Treatment of Neuroinflammation in Alzheimer's Disease

Robert Chu

November 22, 2017

BIOT-511: Molecular Biology, Pharmacology, and Toxicology of Pharmaceuticals
2017 Cohort of Azusa Pacific University's M.S. in Biotechnology Program

Alzheimer's Disease is a progressive neurodegenerative disorder affecting millions of Americans. Clinical biomarkers of Alzheimer's Disease include amyloid-beta plaques and tau neurofibrillary tangles. Large amyloid-beta plaques initiate a cyclical neuroinflammatory response from astrocytes and microglia. The secretase pathways producing amyloid beta monomers have identified as plausible drug targets; however, inhibition of beta and gamma secretases, as demonstrated by verubecestat and semagacestat clinical trials, does not lead to increased cognitive function. To halt the neuroinflammatory response, cytokine pathways must be inhibited without eradicating the ability of astrocytes and microglia to clear amyloid-beta plaques. Masitinib inhibits the cytokine signaling pathway while preventing malignant immune cell connections found in AD brains.

Introduction

More than one century ago, Dr. Alois Alzheimer first described the pathology of an unknown brain disorder, which he named "arteriosclerotic brain atrophy," a disorder we presently know as Alzheimer's Disease (1). Alzheimer's Disease (AD) is a progressive form of dementia affecting approximately six million Americans (2). AD gradually severs cranial neuron synapses, resulting in neurodegeneration, especially in the frontal cortex, which governs higher social functions (3). Biomarkers of AD include amyloid-β (Aβ) overproduction and tau neurofibrillary tangles (NFTs) (4,5). Physiological symptoms include oxidative neuron damage, glial overactivation, and overstimulation of the neuroinflammatory response (6). While the neuroinflammatory response clears cellular debris and foreign particles, this pathway is cyclically overstimulated in AD patients (7). Masitinib attacks the pathophysiology of Alzheimer's Disease with a two-pronged approach (8). This drug inhibits immune signaling pathways and prevents the formation of excess immune cell junctions, both of which lead to the neuroinflammation common in AD brains (9). Masitinib is exiting Phase III clinical trials, demonstrating significant improvements in cognitive function during treatment of neurodegenerative and autoimmune disorders (10).

Background

The neuroinflammatory response in AD is triggered by Aβ overproduction, a derivative of amyloid-precursor protein (APP) (11). APP is commonly cleaved by α-, β-, or γ-secretases in cranial neurons, producing the APP intracellular domains (AICDs), sAPP molecules, and Aβ (12). APP is sequentially cleaved first by α- or β-secretase (BACE) then by γ secretase (GACE). The product of APP cleavage by α- and β-secretase are secreted APP ectodomain α (sAPP-α) and sAPP-β, respectively (13). sAPP-α enhances neuronal development and is critical in neuroregenerative processes; sAPP-β is involved in synaptic pruning, suppresses neuronal development, and enhances astrocytic differentiation (14, 15). GACE then cleaves the two remaining C-terminal fragments (CTFs) into a 50-amino acid AICD (AICD50) and either Aβ

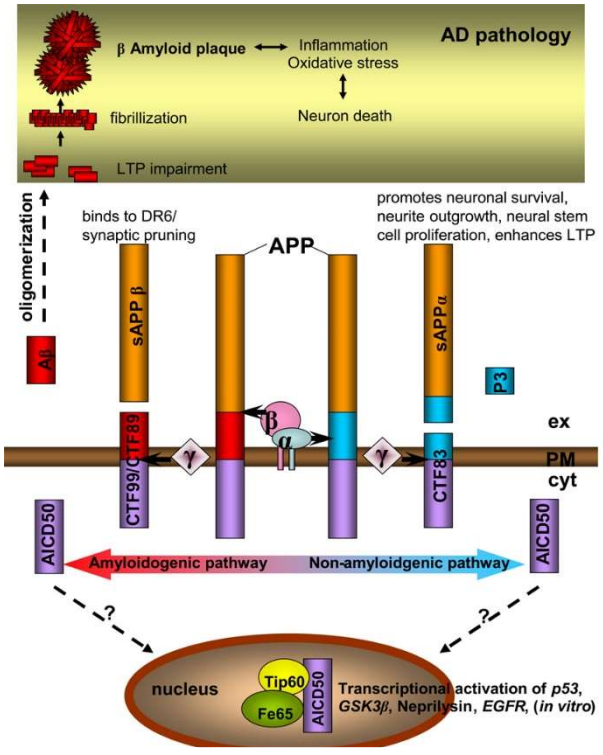

Figure 1. Activity of α-, β-, and γ-secretases on APP and its derivatives. (16)

(from β cleavage) or P3 (from α cleavage) (15). AICD50 is critical in neural cell signaling pathways, specifically those of p53 and other cell growth or proliferation regulators (17). Aβ is then secreted into the extracellular matrix, where monomers polymerize to form plaques (18).

Large Aβ polymers initiate a deadly cycle of inflammatory response factor (IRF) production and Aβ production. Aβ stimulates NFκB activation and extracellular kinase pathways which lead to cytokine or chemokine production (19,20). In response to Aβ deposits, astrocytes upregulate IRF production; IRFs extracellularly upregulate astrocytic IRF and APP production (21, 22). In non-AD brains, resolution occurs when smaller Aβ plaques are cleared with aid from sAPP-α (23). Due to the inability of astrocytes to clear larger Aβ plaques, the Aβ from BACE / GACE cleavage only exacerbates the condition (24). M1-type microglia, which consider Aβ aggregates as pathogens, release pro-inflammatory cytokines which aid in pathogen elimination, but also damage nearby healthy neurons and glial cells (25).

Alternatives

Due to the cyclic nature of neuroinflammation, drugs have been developed to inhibit BACE and GACE activity (26, 27). While these drugs have demonstrated significant improvement during Phase I and II clinical trials, most BACE and GACE inhibitors have failed to clear Phase III clinical trials, due to either lack of improved neural function severe side effects (28). The alternatives considered are verubecestat and semagacestat.

Verubecestat, a BACE inhibitor, was pulled out of Phase II and III clinical trials in February 2017. MERCK stopped the verubecestat trials, citing lack of significant positive results (29). The inhibition of BACE function decreases sAPP-β and AICD50 production, thus decreasing the brain's ability to combat overactive neurons typically found in AD brains (30,31). This drug slows down the rate of Aβ accumulation by BACE inhibition, giving astrocytes and microglia more time to clear larger plaques; however, the loss of sAPP-β may outweigh the benefit of slowed Aβ accumulation. Other BACE inhibitors have shown success in Phase I and II trials, but failed to produce significant improvements in cognitive function in Phase III trials (32,33).

Semagacestat, a GACE inhibitor, was pulled out of Phase III clinical trials in 2010. Eli Lilly halted the semagacestat trials, citing decline of cognitive function (34) Since semagacestat trials demonstrated a decline of cognitive function in patients, some assumptions can be made regarding the effects of GACE inhibitors (35). AICD50 is critical in regulating neural cell growth and proliferation pathways, thus a GACE inhibitor would allow unregulated neuron growth and NFT production (17). If GACE cannot access the AICD50 and P3 precursors, these fragments will also accumulate without regulation, leading to more unusable protein in AD neurons (36). The decline of cognitive function from Eli Lilly's semagacestat trials occurred due to accumulation of unusable protein as well as unregulated neuron growth and proliferation.

Proposed Solution

Masitinib has been used in multiple cancer, neuroinflammatory disease, and autoimmune disease clinical trials (37,38,39). Masitinib, as a Fyn tyrosine kinase blocker and a mast cell-glia axis inhibitor, combats AD pathophysiology with a two-pronged approach (40). Fyn kinases are critical in immune receptor and cytokine signaling pathways while the mast cell-glia axis is critical in neuroinflammatory initiation (41,42). Blocking cytokine immune receptor signaling pathways halts the astrocytic perpetuation of the neuroimmune response. Inhibiting mast cell-glia axis formation lowers the number of microglia and astrocytes contributing to neuroinflammation.

Recommendations

Masitinib passed a Phase II clinical trial in 2011, showing increase in cognitive function (43). France's drug safety committee ANSM halted Phase III clinical trial in 2015 due to deviations from patient safety protocols and toxicity misreports, issuing an audit requiring AB Science to properly report severe adverse events (44,45). In March 2017, AB science finished a major Phase III clinical trial for masitinib in amyotrophic lateral sclerosis (ALS) treatment and presented their positive results at the European Network for the Cure of ALS (ENCALS) conference (46,47). As ALS is also a neurodegenerative disease, masitinib will be a major drug candidate for AD as well (48). AB Science began conducting a Phase 3 clinical study to evaluate the benefits of masitinib in patients with mild-to-moderate AD (10).

Works Cited

1. Drouin, E. and Drouin, G. (2017) The First Report of Alzheimer's Disease. *Lancet Neurol* **9**, 687.

2. 2017 Alzheimer's disease facts and figures. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* **13**, 325-373

3. Bennet, D., Cochran, E., Saper, C., Leverenz, J., Gilley, D., and Wilson, R. (1993) Pathological changes in frontal cortex from biopsy to autopsy in Alzheimer's Disease. *Neurobiol Aging* **6**, 589-96

4. Hardy, J., and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356

5.Wang, Y., Loomis, P. A., Zinkowski, R. P., and Binder, L. I. (1993) A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau. *J Cell Biol* **121**, 257-267

6. Lopategui, C., Herrera, B., and Penton, R. (2014) The role of glial cells in Alzheimer disease: potential therapeutic implications. *Neurologia* **5,** 305-9.

7. Phillips, E., Croft, C., Kurbatskaya, K., O'Neill, M., Hutton, M., Hanger, D., Garwood, C., and Noble, W. (2014) Astrocytes and neuroinflammation in Alzheimer's disease. *Biochem Soc Trans* **5**, 1321-5.

8. Folch, J., Petrov, D., Ettcheto, M., Pedros, I., Abad, S., Beas-Zarate, C., Lazarowski, A., Marin, M., Olloquequi, J., Auladell, C., and Camins, A. (2015) Masitinib for the treatment of mild to moderate Alzheimer's disease. *Expert Rev Neurother* **6**, 587-96.

9. Dubreuil, P., Letard, S., Ciufolni, M., Gros, L., Humbert, M., Casteran, N., Borge, L., Hajem, B., Lermet, A., Sippl, W., Voisset, E., Arock, M., Auclair, C., Leventhal, P., Mansfield, C., Moussy, A., and Hermine, O. (2009) Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor target KIT. *PLoS One* **9**, e7258

10. U.S. National Library of Medicine. (2013) A Phase 3 Study to Evaluate the Safety and Efficacy of Masitinib in Patients With Mild to Moderate Alzheimer's Disease. [online] https://clinicaltrials.gov/show/NCT01872598 (Accessed November 20, 2017)

11. Cai, Z., Hussain, M., and Yan, L. (2014) Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. *Int J Neurosci* **5**, 307-21

12. Epis, R., Marcello, E., Gardoni, F., and Di Luca, M. (2012) Alpha, beta-, and gamma-secretases in Alzheimer's disease. *Front Biosci (Schol Ed)* **4**, 1126-50

13. Zhang, H., Ma, Q., Zhang, Y., and Xu, H. (2012) Proteolytic processing of Alzheimer's β-amyloid precursor protein. *J Neurochem* **120**, 9-21
14. Jiang, J., Wang, Y., Hou, L., Wang, Q., Xu, Z., Sun, Q., and Liu, H. (2013) Distinct roles of sAPP-α and sAPP-β in regulating U251 cell differentiation. *Curr Alzheimer Res* **7**, 706-13
15. Chow, V., Mattson, M., Wong, P., and Gleichmann, M. (2011) An Overview of APP Processing Enzymes and Products. *Neuromolecular Med*. **1**, 1-12
16. Chow, V., Mattson, M., Wong, P., and Gleichmann, M. (2011) An Overview of APP Processing Enzymes and Products, [online] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2889200/ (Accessed November 21, 2017)
17. Alves, D., Sunyach, C., Pardossi-Piquard, R., Sevalle, J., Vincent, B., Boyer, N., Kawarai, T., Giarardot, N., St. George-Hyslop, P., and Checler, F. (2006) Presenilin-dependent gamma-secretase-mediated control of p53-associated cell death in Alzheimer's disease. *J Neurosci* **26**, 6377-85
18. Ueno, M., Chiba, Y., Matsumoto, K., Nakagawa, T., and Miyanaka, H. Clearance of beta-amyloid in the brain. *Curr Med Chem* **35**, 4085-90
19. Behl, C. and Sagara, Y. (1997) Mechanism of amyloid beta protein induced neuronal cell death: current concepts and future perspectives. *J Neural Transm Suppl* **49**, 125-34.
20. Choi, S., Lee, J., Lim, I., Satoh, J., and Kim, S. (2014) Human astrocytes: secretome profiles of cytokines and chemokines. *PLoS One* **4**, e92325
21. Serpente, M., Bonsi, R., Scarpini, E., and Galimberti D. (2014) Innate immune system and inflammation in Alzheimer's disease: from pathogenesis to treatment. *Neuroimmunomodulation* **2-3**, 79-87
22. Verri, M., Pastoris, O., Dossena, M., Aquilani, R., Guerriero, F., Cuzzoni, G., Venturini, L., Ricevuti, G., and Bongiorno, A. (2012) Mitochondrial alterations, oxidative stress and neuroinflammation in Alzheimer's disease. *Int J Immunopathol Pharmacol* **25,** 345-353.
23. Gralle, M., Botelho, M., and Wouters, F. (2009) Neuroprotective secreted amyloid precursor protein acts by disrupting amyloid precursor protein dimers. *J Biol Chem* **22**, 15016-25
24. Atwood, C., Obrenovich, M., Liu, T., Chan, H., Perry, G., Smith, M., and Martins, R. (2003) Amyloid-β: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-β. *Brain Res Rev*, **43**, 1-16

25. Czeh, M., Gressens, P., and Kaindl, A. (2011) The yin and yang of microglia. *Dev Neurosci* **33**, 199-209.
26. Neumann, U., Rueeger, H., Machauer, R., Veenstra, S., Lueoend, R., Tintelnot-Blomley, M., Laue, G., Beltz, K., Vogg, B., Schmid, P., Frieauff, W., Shimshek, D., Staufenbiel, M., and Jacobson, L. (2015) A novel BACE inhibitor NB-360 shows a superior pharmacological profile and robust reduction of amyloid-β and neuroinflammation in APP transgenic mice. *Mol Neurodegener* **10**, 44.
27. Wolfe, M. (2012) γ-Secretase inhibitors and modulators for Alzheimer's disease. *J Neurochem* **120**, 89-98
28. Cummings, J., Lee, G., Mortsdorf, T., Ritter, A., and Zhong, K. (2017) Alzheimer's disease drug development pipeline: 2017. *Alzheimers Dement (N Y)* **3**, 367-384.
29. Merck & Co., Inc. (2017) Merck Announces EPOCH Study of Verubecestat for the Treatment of People with Mild to Moderate Alzheimer's Disease to Stop for Lack of Efficacy. [online] http://investors.merck.com/news/press-release-details/2017/Merck-Announces-EPOCH-Study-of-Verubecestat-for-the-Treatment-of-People-with-Mild-to-Moderate-Alzheimers-Disease-to-Stop-for-Lack-of-Efficacy/default.aspx (Accessed September 24, 2017).
30. Dobrowolska, J., Michener, M., Wu, G., Patterson, B., Chott, R., Ovod, V., Pyatkivskyy, Y., Wildsmith, K., Kasten, T., Mathers, P., Dancho,M., Lennox, C., Smith, B., Gilberto, D., McLoughlin, D., Holder, D., Stamford, A., Yarasheski, K., Kennedy, M., Savage, M, and Bateman, R. (2014) CNS Amyloid-β, Soluble sAPP-α and -β Kinetics During BACE Inhibition. *J Neurosci* **24**, 8336-46.
31. Nistico, R., Salter, E., Nicolas, C., Feligioni, M., Mango, D., Bortolotto, Z., Gressens, P., Collingridge, G., and Pineau, S. (2017) Synaptoimmunology – roles in health and disease. *Mol Brain* **10**, 26.
32. Vandenberghe, R., Rinne, J., Boada, M., Katayama, S., Scheltens, P., Vellas, B., Tuchman, M., Gass, A., Fiebach, J., Hill, D., Lobello, K., Li, D., McRae, T., Lucas, P., Evans, I., Booth, K., Luscan, G., Wyman, B., Hua, L., Yang, L., Brashear, H., and Black, R., for the Bapineuzumab 3000 and 3001 Clinical Study Investigators. (2016) Bapineuzumab for mild to moderate Alzheimer's disease in two global, randomized, phase 3 trials. *Alzheimers Res Ther* **8**, 18.
33. Salloway, S., Sperling, R., Fox, N., Blennow, K., Klunk, W., Raskind, M., Sabbagh, M., Honig, L., Porsteinsson, A., Ferris, S., Reichert, M., Ketter, N., Nejadnik, B., Guenzler, V.,

Miloslavsky, M., Wang, D., Lu, Y., Lull, J., Tudor, I., Liu, E., Grundman, M., Yuen, E., Black, R., and Brashear, H., for the Bapineuzumab 301 and 302 Clinical Trial Investigators. (2014) Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. *N Engl J Med.* **4**, 322-33.

34. Eli Lilly and Company. (2010) Lilly Halts Development of Semagacestat for Alzheimer's Disease Based on Preliminary Results of Phase III Clinical Trials. [online] https://investor.lilly.com/releasedetail.cfm?releaseid=499794 (Accessed November 21, 2017)

35. Doody, R., Raman, R., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, M., Kieburtz, K., He., F., Sun, X., Thomas, R., and Aisen, P. for the Alzheimer's Disease Cooperate Study Steering Committee; Siemers, E., Sethuraman, G., and Mohs, R. for the Semagacestat Study Group. (2013) A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. *N Engl J Med* **369**, 341-50.

36. Potter, R., Patterson, B., Elbert, D., Ovod, V., Kasten, T., Sigurdson, W., Mawuenyega, K., Blazey, T., Goate, A., Chott, R., Yarasheski, K., Holtzman, D., Morris, J., Benzinger, T, and Bateman, R. (2013) Increased *in vivo* Amyloid β-42 production, exchange, and irreversible loss in Presenilin Mutations Carriers. *Sci Transl Med* **5**, 189

37. Deplanque, G., Demarchi, M., Hebbar, M., Flynn, P., Melichar, B., Atkins, J., Nowara, E., Moye, L., Piquemal, D., Ritter, D., Dubreuil, P. Mansfield, C., Acin, Y., Moussy, A., Hermine, O. and Hammel, P. (2015) A randomized, placebo-controlled phase III trial of masitinib plus gemcitabine in the treatment of advanced pancreatic cancer. *Ann Oncol* **6**, 1194-1200

38. Trias, E., Ibarburu, S., Barreto-Nunez, R., Babdor, J., Maciel, T., Guillo, M., Gros, L., Dubreuil, P., Diaz-Amarilla, P., Cassina, P., Martinez-Palma, L., Moura, I., Beckman, J., Hermine, O., and Barbeito, L. (2016) Post-paralysis tyrosine kinase inhibition with masitinib abrogates neuroinflammation and slows disease progression in inherited amyotrophic lateral sclerosis. *J Neuroinflammation* **13**, 177

39. Tebib, J., Mariette, X., Bourgeois, P., Flipo, R., Gaudin, P., Le Loet, X., Gineste, P., Guy, L., Mansfieild, C., Moussy, A., Dubreuil, P., Hermine, O., and Sibilia, J. (2009) Masitinib in the treatment of active rheumatoid arthritis: results of a multicenter, open-label, dose-ranging, phase 2a study. *Arthritis Res Ther* **3**, R95

40. Nygaard, H., van Dyck, C., and Strittmatter, S. (2014) Fyn kinase inhibition as a novel therapy for Alzheimer's disease. *Alzheimers Res Ther* **1**, 8

41. Panicker, N., Saminathan, H., Jin, H., Neal, M., Harischandra, D., Gordon, R., Kanthasamy, K., Lawana, V., Sarkar, S., Luo, J., Anantharam, V., Kanthasamy, A., and Kanthasamy, A. (2015) Fyn Kinase Regulates Microglial Neuroinflammatory Responses in Cell Culture and Animal Models of Parkinsons's Disease. *J Neurosci* **27**, 10058-77.
42. Beghdadi, W., Madjene, L., Benhamou, M., Charles, N., Gautier, G., Launay, P., and Blank, U. (2011) Mast Cells as Cellular Sensors in Inflammation and Immunity. *Front Immunol* **2**, 37.
43. US. National Library of Medicine. (2013) Activity of Masitinib (AB1010) in Mild to Moderate Alzheimer's Disease. [online] https://clinicaltrials.gov/ct2/show/NCT00976118 (Accessed November 21, 2017)
44. Macdonald, G. (2017) AB Science suspends French masitinib trials after ANSM finds deviations. [online] https://www.in-pharmatechnologist.com/Article/2017/05/15/AB-Science-suspends-French-masitinib-trials-after-ANSM-finds-deviations (Accessed November 21, 2017)
45. Globe Newswire. (2015) AB Science: Successful completion of futility test for masitinib in Alzheimer's disease. [online] https://globenewswire.com/news-release/2015/02/09/704560/10119342/en/AB-Science-Successful-completion-of-futility-test-for-masitinib-in-Alzheimer-s-disease.html (Accessed November 22, 2017)
46. Globe Newswire. (2017) AB Science announces positive top-line results of final analysis from study AB10015 of masitinib in amyotrophic lateral sclerosis (ALS). [online] https://globenewswire.com/news-release/2017/03/20/941972/0/en/AB-Science-announces-positive-top-line-results-of-final-analysis-from-study-AB10015-of-masitinib-in-amyotrophic-lateral-sclerosis-ALS.html (Accessed November 22, 2017)
47. Globe Newswire. (2017) AB Science presents phase 3 data for masitinib in amyotrophic lateral sclerosis (ALS) at the European Network for the Cure of ALS (ENCALS) annual meeting [online] https://globenewswire.com/news-release/2017/05/18/987886/0/en/AB-Science-presents-phase-3-data-for-masitinib-in-amyotrophic-lateral-sclerosis-ALS-at-the-European-Network-for-the-Cure-of-ALS-ENCALS-annual-meeting.html (Accessed November 22, 2017)
48. Piette, F., Belmin, J., Vincent, H., Schmidt, N., Pariel, S., Verny, M., Marquis, C., Mely, J., Hugonot-Diener, L., Kinet, J., Dubreuil, P., Moussy, A., and Hermine, O. (2011) Masitinib as an adjunct therapy for mild-to-moderate Alzheimer's disease: a randomized, placebo-controlled phase 2 trial. *Alzheimer Res Ther* **2**, 16