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EDITORIAL

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BACE1 inhibitor drugs for the treatment of Alzheimer's disease: Lessons learned, challenges to overcome, and future prospects†

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Abstract: Alzheimer's disease (AD), first diagnosed over a century ago, remains one of the major healthcare crises around the globe. Currently, there is no cure or effective treatment. The majority of drug development efforts to date have targeted reduction of amyloid-β peptide (Aβ). Drug development through inhibition of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), resulted in promising early clinical studies. However, nearly all small molecule BACE1 inhibitor drugs failed to live up to expectations in later phase clinical trials, due to toxicity and efficacy issues. This commentary aims to provide a brief review of over two decades of BACE1 inhibitor drug development challenges and efforts for treatment of AD and prospects of future BACE1-based drugs.

***Keywords*:** Alzheimer disease, amyloid, BACE1, inhibitor, drug development

Alzheimer's disease (AD) is a neurodegenerative disorder in the brain that leads to progressive impairment of cognition and memory loss (*1*). AD mostly affects elderly patients and there are over 55 million individuals diagnosed with AD around the globe. Currently, there is no cure or disease-modifying treatment (*2*). Thus far, the majority of approved drugs may help to manage symptoms of the disease. The hallmark of AD is the accumulation of neurotoxic amyloid-β peptides (Aβ), mainly Aβ40 and Aβ42, in the brain which is considered a pivotal feature of AD pathogenesis. Among numerous hypotheses explaining the mechanisms underlying AD pathogenesis, the "Amyloid Cascade Hypothesis" introduced in the 1990's remains one of the most widely studied conceptual frameworks for AD drug development (*3,4*). The β-site amyloid precursor protein cleaving enzyme 1 (BACE1) was cloned and identified over two decades ago (*5,6*). The prospect of developing anti-amyloid-β drugs generated huge enthusiasm and anticipation at that time. This initial excitement is understandable as BACE1 is the first protease that initiates production of Aβ in the brain by cleaving the amyloid precursor protein (APP) and forming a soluble *N*-terminal fragment and amyloid-β peptide containing a membrane anchored *C*-terminal fragment (Figure 1). In the following step, γ-secretase cleaves the *C*-terminal fragment, releasing the neurotoxic Aβ peptides of different lengths. Among these, Aβ42 has been recognized as the major cause for onset and progression of AD. Logically, both proteases became important drug development targets against AD. Several γ-secretase inhibitor drug candidates were brought to clinical trials, however they were associated with high toxicity and major side effects. The toxicity appears to be mechanism-based because γ-secretase plays an important role in maintaining important physiological functions (*7*). Since BACE1 is involved in the first step of Aβ production, inhibition of BACE1 was thought to avoid the late stages of AD pathogenesis and issues associated with function-based problems of γ-secretase. However, BACE1 has been shown to process other substrates, which set up potential new challenges (*8,9*).

The design of the first substrate-based potent BACE1 inhibitor, OM99-2, and subsequent determination of the X-ray crystal structure of OM99-2 bound BACE1 was reported in 2000 (Figure 2) (*10,11*). This development set the stage for structure-based evolution of potent and drug-like BACE1 inhibitors. The overall medicinal chemistry development is nothing short of astounding. Today, there are over 250 X-ray crystal structures of BACE1 and inhibitor complexes in the protein database. Also, there exists a large number of patents with many different structural classes (*12,13*). For BACE1 inhibitor clinical development, the challenging problems are to develop drug-like molecules with good selectivity against BACE2 and other aspartic acid proteases. Ideally, such BACE1 inhibitor drugs need to be smaller, have less peptidic features, have efficient blood-brain-barrier penetration, and reduced susceptibility to Pgp-mediated efflux (*14*).

The early development of peptidomimetic small molecule BACE1 inhibitors containing classical

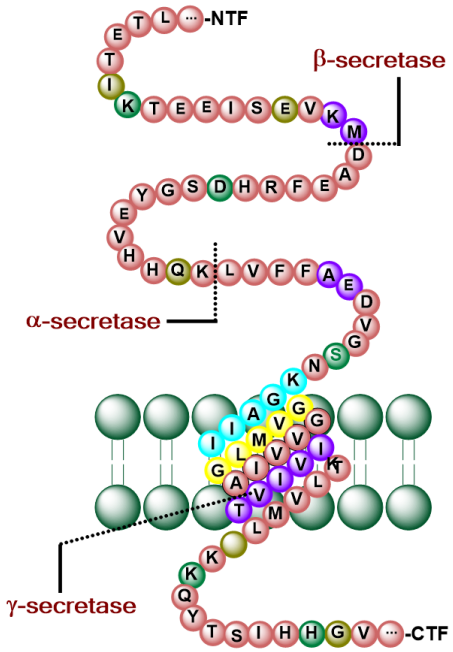

Figure 1. APP cleavage by β- and γ-secretases, common APP mutations in purple. APP, amyloid precursor protein.

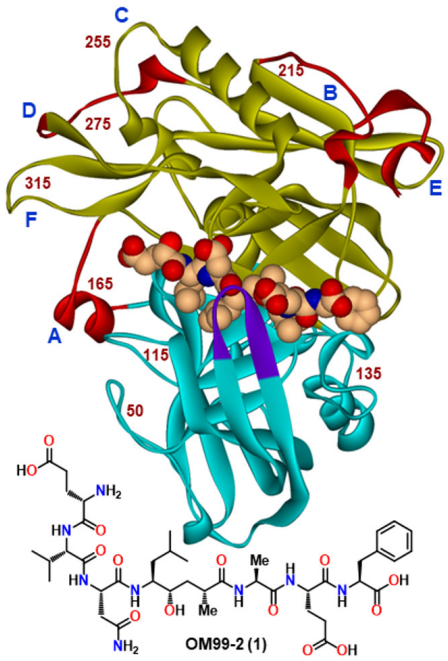

Figure 2. BACE1 inhibitor OM99-2 and Xray structure of BACE1-OM99-2 complex (pdb:1M4H). BACE1, beta-site amyloid precursor protein cleaving enzyme 1.

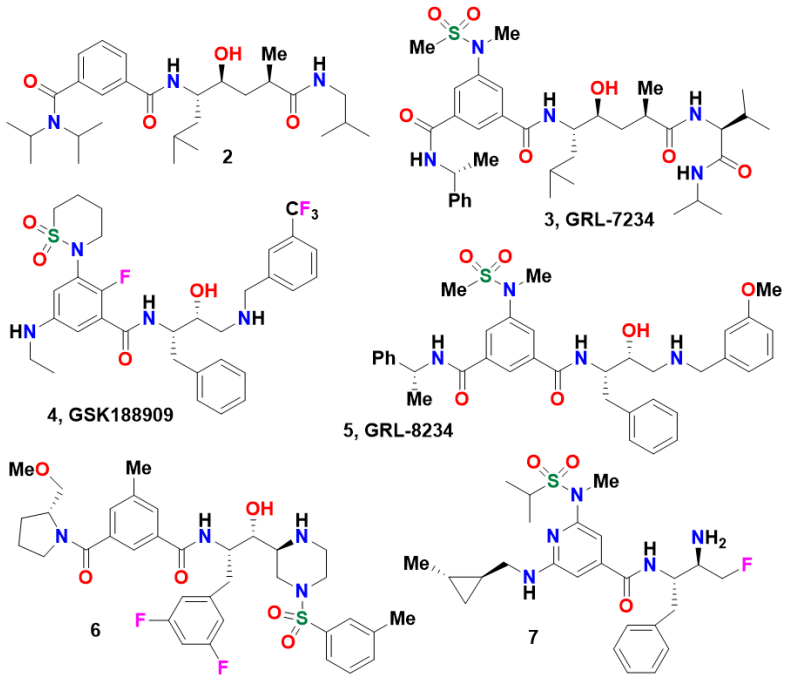

Figure 3. Early evolution of drug-like BACE1 inhibitors with classical dipeptide isosteres. BACE1, beta-site amyloid precursor protein cleaving enzyme 1.

dipeptide isosteres is highlighted in Figure 3. Small molecule BACE1 inhibitor **2** with the Leu-Ala isostere showed good potency IC_{50} = 30 nM, EC_{50}= 3 μM, HEK-293) and cell penetration (*15*). Compound **3** displayed potent BACE1 activity as well as cellular activity (K_i = 1.1 nM, cell EC_{50} = 39 nM). It also showed over 25-fold selectivity against BACE2 and cathepsin D (*16*). Inhibitor **4** with a hydroxy ethylamine isostere (GSK 188909, IC_{50} = 4 nM, cell EC_{50} = 5 nM) showed good selectivity over other aspartic acid proteases and lowered Aβ in transgenic mice (*17*). Inhibitor **5** displayed potent BACE1 activity (K_i = 1.8 nM, cell EC_{50} = 1 nM), modest selectivity against BACE2 and cathepsin D (IC_{50} = 79 nM and 138 nM, respectively), and showed inhibition of Aβ production in transgenic mice (*18*). Intraperitoneal administration of compound **5** with an 8 mg/kg dose

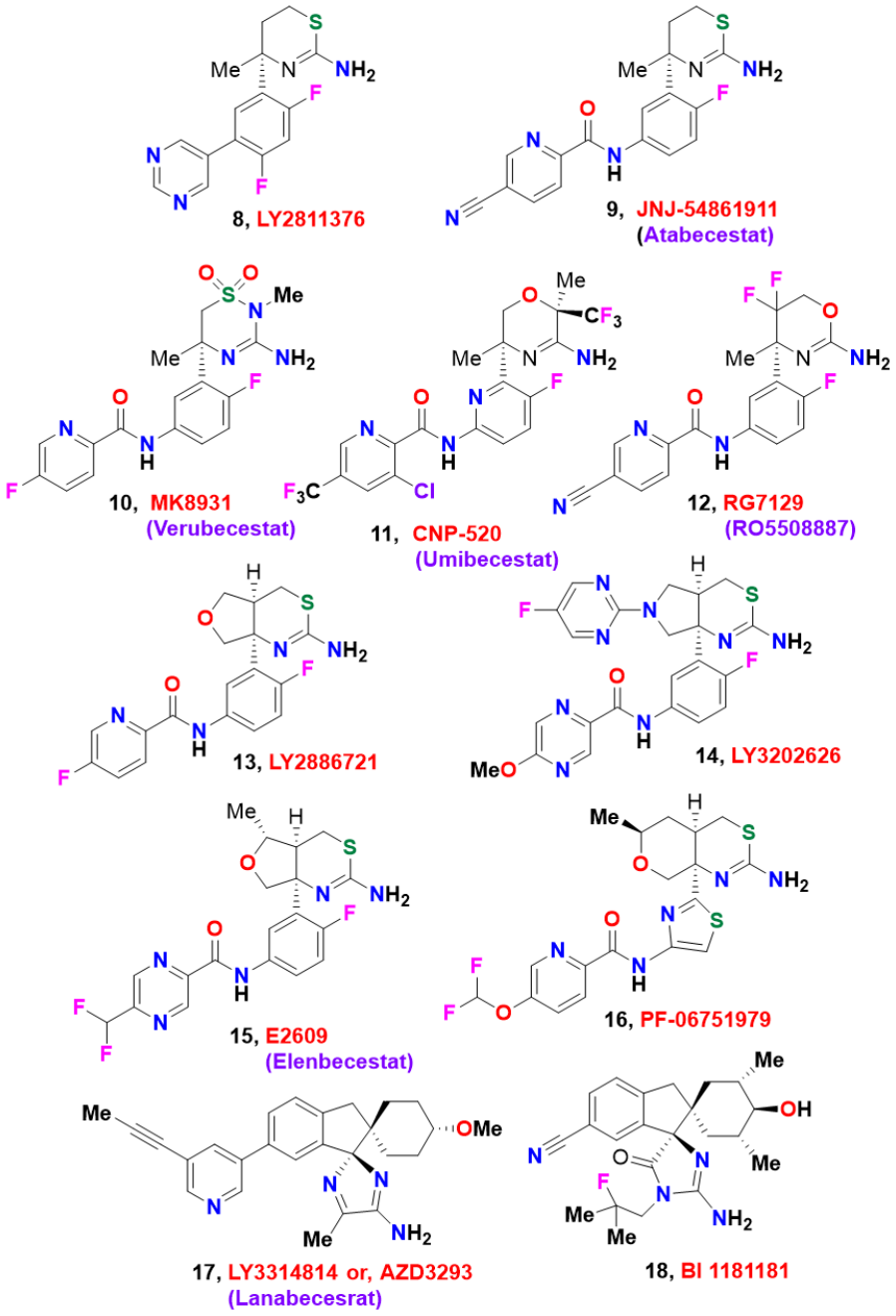

Figure 4. Nonpeptide small molecule BACE1 inhibitors developed for clinical trials. BACE1, beta-site amyloid precursor protein cleaving enzyme 1.

to Tg2579 mice showed 65% reduction of plasma Aβ production after 3 h. Furthermore, this compound has been shown to rescue age-related cognitive decline in transgenic mice. Piperazine derivative **6** was developed as a potent BACE1 inhibitor (IC_{50} = 0.18 nM, cell EC_{50} = 7 nM) (*19,20*). This compound was shown to inhibit peripheral $A\beta_{40}$ in transgenic mice with a single dose. Compound **7** displayed potent *in vitro* activity (cell IC_{50} = 49 nM) and exhibited an *in vitro* $A\beta_{40}$ reduction of 34% in transgenic mice after 3 h using a 50 mg/kg dose (*21*). Also, it showed good drug concentration in the brain (1.9 μM).

To date, no BACE1 inhibitor drug is approved by the FDA. However, fourteen BACE1 inhibitor drugs were evaluated in clinical trials (*13,22*). CTS21166 was the first BACE1 inhibitor reported to have undergone a phase I clinical trial, showing oral bioavailability, CSF penetration, metabolic stability and toxicology profiles. For healthy volunteers receiving 225 mg IV or 200 mg oral doses, CTS21166 exhibited Aβ40 reduction up to 80% in 4-8 h with sustained reduction up to 72 h and showed 40% oral bioavailability in humans. Chemical structure and other information regarding further clinical studies were not reported (*23,24*). Despite steady progress towards drug-like peptidomimetic BACE1 inhibitors, the development of nonpeptide

small molecule heterocyclic BACE1 inhibitors was also pursued. Various high-throughput screening and fragment-based screening of scaffolds were utilized to discover new structural classes that have no peptide features, good metabolic stabilities, and better blood-brain barrier (BBB) penetration ability. These efforts led to the identification of several structural leads which upon medicinal chemistry optimization resulted in the development of potent BACE1 inhibitors with promising pharmacological properties. Several of these BACE1 inhibitors were advanced to clinical development and phase I-III clinical trials were conducted from 2012-2019 (*13,14*). These compound structures are highlighted in Figure 4. One of the interesting features of these clinical agents is the presence of a cyclic amidine functionality. The X-ray structural analysis of BACE1 and inhibitor complexes revealed that the core amidine functionality forms a network of hydrogen bonds with the catalytic aspartates in flap-open conformation of the BACE1. The adjacent fluorobenzene scaffold fills the S1-S2 subsites while the substituted heterocyclic amide derivatives occupy the S3 subsite. Compounds **8**, **9**, **13-16** contain amino-thiazine structural features, compounds **11** and **12** possess amino-oxazine scaffolds while candidates **10** and **18** contain a cyclic guanidine core which interacts with the catalytic aspartate of BACE1. In general, these compounds showed acceptable ADME properties in laboratory animals. In phase I clinical trials, most of these compounds exhibited general tolerability, acceptable safety profiles, BBB penetration, and decline of Aβ levels in CSF. Compound **8** (LY2811376) completed a phase I clinical trial but was discontinued due to toxicological issues. The development of compound **9** (LY2886721) was terminated after phase I and II clinical trials due to abnormal liver enzyme elevation for a number of patients. From 2014-2019, several compounds advanced to phase II and phase III clinical trials with early AD patients. Clinical development of all BACE1 inhibitors, including JNJ-54861911 (**9**), MK-8931(**10**), CNP-520 (**11**), E2609 (**15**) and LY3314814 (**17**), was terminated because the treatment did not exhibit clinical benefit, slow disease progression, nor slow down cognitive decline, and in some cases caused cognitive worsening (*22,25*).

All BACE1 inhibitor Alzheimer's disease clinical trials have been terminated at present; however, this is not the end of BACE drug development. BACE1 remains one of the most promising drug targets for decelerating Aβ production and intervention of AD pathophysiology. Despite major setbacks, the BACE inhibitor clinical trials provided a lot of useful knowledge and a wealth of scientific information for future directions. It is time to scrutinize data, critically analyze pitfalls, and apply the lessons learned toward successful BACE drug development. Many past Alzheimer's drug trials dealt with uncertainties and failures, particularly in the development of immunotherapies. After many obstacles, more recent trials with amyloid reducing-antibodies have been shown to slow down AD progression by targeting fibrillar and pre-fibrillar forms of amyloid aggregates. The recent FDA approval of lecanemab for slowing progression of mild cognitive impairment in early AD patients supports the amyloid hypothesis (*26,27*). BACE1 inhibition in essence provides a complementary pathway by blocking Aβ generation, which ultimately contributes to neurotoxicity and neurodegeneration.

Like the early clinical setbacks with antibody therapies, the early BACE inhibitor drug development efforts also faced numerous concerns including liver toxicity and retinal damage during clinical development. Most recent clinical candidates resolved these issues and compounds with good ADME, BBB properties, and safety profiles have been developed. However, clinical trials presented a new set of problems as these compounds showed no clinical efficacy, undesired side effects, and sometimes cognitive worsening. Are these problems related to BACE1 as a drug target or are they compound specific due to off-target effects? These are important questions that need to be addressed. BACE has two isoforms, BACE1 and BACE2. While BACE2 expression is low in the CNS, its inhibition has been suggested to affect cognition. Also, it is known that BACE processes many other important substrates in the brain, including seizure protein 6, aka sez6-like, neuregulin and NCAML1. Prolonged inhibition of BACE is likely to interfere with structural synaptic plasticity possibly due to diminished processing of sez6 causing disruption of dendritic spine plasticity. Many experts in the field believe a detailed understanding of these BACE1 substrates as well as the physiological role of BACE2 requires further evaluation and understanding (*25,28*). Furthermore, BACE clinical trials resulted in greater than 50% Aβ reduction. Such dramatic reduction may have also contributed towards undesired side effects. Lower doses are likely to reduce side effects. If a low dose reduces Aβ production to a low percentage and slows down the AD onset for a few years, still that would be a significant clinical achievement.

Interestingly, all recent clinical BACE inhibitors showed only marginal selectivity against these two BACE isoforms. It is possible that the lack of selectivity may have contributed to some of the observed side effects. Also, most of the clinical candidates belong to the same structural class as they possess some core similarities, particularly the presence of a cyclic amidine as well as the P1-P2 fluorobenzene scaffold as can be seen in Figure 4. Thus, medicinal chemistry development of structurally more diverse and highly selective BACE1 inhibitors may shed light on BACE1 as a drug target as well as off-target effects related to specific features of compounds. Successful development and use of aspartic acid protease inhibitor drugs has been

accomplished for the treatment of other ailments. It is relevant that approved protease inhibitor drugs for HIV/AIDS and renin inhibitors for treatment of hypertension contain classical hydroxyethylene or hydroxyethylamine dipeptide isosteres at the active site. These drugs have been used for decades as chronic therapies. Since the early development of highly selective BACE1 inhibitors with desirable safety and efficacy in animals has been demonstrated, it may also be appropriate to develop the next generation of selective BACE1 inhibitors utilizing these time-tested classical dipeptide isosteres. These approaches are currently under development and show promise toward highly selective BACE1 inhibitors for disease-modifying treatment of AD. We strongly believe that it is too early to give up on the challenges of the BACE1 target.

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† In memory of Dr. Jordan Tang, a brilliant pioneer and scholar with a kind heart.

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