/N-) for primary or secondary prevention of progression to later stages of AD. Therefore, to facilitate the demonstration of disease modification, the primary outcomes of future BACE1 inhibitor clinical trials should not solely rely on cognitive endpoints, but should also include biomarkers that assess A/T/N status. Such a therapeutic strategy has been shown to be successful with the development of the statin drugs, wherein lowering serum cholesterol as the primary outcome was indicated after epidemiological studies linked cholesterol to

cardiovascular and cerebrovascular disease. An analogous situation now exists for lowering cerebral Aβ and AD.

Lastly, there needs to be a mechanism that will allow a potentially safe level of BACE1 inhibition to be tested in a prevention setting with appropriate incentives for drug makers and reassurances to regulatory and ethical agencies to approve these trials. Particularly, primary prevention studies, which aim to prevent Aβ related pathological changes from developing in high risk populations, will require exposure to drug for many years to prove efficacy. Even for drugs with a very good safety profile, the incentive for pharmaceutical companies to conduct long duration prevention trials is low because of the diminished likelihood of financial benefit (REF: PMID: 21262461). Therefore, alternative funding mechanisms (philanthropic, governmental, patient advocacy groups), longer patent life times or prolonged regulatory exclusivity after drug approval (e.g. 10-15 years) that recognize the urgent need for prevention therapies but also the long duration of AD prevention trials as well as a regulatory environment that could provide guidance on alternative approval pathways (e.g., accelerated approval based on biomarker outcomes, orphan drug status) will be necessary to consider moving forward with prevention trials testing BACE1 inhibitors. Additionally, at least in the United States, broader policy changes that recognize the critical need for AD prevention therapies could establish a specific designation for these types of trials which could facilitate alternative regulatory pathways for therapies tested in prevention studies. Fortunately, there are prevention platforms ready to implement these types of studies in populations at high risk for developing AD (e.g., Anti Amyloid Treatment in Asymptomatic Alzheimer's study, Alzheimer Prevention Initiative, Dominantly Inherited Alzheimer Network Trials Unit)[48-50].

Conclusion

Strong inhibition of cleavage of the many substrates of BACE1 and the concomitant side effects pose a formidable challenge, but BACE inhibitors exhibit exquisite sensitivity for titrating Aβ levels in the CNS and thus may offer a powerful, practical, and simple (compared to intravenous infusion) approach for AD prevention. Long-term exposure to BACE1 inhibition at

appropriate dosages below 50% in the preclinical population may lower Aβ enough to delay AD symptom onset with an acceptable side effect profile. Thus, we propose that as long as no other drugs are available to treat or even prevent AD, BACE1 should remain a drug target, primarily for AD prevention instead of therapy. Additionally, if an acceptable dose is identified, it might be an important component of combination approaches targeting multiple AD pathologies. The clinical and preclinical studies suggested in Table 2 will help us to better understand the adverse effects of BACE1 inhibitors and their reversibility, to determine whether an appropriate, preventive low dose-inhibition of BACE1 is meaningful and to decide whether BACE1 inhibition remains a viable option for AD prevention. The case of the cholesterol-lowering statins is instructive: these drugs, some of the most widely prescribed in the world, were nearly abandoned because of concerns about long-term side-effects. In the face of challenges, even those of perceived risk, persistent preclinical and clinical research eventually led to the discovery of safe and effective statin doses for the prevention of cardiovascular disease[51]. Following the discontinuation of a drug program, there is little incentive for a pharmaceutical company to pursue further research for that compound. Therefore, one way of advancing the field is for academic and AD advocacy groups to work together and encourage our pharmaceutical partners to continue studying the important questions highlighted in the previous sections of this commentary and making their compounds available for researcher-initiated small scale clinical trials testing some of the above discussed hypotheses. The goal is the same for all of us, namely the treatment or prevention of AD. This is a severe disease and we should be ready to accept more risks, in concert with patients and their caregivers, to explore all aspects of a potential treatment. Learning from the BACE1 inhibitor trials will require us to change the way we collaborate and share information and clinical samples. We want to open the discussion to identify how we move forward to avoid or mitigate similar premature discontinuation of development of promising drugs for Alzheimer's disease in the future[13].

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RJB is a cofounder and serve on the scientific advisory board for C2N Diagnostics LLC. Washington University also holds equity in C2N Diagnostics. C2N has licensed certain anti-tau antibodies to AbbVie for therapeutic development.

Washington University, Randall Bateman, and David Holtzman have equity ownership interest in C2N Diagnostics and receive royalty income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C2N Diagnostics. RJB receives income from C2N Diagnostics for serving on the scientific advisory board. Washington University, with RJB as co-inventor, has submitted the US nonprovisional patent application “Methods for Measuring the Metabolism of CNS Derived Biomolecules In Vivo” and provisional patent application “Plasma Based Methods for Detecting CNS Amyloid Deposition”.

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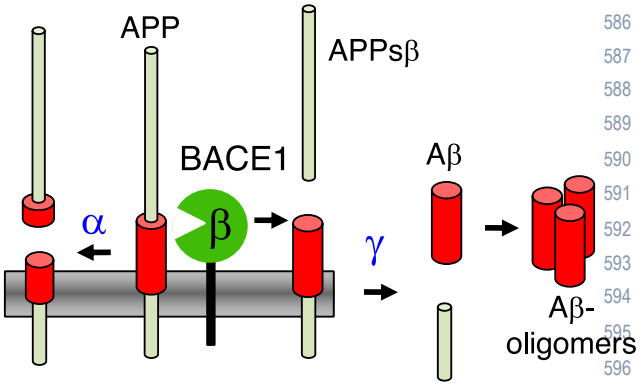
Box 1: Role of BACE1 in Aβ generation



Proteolytic generation of Aβ. The Aβ peptide is an approximately 40 amino acid long proteolytic cleavage product of the amyloid precursor protein (APP). The protease BACE1 (also known as β-secretase) cleaves APP at the N-terminus of the Aβ domain (Vassar Science 99; Yan et al. 1999; Sinha et al. 1999; Husain et al., 1999). Subsequently, γ-secretase cleaves at the C-terminal end of Aβ, leading to Aβ secretion (PMID: 32616437, PMID: **32418657,** PMID: 29976761). γ-secretase can cleave distinct peptide bonds, generating Aβ peptides of different lengths. The cleavage position of γ-secretase matters, because longer Aβ peptides (e.g. Aβ42, Aβ43) are more prone to aggregation than shorter Aβ peptides (e.g. Aβ40) and are the predominant Aβ peptides found in the amyloid plaques in AD patients. In an alternative pathway, the α-secretase ADAM10 cleaves APP within the Aβ domain (Lammich PNAS99, Kuhn EMBOJ10, Jorissen J Neurosci 10), which precludes Aβ

generation. Additional proteases, such as δ- and ε-secretase (REF PMID: 26322584, PMID: 26549211), also cleave APP, but are not shown in this scheme for simplicity.

Box 2: Chances and challenges of anti-amyloid therapy for use in long-term prevention

Class of anti-amyloid therapy	Support for use in long-term prevention	Limitation for long-term prevention
Secretase inhibitors (gamma and beta-secretase inhibitors); secretase modulators (gamma- secretase modulators)	Oral administration; well established pharmacokinetic/pharmacodynamic profiles to target a precise level of inhibition; ease of use; cost of production; ease of distribution; newer gamma-secretase inhibitors/modulators may avoid major side effects of previously tested drugs.	Current side effect profile.
Active vaccination	limited number of doses needed; broad distribution.	Lack of sufficient pharmacodynamic engagement with currently tested therapies; immunogenicity profiles remain uncertain (chronic immune response uncertain).
Passive immunotherapy	Strong pharmacodynamic effect; possible clinical benefit.	Cost of production; monthly infusions limit access; need for frequent MRI monitoring during initial titration.
Amyloid Precursor Protein/ BACE RNA directed therapies (small interfering (silencing) RNA and antisense oligonucleotide)	Precision targeting of amyloid precursor protein (APP) or related secretase activity); potential for quarterly or biannual dose administration.	Clinical data currently limited; need for intrathecal administration; cost; potential for similar side effect profile if targeting BACE; optimal level of APP suppression unknown (physiologic function of amyloid protein not completely understood).

A detailed description of chances and limitations of the different non-BACE1-targeting approaches was recently published (PAnza Nat REvNeurol 2019; Scheltens Lancet 2021, Cummings et al ALzheimers and Dementia 2019, Lewcock Neuron 2020 PMID: 33096024/)

Box 3: Physiological functions of BACE1 in adult mice

Cleavage of several BACE1 substrates is required primarily during development[52] and may not be relevant as a cause of side effects in elderly humans. Examples of physiological substrates with functions during development are type III neuregulin 1 (Nrg1) during postnatal myelination in mice [41,53-55] (take out here REF 53 and put in instead PMID: 17099708), Jagged1 during hippocampal astro- and neurogenesis (Hu, X et al. 2013, Cell Rep 4, 40). However, at least five substrates and their cleavage products have been associated with phenotypes in mice when BACE1 was pharmacologically or genetically inhibited in adulthood, IgNrg1 (motor defects)[53], SEZ6 (altered neuronal connectivity and long term potentiation (LTP))[56,57], CHL1 (structural changes in hippocampus and olfactory bulb)[43,52,58], Nrg3 (altered synaptogenesis)[59,60], APP/Aη-α[61]. Additional phenotypes may potentially be caused by other BACE1-dependent cleavage fragments of APP (e.g. Aβ) or other BACE1 substrates, where physiological functions are not yet well established.

Table 1: Outcomes of BACE1 inhibitors in clinical trials

Compound and company	Potency and selectivity (BACE1/BACE2)	Doses	Extent of Aβ lowering in CSF	Phase and population tested	Discontinued	Cognition change (and primary cognitive test(s)?	Reason for termination	References
Atabecestat (JNJ-54861911) ***Janssen/Shionogi***	Not published	5 mg, 25 mg	CSF Aβ40: 67–68% (10 mg dose) 87–90% (50 mg dose)	Phase 2/3, EARLY **Asymptomatic** at risk of AD	May 2018	Cognitive worsening: Preclinical Alzheimer Cognitive Composite (PACC Score)	Stopped because of hepatotoxicity	[9,62,63] NCT: 02569398
Elenbecestat (E2609) ***Biogen/Eisai***	BACE1 IC_{50} 7 nM (in cellular assay) BACE1 K_d 19 nM BACE2 K_d 67 nM **(BACE1>BACE2)**	50 mg	CSF: 70%	Two Phase 3 trials, MISSION AD 1 and 2 Biomarker-confirmed **MCI** due to AD/**prodromal AD**	September 2019	NA: Alzheimer's Disease Composite Score	Stopped for unfavourable risk/benefit ratio	[64,65] NCT: 03036280
Lanabecestat (AZD3293 , LY3314814) ***AstraZeneca/ Eli Lilly***	BACE1 IC_{50} 0.6 nM BACE2 IC_{50} 0.9 nM **(BAC1~BACE2)**	20 mg, 50 mg	CSF Aβ40: 58.0% (20 mg dose) 73.3% (50mg dose) CSF Aβ42: 51.3% (20 mg dose) 65.5% (50mg dose)	Phase 2/3, AMARANTH **MCI due to AD or mild AD** worsening in the past six months, and MMSE >21	June 2018	No change: Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog13)	Stopped for futility	[15,66,67] NCT: 02245737
		20 mg, 50 mg	insufficient CSF samples for meaningful analysis	Phase 3, DAYBREAK-ALZ **Mild AD dementia**, probable AD with a biomarker evidence of brain amyloid and an MMSE of 10-26	June 2018	No consistent change; trend for worsening of 20 mg but not 50 mg dose: Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog13)	Stopped for futility	NCT:02783573

LY3202626 ***Eli Lilly***	BACE1 IC_{50} 0.61 nM BACE2 IC_{50} 0.87 nM **(BAC1~BACE2)**	3 mg, 12 mg	CSF Aβ40: 50% (1mg dose) 75% (6 mg dose) 90% (26 mg dose)	Phase 2, NAVIGATE-AD **Mild AD dementia** as diagnosed by a positive florbetapir scan, and an MMSE of 20-26	August 2018	Trend for worsening in 3 mg dose: Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog13)	Stopped for futility	[68,69] NCT: 02791191
				Phase 2, TRAILBLAZER in combination with Donanemab Memory decline, meet CogState Brief Battery cutoff and positive tau PET scan	October 2018		Stopped for safety and efficacy concerns	NCT: 03367403
Umibecestat (CNP520) ***Amgen/Novartis***	BACE1 IC_{50} 11 nM BACE2 IC_{50} 30 nM **(BAC1>BACE2)**	15 mg, 50 mg	>60% (10 mg dose) >80% (35 mg dose) >90% (85 mg dose)	Phase 2/3, GENERATION 1 and 2 **Cognitively normal**, homozygous for ApoE4	July 2019	Cognitive worsening: RBANS	Stopped for unfavourable risk/benefit ratio	[19,33,70] NCT: 02565511
Verubecestat (MK-8931) ***Merck***	BACE1 K_i 2.2 nM BACE2 K_i 0.38 nM **(BAC1<BACE2)**	12 mg, 40 mg	CSF Aβ40: 71.1% (12 mg dose) 80.6% (40mg dose) CSF Aβ42: 62.7% (12 mg dose) 76.4% (40mg dose)	Phase 2/3, EPOCH **Mild to moderate AD**	February 2017	Trend for worsening: Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) Score	Stopped for futility	[8,14,30,64] NCT:01739348 NCT01953601
		12 mg, 40 mg	CSF Aβ40: 66.6% (12 mg dose) 88.1% (40mg dose) CSF Aβ42: 60.2% (12 mg dose) 81.0% (40mg dose)	Phase 3, APECS **Prodromal AD, MCI due to AD** with positive amyloid PET scan, but not	February 2018	Cognitive worsening	Stopped for futility	

				functionally impaired				
PACC- Preclinical Alzheimer Cognitive Composite: Free and Cued Selective Reminding Test; Delayed Paragraph Recall test; Wechsler Adult Intelligence scale; and Mini Mental State Examination (MMSE); Repeatable Battery for the Assessment of Neurological Status (RBANS).								

Table 2: Next steps for low-dose BACE inhibition for AD prevention and go/no-go decisions

Preclinical experiments	Clinical experiments
1. Correlate cleavage of known BACE1 substrates with cognitive worsening and brain/hippocampus volume reduction in mice treated with different doses and durations of BACE1 inhibitor. 2. Identify substrate correlates of cognitive impairment and brain/hippocampus volume reduction associated with BACE1 inhibition in mice. 3. Manipulate substrate(s) genetically and/or pharmacologically up and down to demonstrate predicted cognitive and brain/hippocampus volume effects in mice. 4. Perform in vitro and in vivo experiments (biochemical, electrophysiological, histological) to determine molecular and cellular mechanisms of substrate that is most likely responsible for cognitive worsening and brain/hippocampus volume reduction. 5. Verify in CSF of humans treated with BACE1 inhibitor that substrate correlate is altered in expected manner to account for cognitive worsening and brain/hippocampus volume reduction.	1. Determine whether reversibility of cognitive worsening and hippocampal volume loss observed for umibecestat are also seen for the other BACE1 inhibitors. 2. Proteomic analyses of CSF from participants of BACE1 inhibitor phase 3 clinical trials to correlate changes in one or more known BACE1 substrates with cognitive worsening and brain/hippocampus volume reduction. 3. Unbiased proteomic analysis of CSF from participants treated with BACE1 inhibitor compared to placebo to determine other unknown proteins that correlate with cognitive worsening and brain/hippocampus volume reduction, which may inform mechanism. 4. Determine dose-response relationship between Aβ lowering and substrate processing. Use CSF samples from small size (e.g., n=10-20/dose), short duration (e.g., 3 months) phase 1 or 2 studies of healthy elderly treated with three low-doses of BACE1 inhibitor (e.g., 12%, 25%, 50% Aβ lowering) and placebo and analyze BACE1 substrates in CSF. If required, carry out new short-term phase 1 trial with adjusted low dose.

	5. Based on results from #4, conduct medium size (e.g., n=100-200/dose), medium duration (e.g., 6 months) study of unimpaired Aβ-positive elderly or young, cognitively unimpaired DIAD mutation carriers treated with two low doses of BACE1 inhibitor that shows minimal changes in cognition-relevant BACE1 substrate(s), compared to placebo. Correlate cognition and brain/hippocampus volume with CSF BACE1 substrates and Aβ. 6. Based on results from #5, conduct large (e.g., n=1000-2000), long duration (e.g., 4-5 years) study of unimpaired Aβ-positive elderly treated with one low dose of BACE1 inhibitor that shows minimal changes in cognition-relevant BACE1 substrate(s), cognition, and brain/hippocampus volume with CSF BACE1 substrates and Aβ, compared to placebo; or long duration (e.g. 7-10 years) cognitively unimpaired DIAD mutation carriers in a primary prevention paradigm with natural history data as comparator population.

References

1. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO molecular medicine* 2016; **8**(6): 595-608.
2. De Strooper B, Karran E. The Cellular Phase of Alzheimer's Disease. *Cell* 2016; **164**(4): 603-15.

3. Hussain I, Powell D, Howlett DR, et al. Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Molecular and cellular neurosciences* 1999; **14**(6): 419-27.
4. Sinha S, Anderson JP, Barbour R, et al. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 1999; **402**(6761): 537-40.
5. Vassar R, Bennett BD, Babu-Khan S, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999; **286**(5440): 735-41.
6. Yan R, Bienkowski MJ, Shuck ME, et al. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 1999; **402**(6761): 533-7.
7. Das B, Yan R. A Close Look at BACE1 Inhibitors for Alzheimer's Disease Treatment. *CNS drugs* 2019; **33**(3): 251-63.
8. Egan MF, Kost J, Voss T, et al. Randomized Trial of Verubecestat for Prodromal Alzheimer's Disease. *The New England journal of medicine* 2019; **380**(15): 1408-20.
9. Henley D, Raghavan N, Sperling R, Aisen P, Raman R, Romano G. Preliminary Results of a Trial of Atabecestat in Preclinical Alzheimer's Disease. *The New England journal of medicine* 2019; **380**(15): 1483-5.
10. Knopman DS. Lowering of Amyloid-Beta by beta-Secretase Inhibitors - Some Informative Failures. *The New England journal of medicine* 2019; **380**(15): 1476-8.
11. Doody RS, Raman R, Farlow M, et al. A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. *New England Journal of Medicine* 2013; **369**(4): 341-50.
12. Coric V, van Dyck CH, Salloway S, et al. Safety and tolerability of the gamma-secretase inhibitor avagacestat in a phase 2 study of mild to moderate Alzheimer disease. *Archives of neurology* 2012; **69**(11): 1430-40.
13. De Strooper B. Lessons from a failed gamma-secretase Alzheimer trial. *Cell* 2014; **159**(4): 721-6.
14. Egan MF, Kost J, Tariot PN, et al. Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease. *N Engl J Med* 2018; **378**(18): 1691-703.
15. Wessels AM, Tariot PN, Zimmer JA, et al. Efficacy and Safety of Lanabecestat for Treatment of Early and Mild Alzheimer Disease: The AMARANTH and DAYBREAK-ALZ Randomized Clinical Trials. *JAMA Neurology* 2019.
16. Zuhl AM, Nolan CE, Brodney MA, et al. Chemoproteomic profiling reveals that cathepsin D off-target activity drives ocular toxicity of beta-secretase inhibitors. *Nature communications* 2016; **7**: 13042.
17. Cai J, Qi X, Kociok N, et al. beta-Secretase (BACE1) inhibition causes retinal pathology by vascular dysregulation and accumulation of age pigment. *EMBO molecular medicine* 2012; **4**(9): 980-91.
18. Company ELa. Lilly Voluntarily Terminates Phase II Study for LY2886721, a Beta Secretase Inhibitor, Being Investigated as a Treatment for Alzheimer's Disease. 2013.

19. Neumann U, Ufer M, Jacobson LH, et al. The BACE-1 inhibitor CNP520 for prevention trials in Alzheimer's disease. *EMBO molecular medicine* 2018.
20. Voytyuk I, Mueller SA, Herber J, et al. BACE2 distribution in major brain cell types and identification of novel substrates. *Life science alliance* 2018; **1**(1): e201800026.
21. Farzan M, Schnitzler CE, Vasilieva N, Leung D, Choe H. BACE2, a beta -secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. *Proceedings of the National Academy of Sciences of the United States of America* 2000; **97**(17): 9712-7.
22. Yan R, Munzner JB, Shuck ME, Bienkowski MJ. BACE2 functions as an alternative alpha-secretase in cells. *The Journal of biological chemistry* 2001; **276**(36): 34019-27.
23. Rochin L, Hurbain I, Serneels L, et al. BACE2 processes PMEL to form the melanosome amyloid matrix in pigment cells. *Proceedings of the National Academy of Sciences of the United States of America* 2013; **110**(26): 10658-63.
24. Esterhazy D, Stutzer I, Wang H, et al. Bace2 is a beta cell-enriched protease that regulates pancreatic beta cell function and mass. *Cell metabolism* 2011; **14**(3): 365-77.
25. Stutzer I, Selevsek N, Esterhazy D, Schmidt A, Aebersold R, Stoffel M. Systematic proteomic analysis identifies beta-site amyloid precursor protein cleaving enzyme 2 and 1 (BACE2 and BACE1) substrates in pancreatic beta-cells. *J Biol Chem* 2013; **288**(15): 10536-47.
26. Kuhn PH, Koroniak K, Hogl S, et al. Secretome protein enrichment identifies physiological BACE1 protease substrates in neurons. *Embo j* 2012; **31**(14): 3157-68.
27. Zhou L, Barao S, Laga M, et al. The neural cell adhesion molecules L1 and CHL1 are cleaved by BACE1 protease in vivo. *J Biol Chem* 2012; **287**(31): 25927-40.
28. Hemming ML, Elias JE, Gygi SP, Selkoe DJ. Identification of beta-secretase (BACE1) substrates using quantitative proteomics. *PLoS One* 2009; **4**(12): e8477.
29. Cebers G, Lejeune T, Attalla B, et al. Reversible and Species-Specific Depigmentation Effects of AZD3293, a BACE Inhibitor for the Treatment of Alzheimer's Disease, Are Related to BACE2 Inhibition and Confined to Epidermis and Hair. *The journal of prevention of Alzheimer's disease* 2016; **3**(4): 202-18.
30. Kennedy ME, Stamford AW, Chen X, et al. The BACE1 inhibitor verubecestat (MK-8931) reduces CNS beta-amyloid in animal models and in Alzheimer's disease patients. *Science translational medicine* 2016; **8**(363): 363ra150.
31. Shimshek DR, Jacobson LH, Kolly C, et al. Pharmacological BACE1 and BACE2 inhibition induces hair depigmentation by inhibiting PMEL17 processing in mice. *Scientific reports* 2016; **6**: 21917.
32. Egan MF, Mukai Y, Voss T, et al. Further analyses of the safety of verubecestat in the phase 3 EPOCH trial of mild-to-moderate Alzheimer's disease. *Alzheimers Res Ther* 2019; **11**(1): 68.

33. Lopez Lopez C, Tariot PN, Caputo A, et al. The Alzheimer's Prevention Initiative Generation Program: Study design of two randomized controlled trials for individuals at risk for clinical onset of Alzheimer's disease. *Alzheimer's & dementia (New York, N Y)* 2019; **5**: 216-27.
34. Fagan T. Umibecestat-Driven Cognitive Decline Is Reversible, 2020.
35. Rogers MB. Bump in the Road or Disaster? BACE Inhibitors Worsen Cognition. 2018. https://www.alzforum.org/news/conference-coverage/bump-road-or-disaster-bace-inhibitors-worsen-cognition.
36. Rogers MB. Picking Through the Rubble, Field Tries to Salvage BACE Inhibitors. 2019. https://www.alzforum.org/news/conference-coverage/picking-through-rubble-field-tries-salvage-bace-inhibitors.
37. McConlogue L, Buttini M, Anderson JP, et al. Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice. *The Journal of biological chemistry* 2007; **282**(36): 26326-34.
38. Cao L, Rickenbacher GT, Rodriguez S, Moulia TW, Albers MW. The precision of axon targeting of mouse olfactory sensory neurons requires the BACE1 protease. *Scientific reports* 2012; **2**: 231.
39. Dominguez D, Tournoy J, Hartmann D, et al. Phenotypic and biochemical analyses of BACE1- and BACE2-deficient mice. *The Journal of biological chemistry* 2005; **280**(35): 30797-806.
40. Laird FM, Cai H, Savonenko AV, et al. BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2005; **25**(50): 11693-709.
41. Hu X, Das B, Hou H, He W, Yan R. BACE1 deletion in the adult mouse reverses preformed amyloid deposition and improves cognitive functions. *J Exp Med* 2018; **215**(3): 927-40.
42. Lombardo S, Chiacchiaretta M, Tarr A, et al. BACE1 partial deletion induces synaptic plasticity deficit in adult mice. *Sci Rep* 2019; **9**(1): 19877.
43. Barao S, Gartner A, Leyva-Diaz E, et al. Antagonistic Effects of BACE1 and APH1B-gamma-Secretase Control Axonal Guidance by Regulating Growth Cone Collapse. *Cell reports* 2015; **12**(9): 1367-76.
44. Jonsson T, Atwal JK, Steinberg S, et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 2012; **488**(7409): 96-9.
45. Martiskainen H, Herukka SK, Stancakova A, et al. Decreased plasma beta-amyloid in the Alzheimer's disease APP A673T variant carriers. *Ann Neurol* 2017; **82**(1): 128-32.
46. Maloney JA, Bainbridge T, Gustafson A, et al. Molecular mechanisms of Alzheimer disease protection by the A673T allele of amyloid precursor protein. *The Journal of biological chemistry* 2014; **289**(45): 30990-1000.
47. Das P, Verbeeck C, Minter L, et al. Transient pharmacologic lowering of Abeta production prior to deposition results in sustained reduction of amyloid plaque pathology. *Molecular neurodegeneration* 2012; **7**(1): 39.

48. Mills SM, Mallmann J, Santacruz AM, et al. Preclinical trials in autosomal dominant AD: implementation of the DIAN-TU trial. *Revue neurologique* 2013; **169**(10): 737-43.
49. Reiman EM, Langbaum JB, Fleisher AS, et al. Alzheimer's Prevention Initiative: a plan to accelerate the evaluation of presymptomatic treatments. *Journal of Alzheimer's disease : JAD* 2011; **26 Suppl 3**: 321-9.
50. Sperling RA, Rentz DM, Johnson KA, et al. The A4 study: stopping AD before symptoms begin? *Science translational medicine* 2014; **6**(228): 228fs13.
51. Brown MS, Goldstein JL. A tribute to Akira Endo, discoverer of a "Penicillin" for cholesterol. *Atherosclerosis Supplements* 2004; **5**(3): 13-6.
52. Ou-Yang MH, Kurz JE, Nomura T, et al. Axonal organization defects in the hippocampus of adult conditional BACE1 knockout mice. *Sci Transl Med* 2018; **10**(459).
53. Cheret C, Willem M, Fricker FR, et al. Bace1 and Neuregulin-1 cooperate to control formation and maintenance of muscle spindles. *Embo j* 2013; **32**(14): 2015-28.
54. Fleck D, van Bebber F, Colombo A, et al. Dual cleavage of neuregulin 1 type III by BACE1 and ADAM17 liberates its EGF-like domain and allows paracrine signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2013; **33**(18): 7856-69.
55. Willem M, Garratt AN, Novak B, et al. Control of peripheral nerve myelination by the beta-secretase BACE1. *Science (New York, NY)* 2006; **314**(5799): 664-6.
56. Zhu K, Xiang X, Filser S, et al. Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1 Inhibition Impairs Synaptic Plasticity via Seizure Protein 6. *Biological psychiatry* 2018; **83**(5): 428-37.
57. Pigoni M, Wanngren J, Kuhn PH, et al. Seizure protein 6 and its homolog seizure 6-like protein are physiological substrates of BACE1 in neurons. *Mol Neurodegener* 2016; **11**(1): 67.
58. Hitt B, Riordan SM, Kukreja L, Eimer WA, Rajapaksha TW, Vassar R. beta-Site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1)-deficient mice exhibit a close homolog of L1 (CHL1) loss-of-function phenotype involving axon guidance defects. *The Journal of biological chemistry* 2012; **287**(46): 38408-25.
59. Muller T, Braud S, Juttner R, et al. Neuregulin 3 promotes excitatory synapse formation on hippocampal interneurons. *Embo j* 2018; **37**(17).
60. Wang YN, Figueiredo D, Sun XD, et al. Controlling of glutamate release by neuregulin3 via inhibiting the assembly of the SNARE complex. *Proceedings of the National Academy of Sciences of the United States of America* 2018; **115**(10): 2508-13.
61. Willem M, Tahirovic S, Busche MA, et al. eta-Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature* 2015; **526**(7573): 443-7.
62. Donohue MC, Sperling RA, Salmon DP, et al. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. *JAMA Neurol* 2014; **71**(8): 961-70.

63. Timmers M, Streffer JR, Russu A, et al. Pharmacodynamics of atabecestat (JNJ-54861911), an oral BACE1 inhibitor in patients with early Alzheimer's disease: randomized, double-blind, placebo-controlled study. *Alzheimers Res Ther* 2018; **10**(1): 85.
64. Wang J, Logovinsky V, Hendrix S, et al. ADCOMS: A composite clinical outcome for prodromal Alzheimer's disease trials. *Journal of Neurology, Neurosurgery & Psychiatry* 2016; **87**: jnnp-2015.
65. Lynch SY, Kaplow, J., Zhao, J., Dhadda, S., Luthman, J. and Albala, B. Elenbecestat, a BACE inhibitor: results from a Phase 2 study in subjects with mild cognitive impairment and mild-to-moderate dementia due to Alzheimer's disease.; 2018: Wiley; 2018. p. P1623.
66. Mohs RC, Knopman D, Petersen RC, et al. Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's Disease Assessment Scale that broaden its scope. The Alzheimer's Disease Cooperative Study. *Alzheimer disease and associated disorders* 1997; **11 Suppl 2**: S13-21.
67. Eketjall S, Janson J, Kaspersson K, et al. AZD3293: A Novel, Orally Active BACE1 Inhibitor with High Potency and Permeability and Markedly Slow Off-Rate Kinetics. *Journal of Alzheimer's disease : JAD* 2016; **50**(4): 1109-23.
68. McKinzie D, May P, Boggs L, et al. NONCLINICAL PHARMACOLOGICAL CHARACTERIZATION OF THE BACE1 INHIBITOR LY3202626. Alzheimer's Dement; 2016. p. P432-P3.
69. Willis B, Lowe S, Daugherty L, et al. PHARMACOKINETICS, PHARMACODYNAMICS, SAFETY, AND TOLERABILITY OF LY3202626, A NOVEL BACE1 INHIBITOR, IN HEALTHY SUBJECTS AND PATIENTS WITH ALZHEIMER'S DISEASE. Alzheimer's Dement; 2016; 2016. p. P418.
70. Hsiao CC, Rombouts F, Gijsen HJM. New evolutions in the BACE1 inhibitor field from 2014 to 2018. *Bioorg Med Chem Lett* 2019; **29**(6): 761-77.