


INSIGHTS

A promising new γ -secretase modulator for Alzheimer's disease

Justyna A. Dobrowolska Zakaria  and Robert J. Vassar 

Effective and safe treatments for Alzheimer's disease (AD) have been an elusive target for scientists who have been working tirelessly to gain control over a disease that is affecting millions of people, with continually rising case numbers as the population ages. However, in this issue of *JEM*, Rynearson et al. (2021). *J. Exp. Med.* <https://doi.org/10.1084/jem.20202560>) present a beacon of hope for this field with a preclinical evaluation of a potent and robust γ -secretase modulator (GSM).

One of the main neuropathological hallmarks of Alzheimer's disease (AD) are amyloid- β ($A\beta$) plaques that accumulate in the brains of those afflicted with this disease. The amyloid hypothesis posits that increased production and/or decreased clearance of $A\beta$ initiates a cascade of events that results in the accumulation of these plaques and eventually leads to neurofibrillary tangles, synaptic loss, and neuronal death manifesting as AD (Tanzi and Bertram, 2005). $A\beta$ peptides of varying length are formed when the amyloid precursor protein (APP) is cleaved first by β -secretase (BACE1) and subsequently by γ -secretase. The length of $A\beta$ formed is dependent on the position at which γ -secretase cuts the protein; the most predominant peptide formed is $A\beta_{40}$. The toxic variant that more readily aggregates into plaques is $A\beta_{42}$ (Tanzi and Bertram, 2005), and evidence from rodent and human studies shows that there is an increase in the ratio of $A\beta_{42}/A\beta_{40}$ in the disease setting (Jankowsky et al., 2004; Kwak et al., 2020). Shorter $A\beta$ peptides, like $A\beta_{37}$ and $A\beta_{38}$, are nontoxic and are found to a lesser extent.

In this issue, Rynearson et al. (2021) present the results of a very thorough preclinical evaluation of several γ -secretase modulators (GSMs) from the pyridazine-derived class. Their goal was to find a GSM that could safely and effectively shift where

the γ -secretase cleaved the C-terminal fragment of APP, so that less of the toxic $A\beta_{42}$ would be formed. One of the compounds presented, compound 2, showed promise for further clinical evaluation, but compound 3 may also be a viable option to pursue in the future. They tested these compounds in several animal models and with short (acute), medium (sub-chronic), and long-term (chronic) treatment. Initially, the researchers tested levels of plasma and brain $A\beta_{40}$ and $A\beta_{42}$ in mice that had received a 9-d oral treatment of compounds 2 and 3 at varying doses (10–50 mg/kg) and found that there was a dose-dependent decrease in $A\beta_{40}$ for both compounds. Brain $A\beta_{42}$ was below detection even at the lowest dose, and plasma $A\beta_{42}$ was significantly lowered starting from the lowest dose as well. Next, the researchers investigated the long-term treatment of compound 2 (the most promising of the compounds from this portfolio) in the presenilin APP (PSAPP) mouse model to determine its effects on $A\beta$ deposition, how well this drug was tolerated, and whether it was safe in longer exposure settings. PSAPP mice have almost no $A\beta$ deposition at 3 mo, but by 6 mo they have a significant number of plaques. Thus, by using this model, the researchers were able to test their compound in two scenarios: 1) would the drug prevent the formation of plaques (i.e., a prophylactic treatment



Insights from Justyna A. Dobrowolska Zakaria and Robert J. Vassar.

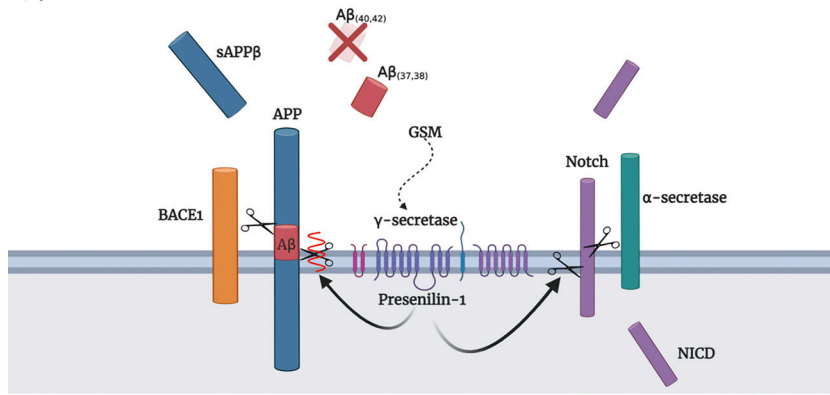
and 2) could the drug stop or reverse the accumulation of plaques after they had already started to form (disease-modifying treatment). Mice in the prophylactic group were 3 mo old, and those in the disease-modifying group were 6 mo old. All mice were treated for 3 mo. Analyses of the brains and plasma in the prophylactic group showed $A\beta_{40}$ and $A\beta_{42}$ were significantly decreased and $A\beta_{38}$ was increased. In the disease-modifying group, $A\beta_{42}$ was significantly decreased as well, and there were changes to $A\beta_{40}$ and $A\beta_{38}$ like in the prophylactic group, but these tests did not meet statistical significance. The authors performed necropsies of the mice that showed there was no obvious toxicity that had occurred as a result of the long-term treatment. They also tested the mutagenic potential of the drug in rats, and their tests confirmed it was not mutagenic. In both

Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL.

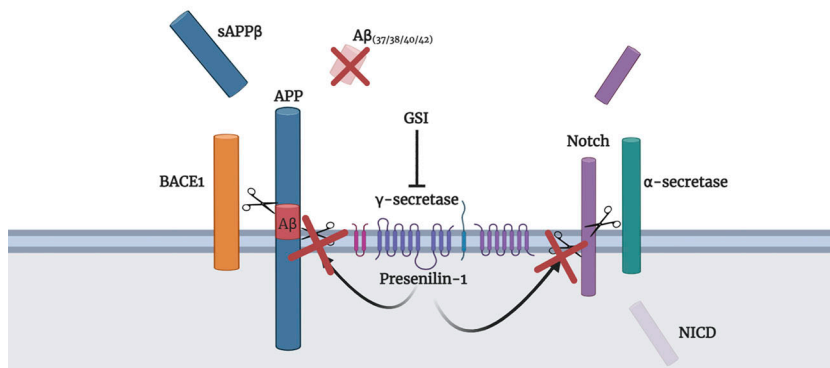
Robert J. Vassar: r-vassar@northwestern.edu.

© 2021 Dobrowolska Zakaria and Vassar. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

A) γ -secretase modulation



B) γ -secretase inhibition



(A) GSMs, such as compound 2, affect the presenilin-1 component of γ -secretase so that it cleaves APP preferentially to produce $A\beta_{37}$ and $A\beta_{38}$, thus decreasing production of the toxic $A\beta_{42}$. The goal of modulators is to not disturb γ -secretase proteolytic cleavage of Notch and over 100 other substrates of γ -secretase that have diverse and important physiological functions. (B) γ -secretase inhibitors (GSIs) are successful at decreasing all $A\beta$ species, including the toxic form, by blocking γ -secretase cleavage of APP. However, this blanket inhibition has the undesired effect of blocking important and necessary physiological processing of Notch and many other substrates. Notch intracellular domain (NICD), for example, is critical in determinations of cell fate not only in embryonic development, but also in adult organisms, and perturbing Notch proteolysis has toxic effects with cancer implications. Figure created with BioRender.com.

mice and rats treated with compound 2, $A\beta_{40}$ and $A\beta_{42}$ were positively correlated when measured in plasma, cerebrospinal fluid (CSF), or brain, indicating that, when determining effects of GSMs, $A\beta$ peptides in biological fluids can be used as a surrogate biomarker of changes of these peptides in the brain.

Following these studies, the researchers next assessed effects of compound 2 on $A\beta_{40}$ and $A\beta_{42}$ in a nonhuman primate (NHP) model of cynomolgus macaques over a range of 10–200 mg/kg of dosing, resulting in verified dose-dependent drug exposures. The maximum lowering (60–70%) of these two plasma $A\beta$ peptides was achieved at the lowest dose of 10mg/kg, which was consistent with their rodent data. Unlike in rodents, there were no $A\beta_{38}$ changes in NHPs. Based on all the animal model data and

human pharmacokinetic parameters, the researchers extrapolated that the 50% effective dose for humans would be 100 mg/d and this would have a 130-fold safety margin (Ryneronson et al., 2021). Testing this compound on human participants is dependent on the results of the FDA's review of the authors' completed investigational new drug safety and toxicity studies.

Over the past two decades, the vast majority of candidate therapeutics for AD have failed (Cummings et al., 2014). Some clinical trials have targeted BACE1 and γ -secretase using inhibitors of these proteases to prevent the formation of $A\beta$ altogether. However, these two secretases both cleave other proteins, some with very important physiological functions, so having a unilateral decrease of their proteolytic activities has

had unintended and unsafe effects, causing some of these clinical trials to be halted due to safety concerns (Coric et al., 2015; Egan et al., 2019; Henley et al., 2019). In this respect, GSMs, such as compound 2, hold an advantage, because unlike γ -secretase inhibitors, they still allow for γ -secretase to function as a protease of other proteins (Wagner et al., 2012) and do not present with any adverse effects on Notch signaling (Kounnas et al., 2010). Their mechanism of action is more targeted, with the goal being to alter the way APP is cut, thus shifting the cleavage to favor formation of the nontoxic $A\beta_{37}$ and $A\beta_{38}$ over the formation of $A\beta_{42}$ (see figure).

Additionally, in recent years it is becoming clearer that treatment of AD, particularly when focusing on decreasing production or increasing clearance of $A\beta$, may need to occur presymptomatically. In 2012, a pivotal study reported that humans with genetic mutations in APP or presenilin-1 (a component of γ -secretase) that lead to development of AD at an early age have amyloid deposits in their brains up to 15 yr before symptom onset (Bateman et al., 2012). Moreover, their CSF $A\beta_{42}$ concentrations started to decline even earlier than that: 25 yr before expected symptom onset! By the time a patient has noticeable symptoms, there is already a significant amyloid plaque burden throughout the brain that has caused a myriad of neurodegeneration. Most likely by this point, the trajectory of the disease is too far along, and continued neurodegeneration cannot be effectively halted by amyloid-targeted therapies, even if the therapies do decrease the patient's current amyloid burden. Since Bateman's report, particularly in the last several years, there has been a more marked shift in AD amyloid-based clinical trials away from patients with mild-to-moderate AD and toward targeting asymptomatic AD populations with no or low amyloid deposition (primary or secondary prevention, respectively) by identifying risk factors and biomarkers and enrolling those patients in studies (Crous-Bou et al., 2017). One large trial, the Dominantly Inherited Alzheimer Network Trials Unit, has enrolled presymptomatic mutation carriers that will eventually develop AD (Moulder et al., 2013). Additionally, secondary prevention trials, such as the Alzheimer's Prevention Initiative, Anti-Amyloid Treatment in Asymptomatic

Alzheimer's Disease, and EARLY (A5) have emerged (Reiman et al., 2011; Sperling et al., 2014). These trials target older individuals that may be more predisposed to developing AD, for example, because they have one or two alleles of ApoE4 (which may enhance deposition or reduce clearance of A β). Participants of these trials undergo brain positron emission tomography imaging that indicates amyloid deposits are present even though they are still asymptomatic.

The holy grail of stopping AD lies with "going earlier" into the prevention paradigm and testing potential therapeutics at a point in the disease where they stand a chance at being effective. Owing to this, compound 2 is an exciting potential new therapeutic in that preclinically it has shown that it can be used in rodents to prevent A β accumulation when administered before any initial A β is found in the brain. This is, therefore, a promising potential therapeutic to test in primary prevention trials. Further, compound 2 has also reduced A β ₄₂ in rodents that already have plaque deposition, thus suggesting the possibility of it being tested in secondary prevention trials, as well.

Compound 2 also may be a safer alternative to other therapeutics in its potential to avoid undesired effects on other γ -secretase substrates. Any AD therapeutic will require a high safety margin for chronic treatment, as it is becoming apparent that treatment to prevent AD would have to commence years, if not decades, before symptom onset, and most likely continue for the duration of the patient's life. In this respect, compound 2 shows promise as well. Additionally, it is quite robust in reducing A β ₄₂, as even in the lowest doses tested in NHPs ~60% of the peptide was eliminated. From studies of an AD-protective APP mutation, A673T, we know that only a ~30% reduction in amyloidogenic peptides is necessary to prevent the disease (Jonsson et al., 2012). Incorporating lower doses to achieve optimally safe A β ₄₂ reduction could further eliminate any potential unexpected adverse effects.

Rynearson et al. (2021) present a well-executed study that contributes significantly to the efforts of the AD field to discover successful disease-modifying AD therapies, of which none yet exist. We look forward to learning more about compound

2's therapeutic potential in humans if it is approved by the FDA.

References

- Bateman, R.J., et al. 2012. *N. Engl. J. Med.* <https://doi.org/10.1056/NEJMoal202753>
- Coric, V., et al. 2015. *JAMA Neurol.* <https://doi.org/10.1001/jamaneurol.2015.0607>
- Crous-Bou, M., et al. 2017. *Alzheimers Res. Ther.* <https://doi.org/10.1186/s13195-017-0297-z>
- Cummings, J.L., et al. 2014. *Alzheimers Res. Ther.* <https://doi.org/10.1186/alzrt269>
- Egan, M.F., et al. 2019. *N. Engl. J. Med.* <https://doi.org/10.1056/NEJMoal812840>
- Henley, D., et al. 2019. *N. Engl. J. Med.* <https://doi.org/10.1056/NEJMc1813435>
- Jankowsky, J.L., et al. 2004. *Hum. Mol. Genet.* <https://doi.org/10.1093/hmg/ddh019>
- Jonsson, T., et al. 2012. *Nature.* <https://doi.org/10.1038/nature11283>
- Kounnas, M.Z., et al. 2010. *Neuron.* <https://doi.org/10.1016/j.neuron.2010.08.018>
- Kwak, S.S., et al. 2020. *Nat. Commun.* <https://doi.org/10.1038/s41467-020-15120-3>
- Moulder, K.L., et al. 2013. *Alzheimers Res. Ther.* <https://doi.org/10.1186/alzrt213>
- Reiman, E.M., et al. 2011. *J. Alzheimers Dis.* <https://doi.org/10.3233/JAD-2011-0059>
- Rynearson, K.D., et al. 2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20202560>
- Sperling, R.A., et al. 2014. *Sci. Transl. Med.* <https://doi.org/10.1126/scitranslmed.3007941>
- Tanzi, R.E., and L. Bertram. 2005. *Cell.* <https://doi.org/10.1016/j.cell.2005.02.008>
- Wagner, S.L., et al. 2012. *Arch. Neurol.* <https://doi.org/10.1001/archneurol.2012.540>