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Alzheimer's Disease: A Challenge in the Face of Modern Era

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Abstract

Alzheimer's disease (AD) is a devastating disease which affects the mental capacity and hence the standard of life of patients suffering from this disease. The accumulation of β-amyloid plaques and tau tangles has been found to be central to the disease. Over the years, research on β-secretase has become known as a hopeful target for the exploration of AD. A number of drugs have been approved by the US-FDA which meliorates only some of the symptoms of AD, but do not help in modifying the principal disease mechanisms.

Keywords: Alzheimer, β-amyloid, β-secretase, BACE1, Blood-brain barrier (BBB)

1. Introduction

About a century and a decade ago, a psychiatrist named Alois Alzheimer brought to light abnormal protein deposits in the brain of one of his patients, Auguste Deter, who showed symptoms of being in disorientation along with progressive memory loss and

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focal neurological deficits. It was at this point in history when the research on a disease, which would affect 115.4 million people by2050,began [1]. A few years down the line, a psychiatrist, Emil Kraepelin named this condition as Alzheimer's disease (AD). It is described by the accrual of β-amyloid plaques in the extracellular spaces as well as along the fortifications of the blood vessels and neurofibrillary tangles of the protein tau inside neurons. The buildup of β-amyloid in the extracellular spaces is believed to block communication between neurons, and unusually high levels of tau tangles inside the neurons are speculated to obstruct with the transport of nutrients and other essential molecules within the cell, both eventually leading to fatality of cells. It is an incessantly progressing neurodegenerative disorder which impairs wide areas of the hippocampus and the cerebral cortex. The disease, which has a clinical phase of about 8-10 years, has a pre-clinical prodromal phase which extends over two decades. There are broadly 2 types of AD: early onset with the mean age of 45 years and late onset (also known as sporadic AD) with an average onset age of 80 years. The Early onset AD (which makes up <1% cases of AD and is associated with the familial forms of autosomal dominant inheritance) is attributed to mutations in the gene encoding the amyloid precursor protein (located on chromosome 21), PSEN 1 and PSEN 2.Multiple hypotheses have been speculated in a bid to explain the cause of the disease but the most accepted one is the amyloid cascade hypothesis which projects the agglomeration of misfolded β-amyloid as the root cause. B-amyloid results from the proteolytic cleavage of the transmembrane protein APP by β-secretase followed by cleavage with γ-secretase leading to the formation of Aβ 42 and Aβ 40 in the endocytic regions[2]. Neurofibrillary tangles are nothing but an aggregation of straight or paired helical filaments, twisted ribbons and other conformations of the microtubule protein tau.

1.1 Role of genetic variation

Genetic variation plays a key role in the pathogenesis of AD. Heritability with about 58-79% of variation in the phenotype is estimated to be caused by genetic factors. Among the early-onset AD cases, 60% have various cases within the family and 13% of these have autosomal dominant inheritance pattern [3].

In case of late onset AD, the leading risk factor is apolipoprotein E (APOE) ε4 allele. It confers an increase in risk of AD by about 4-fold in carriers in comparison to non-carriers. The population attributable fraction i.e., the proportional decrease in mortality of the non-APOE genes is found to be as much as 35%.Genetic variations like copy number variations (CNV) also play a significant part in the progress of AD as simple addition or loss)may affect one or more genes altering their functions. In a study conducted on families with autosomal dominant inheritance, a duplication of APP locus was observed wherein an evidently higher mRNA expression level was observed[3]. An investigation of the role of CNVs in late onset AD identified duplication in the CR1 region, producing different CR1 isoforms (CR1-F and CR1-S)and considerable association was observed in the carriers of CR1-S. In another study a chromosomal region including a cluster of olfactory receptors were found to be associated with age of onset of AD.

1.2 β-secretase as a therapeutic target

β-secretase also known as memapsin-2 was discovered in 1999 by serendipity by the research collaboration of Drs. Jordan Tang and Arun Ghosh[4-6]. When the research on aspartic acid proteases was more than several decades old, the existence of new ones, waiting to be discovered was thought to be an unlikely case. The fact that the human body had very few places acidic enough for aspartic proteases to thrive supported his reasoning. However, certain sequences having characteristics similar to that of aspartic proteases emerged on further research and thus in September 1999 cloning and a few properties of memapsin were reported.

Following the discovery β-secretase, the next step was to find a substrate for this enzyme. Expression levels of β-secretase was found to be high in the brain, pancreas and testis. The misunderstanding that the brain cells did not revive easily caused the researchers to focus their research on the brain and thus they synthesized peptides having cleavage sites similar to that of brain membrane proteins leading to the synthesis of APP. The cleavage of this was carried out by memapsin which led to the conclusion

that memapsin-2 was the β-secretase which cleaved APP thus leading to the formation of Aβ.

Following the cloning, kinetic and specificity studies, efforts were aimed at designing an inhibitor based upon transition state mimetic concept with non-hydrolysable dipeptide isostere wherein they zeroed in on Leu-Ala hydroxy ethylene isostere. S-hydroxystereochemistry was preferred over R- hydroxy stereochemistry due to the former's prevalence in transition state inhibitors of other proteases as in the case of HIV-1 protease.

Using solid phase peptide synthesis, a leu-ala isostere with an Fmoc protection of the N-terminus and a tert-butyl dimethyl silyl protection of the hydroxy group was synthesized in the research lab of Dr Ghosh after great difficulties which lead to the discovery of highly potent memapsin 2 inhibitors, OM99-1 and OM99-2.. OM99-2 was found to have a K_i of 1.6nM against memapsin[4].

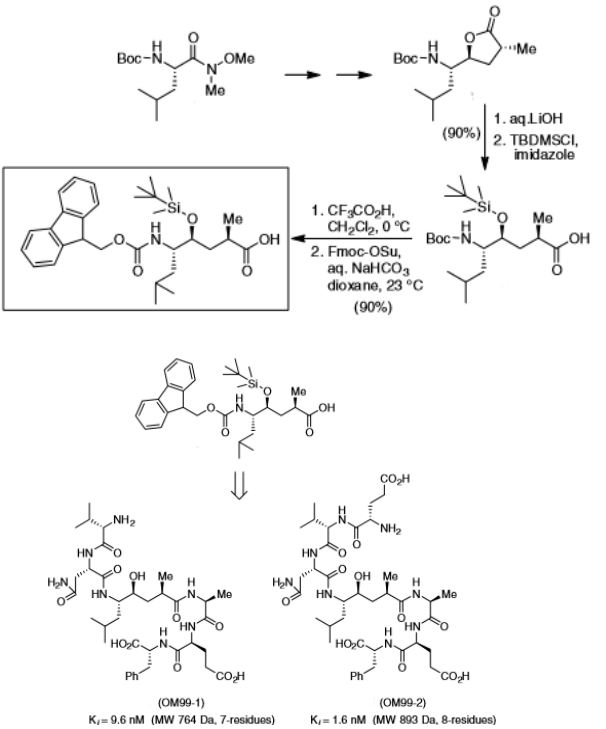

Figure 1: Top - Synthetic scheme showing protection group incorporation; Bottom – structures of OM99-1 & OM99-2 derived from the protected intermediate [4]

Further lead optimization were performed incorporating isosteres and heterocyclic motifs in order to improve the cell potency as well as *in vivo* pharmacokinetic activities. A novel molecule having a 3,5-dimethylpyrazole functional group was synthesized to get a 37nM active compound. This ligand was further modified by attaching an isobutyl side chain giving rise to a strong inhibitor with Ki of 3.3.nM(Fig-2). Furthermore, memapsin-2 inhibitors containing dihydroquinzolin-2-amine group were also investigated displaying a K_i of 103nM and a cellularIC_{50} value of 1uM(Fig-2)[5]

Figure2: Small molecules inhibitors of Memapsin-2

Structure-activity relationship (SAR) studies carried out led to the conclusion that S_1 and S_3 subsites are hydrophobic pockets with the preferences for Leu or Val and Phe, Leu or Met respectively and S_2 subsite is a hydrophilic pocket which favors Asp, Asn and Met. Further optimization of the above inhibitors gave rise to OM00-3 which was found to be 5-fold more potent against BACE1 than its predecessor. S_1'- S_4' subsites showed fewer restrictions while the non-prime side demonstrated greater tolerability [4, 5]. Hydrophobic groups were fixed at the C- terminus of a tetrapeptidehomostatine scaffold with hydrophobic groups and introduced polar substituents. This gave MMI-138 which was 60-fold more selective towards BACE1 over BACE2[6].

Figure3: Structures of potent inhibitors of Mempsin-2

A comparative study of inhibitors with homostatine and statine cores revealed the former to be more effective against BACE1 which led to the bulk of research being carried out on it[7].

1.3 Structure of memapsin-2 (β-secretase)

X-ray crystal structure ofmemapsin-2 in complex with the potent ligand OM99-2 disclosed a bilobal folding of eukaryotic aspartic proteases where the substrate binding cleft is positioned between the C- and N- terminal lobes[8]. The aspartic acid residues at the active site form hydrogen bonds similar to structures of other aspartic proteases. It has a number of surface loops and helix-like insertions where four of the insertions exist in close proximity forming an extension of the cleft that interacts with certain subsites. In general, it was found that the active site of memapsin is more exposed and accessible in comparison to other aspartic proteases. After investigating the structure of β-secretase, that of the inhibitor bound peptide was undertaken which after multiple trial and errors revealed itself. It was found that the inhibitor OM99-2 has strong bonds with six subsites on the protease.

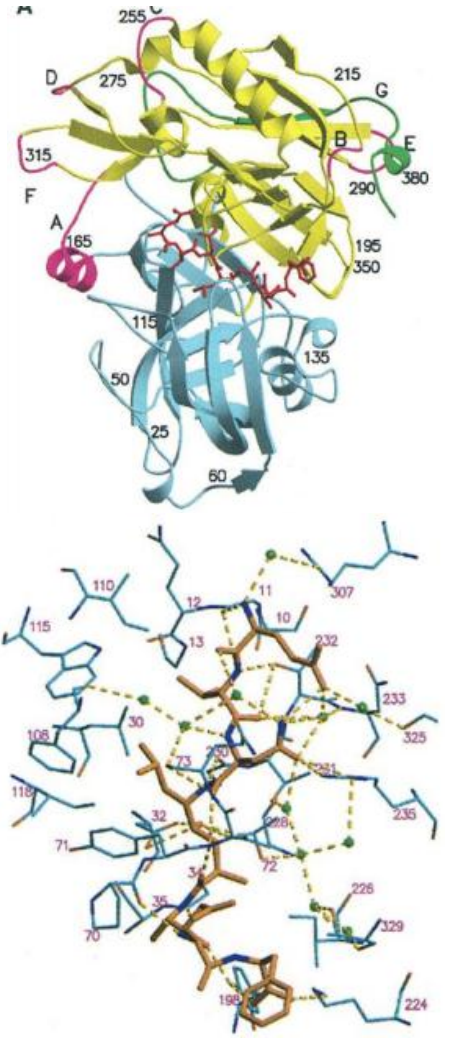

Figure 4: Left - The x-ray crystal structure of memapsin-2 bound to ligand OM99-2. Right - Stereo presentation of interactions between inhibitor OM99-2 (orange) and memapsin 2 (light blue)[8]

Though the discovery of the pseudopeptide inhibitor was pivotal in drug development, its therapeutic applications are limited which can be attributed to its hugemass, the occurrence of several peptide bonds and toxic characteristics. SAR studies of OM99-2 carried out with the objectives of lower molecular weight, peptide properties, toxicities and higher binding affinity lead to several inhibitors with sub nanomolar BACE1 inhibitory constants and significantly high

selectivity against memapsin-1 and cathepsin D[9]. The research further investigated into the synthesis of macrocyclic inhibitors as it was expected that they would be more selective due to restricted bond rotations[4].

1.4 Peptidomimetic inhibitors

To improve membrane permeability and to further lower molecular weight, the focus was turned to peptidomimetic inhibitors. A pharma company named Elan/Pharmacia claimed patent for both statine as well as homostatine containing peptidomimetics. From an array of statins boundto C-terminal amides obtained from substituted benzylamines and amino methylcyclohexanes, the focus was on S_2 and S_3 subsites to be occupied by N-terminal derivatives, the significant ones being isophthalimides, aminothiazoles and sulfones. Polar variations of the P_1' side chain were also examined for greater selectivity over cathepsin D which gave compound-1.Further alterations of the nonprime side isophthalimide moiety using more polar substituents at the S_2 gave rise to exceptional selectivity of BACE1 over BACE2 and cathepsin D. An example of one such inhibitor is compound-2.

Figure 5: Structures of Peptidomimetic inhibitors

A bulk of research has also been carried out on hydroxy ethylamine-based peptidomimetic BACE1 inhibitors.

1.5 Non-peptide BACE1 inhibitors

Small molecule non-peptide inhibitors were synthesized to develop inhibitors with little peptide character and greater metabolic stability and blood-brain barrier penetration. A no. of such inhibitors were developed which were based on moieties like acyl

guanidine, 2-aminopyridine, aminoimidazole, amino/ iminohydantoin, aminothiazole and aminooxazoline etc. An acyl guanidine-based inhibitor 1 was found to have a BACE1 IC_{50} of 3.7μM and the crystal structure of the complex showed that the acyl guanidine group formed 4 hydrogen bonds with the catalytic aspartic acid residues. Further investigation gave another inhibitor 2 with an IC_{50} value of 110nM. It showed 3-fold selectivity over BACE2 and 54-fold-selectivity over cathepsin D. It was also found that when 3-N-methyl-methylsulfonamide was substituted at the isophthalyl group the interactions with the hydrophilic residues of S_2 increased allowing shortening of the prime side of hydroxy ethylene-based indicators. This gave GRL-8234 which lowered plasma concentration of Aβ40 and Aβ42 in transgenic mouse by about 65%[9].

Figure 6: Non-peptide based BACE1 inhibitors

1.6 Alzheimer's diseaseand cognitive impairment [10]

Monocyte chemo attractant protein-1 (MCP-1) also known as chemokine CCL2 is a cytokine which belongs to CC chemokine family. Cytokines and chemokines have profound effect on AD due to their high concentration found in peripheral blood as well as

cerebrospinal fluid (CSF). In humansMCP-1 is produced in the endothelial cells, fibroblast, epithelial cells, smooth muscles, mesangial cell, monocyte, and microglia. However, monocytes/ macrophages constitute the major source of MCP-1.The main functions of MCP-1 are migration and infiltration of monocytes, memory T lymphocytes and natural killer cells. MCP-1 is responsible for inflammatory process in AD which depends on accumulations of beta-amyloid and microglia,

MCP-1 is required for the beta-amyloid clearance, myelin degradation and neuronal loss. In patients suffering from mild cognitive impairement and mild AD, the level of MCP-1 is found to be quite high in serum and CSF. There are two conflicting results from genetic studies regarding the association between CCL2 genotype and risks of AD. According to one study, the single nucleotide polymorphism (SNP) at the CCL2 promoter did not match up with the threat or clinical outcomes of AD. While the results of other genetic study proved that MCP-1 levels have no direct influence on CCL2 promoter polymorphism [10].

By conducting the two year follow up study on patients suffering from MCI and AD, it was found that the plasma MCP-1 level in AD patients (245.6 ± 102.8 micro g/ ml) is higher than MCI patients (204 ± 67.8 micro g /ml). The AD patients are again of different category - very mild AD patients, mild AD patients and severe AD patients. Amongst them severe AD patients (342.8 ± 157.1 micro g/ml) has highest concentration of MCP-1[18].The mouse models of AD expressed the deficiency of CCR2 to be responsible for the acceleration in the progression of early disease. The neuroinflammation in AD and MCP-1 in peripheral blood may draw blood derived monocyte to migrate into brain. To increase the diagnostic accuracy the study can be conducted with the help of biomarkers like beta- amyloid deposition and tau- mediated neuronal degeneration[11-12].

Blood-brain glucose transport in Alzheimer's disease (AD) [13]

Glucose transporter 1 (GLUT1) also known as solute carrier family 2 is a uniporter protein which is encoded by SLC2A1 gene. It is the first glucose transporter characterized and helps in transportation of glucose across the plasma membranes of mammalian cells. Other

than GLUT1, there are many glucose transporters including GLUT2, GLUT3, GLUT4 and GLUT4. GLUT1 is major receptor in the case of vitamin C as well as glucose uptake. GLUT1 is mostly found in fetal tissues. In case of adults, high concentration of GLUT1 is found in erythrocytes and also in endothelial cells of barrier tissues such as blood - brain barrier (BBB). The facilitated diffusion of glucose across the membranes of endothelial cells of BBB is responsible for the glucose to enter into the brain tissue from plasma. Therefore, in order to maintain homeostasis, GLUT1 acts as a key regulator of glucose transfer into and out of brain. Thus, the level of GLUT1 levels in cerebral micro vessels gives us an idea about the brain glucose uptake. The deposition of beta-amyloid (Aβ) and hyper phosphorylated tau -proteins are responsible for the impairment of neurovascular regulation, BBB integrity and expression of glucose transporters at BBB in AD. The onset of symptoms of AD is marked by the loss of glucose transporters from BBB. The glucose transport across BBB has been the rate determining step when it comes to the glucose metabolism in AD (the number of glucose transporter is found to decrease in AD patients). Hence it is crucial to study the physiological impact of BBB glucose transport on AD progress and evolution as well as the prevention of further neurovascular degeneration.

GLP-1 which is an incretin hormone helps to decline cerebral metabolic rate of glucose as well as increase the number of glucose transporters in AD patients. The blood brain glucose capacity (T_{max}) is inversely related with the duration of AD and the cerebral metabolic rate for glucose (CMR_{glu}) is positively co-related with cognition is observed in treatment of AD with liraglutide or placebo (substance/ treatment with no active therapeutic effect). Liraglutide is derivative of human incretin glycogen like peptide-1 which is used as glucagon-like peptide-1 receptor against binding to same receptor. Recent studies have shown that reduced expression of GLUT1 in BBB leads to huge progression of neuropathology and that BBB breakdown is resulted from the GLUT1 deficiency. Glucose metabolism is well explained by two important factors - T max and CMR_{glu}. T_{max} step is based on the density and activity of transporter whereas the CMR_{glu} step is concerned with the maximum velocity (V_{max}) of hexokinase in brain

tissue. Presence of substrate in the tissue at a concentration which is stable with the V_{max} is necessary for the phosphorylation of glucose.

Glucose transfer across the BBB is according to the glucose fluxes which exists in both the membranes (capillary endothelium) and was brought about by the glucose transporter GLUT1. The glucose fluxes are adjusted by

- Changes in concentration gradients in the direction of the tissue which results in difference between the fluxes across the endothelial membranes.
- Changes in affinity of transporters.
- Changes in number or density of transporters

From the above stated methods, the magnitude of the maximum transport capacity (Tmax) is determined by the last method. Behavioral deficits which are associated with the development of neuropathology and synaptic dysfunction which is initiated by hyper phosphorylated tau proteins results from reduced availability of glucose.

The increase in the T_{max} value was elucidated by the boost in the number of GLUTS which was due to the effect of liraglutide or by the postprandial insulin levels. Liraglutide increases the GLUTS levels in the periphery by adenosine monophosphate activated protein kinase (AMPK) dependent mechanism irrespective of insulin level. The astrocytic insulin receptors modulate GLUT1 expressions which cause an increase in GLUT1 protein level across the membranes. Hence glucose transport is potentially an essential therapeutic target treatment on AD.

Impairments in spatial memory and degeneration of hippocampal cholinergic synapses [14]

Choline acetyltransferase (ChAT) is a transferase enzyme which conducts the synthesis of neurotransmitter acetylcholine. The transfer of an acetyl group from co-enzyme acetyl-co-A to choline and later on yielding acetylcholine was catalyzed by ChAT. ChAT is mostly found in abundance at cholinergic neurons - both central nervous systems(CNS) and peripheral nervous system(PNS). The presence of ChAT at nerve cell classifies them into the category of

'cholinergic neuron'. Depending upon the neurotransmitter involved there are various types of transmission. Cholinergic transmission involves the release of neurotransmitter acetyl choline and its activation of the postsynaptic receptor. Early stage of AD is marked by the degeneration of ChAT neurons in the vertical diagonal band of Broca. ChAT directly provides newly generated immature neurons (NGIs) in the dorsal hippocampus (dNGIs) and manage both dNGIs survival and spatial pattern separation. Amyloid- β plaques, cholinergic synaptic transmission, dNGIs survival and spatial pattern separation are all impaired by the onset of AD. The decay in cholinergic synaptic transmission is effectively reduced by the activation of vChATs with theta brust stimulation (TBS) thereby protecting spatial pattern separation impairments in AD patients. The impairment in spatial pattern separation is mainly caused by the degeneration of cholinergic synaptic transmission that regulates the dNGIs survival.

Deposition of amyloid- β has served as major hallmark in the brains of AD which is associated with the loss of central neurons. Spatial memory is a part of memory which is associated with recording information about one's environment and spatial orientation. A person's spatial memory is essential to navigate around a familiar city. Spatial memory has representations within working short term as well as long term memory. In AD patients, the impairment of spatial memory is associated with decrease in excitatory glutamatergic terminals. The degeneration of thousands of synapses in brain is observed in early stages of this particular disease,vChAT has an important role in a range of cognitive activities such as attention, learning and memory and consciousness. The functional synaptic connection with dNGIs is initiated by vChAT. vChAT also help in the regulation of pattern separation associated with spatial memory. Increasing the Act levels with the help of pharmacological inhibition of acetylcholinesterase improves behavioral performance in AD patients. While the experiment approach leads to the damage and possibly the deletion of non-cholinergic pathways or non-specific activation of cholinergic synapses. Muscarinic acetylcholine receptor otherwise known as M_1 receptor mediates cholinergic transmission in dNGIs. Several receptors including α7- and β2-

containing acetylcholine receptors are associated with the proliferation as well as the survival of NGIs.

1.7 Drug Trials

Clinical trial of Solanezumab for mild dementia due to AD[15-16]

Aβ plaques and neurofibrillary tangles are characteristic of AD. To increase the clearance from the brain of soluble Aβ, solanezumab, a humanized monoclonal antibody was designed since the former may lead to toxic effects in the synapses and may in turn cause the deposition of fibrillary amyloid. Patients with mild dementia due to AD were subjected to a double-blind, placebo-controlled, phase 3 trial, defined as a Mini–Mental State Examination (MMSE) ion) and with amyloid deposition shown by means of florbetapir positron-emission tomography or Aβ1-42 measurements in cerebrospinal fluid. Patients were given solanezumab intravenously. A significant benefit was not shown in patients with mild AD upon administering this phase 3 trial of solanezumab intravenously at a dose of 400 mg, compared to placebo, in reducing cognitive decline.

Because of failure of the primary outcome in the analysis, significance testing of the secondary measures was not reported. When compared with the results of earlier phase 3 trials of solanezumab that involved patients with more advanced disease, the current trial of only patients with mild AD was expected to produced outcomes of at least the same magnitude or greater as those seen in the previous trials. Significant differences were not observed between the solanezumab group and the placebo group with regard to serious adverse events. The completion rate of 85.6% in the current trial was high, with limited early withdrawals from the trial.

Insufficient peripheral reductions in soluble free Aβ concentrations and the pathobiological events are the possible reasons why there was no reduction in cognitive decline even after administering various doses of solanezumab. A high level of peripheral target engagement was seen when the solanezumab dose was administered in this trial. However, there was no clinical efficacy for this trial. A lower dose of solanezumab (400 mg) was found to be insufficient to produce a meaningful effect.

A measurement of the cerebrospinal fluid concentrations gives information about the Solanezumab penetration into the central nervous system (CNS). It was observed that penetration of CNS antibody in the brain was too low so as to neutralize enough interstitial fluid Aβ in turn producing a clinically meaningful effect using this dose. However, the pathological changes in the mild stage were not amenable to treatment with a drug targeting soluble Aβ.Solanezumab was basically designed for the disease that results from the overproduction of or reduced clearance of Aβ (or both). Solanezumab would not be expected to slow disease progression.

Thus, the results of trial 3 did not show any benefits of solanezumab on the primary outcome of cognitive decline and did not reproduce the secondary analyses of the first and second trials. The further trials with different doses and timing of solanezumab may require examination. Several strategies have now been figured out, which come with a good rationale ensuring treatment targets for Alzheimer's disease. These anti-amyloid treatments include - passive and active immunotherapies, anti-aggregation approaches and γ and β secretase inhibitors.

Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease[17]

For the treatment of AD, a humanized anti-amyloid beta monoclonal antibody named Bapineuzumab is in clinical development. Two double-blind randomized, placebo controlled, phase 3 trials were conducted for the patients suffering from mild to moderate AD. Just like placebo, bapineuzumab was also administered intravenously.

Upon comparing the primary outcomes, there were no significant differences between the groups. It was observed that, amyloid related imaging abnormalities with the edema among the patients who were administered with bapineuzumab increased on increasing the dosage. Hence it was observed that the clinical outcome in patients with AD was not improved upon administering Bapineuzumab.

There was no significant difference between the groups administered with bapineuzumab and placebo with respect to their primary end points and clinical end points. Only a limited number

of doses of bapineuzumab were used for trials. The abnormalities can be related to the antibody effects on amyloid in the cerebral arterioles, according to the recent research in transgenic mice. A decreased rate in the accumulation of the amyloid in the brain was observed in the patients who received bapineuzumab upon using a marker of neurodegeneration, but it also indicated that bapineuzumab alters Aβ accumulation.

A Phase 3 Trial of Semagacestat for Treatment of AD[18]

The sequential action of β- and γ-secretase on the amyloid precursor protein results in the production of cortical amyloid beta (Aβ) protein plaques which are characteristic of AD.Like in case of the previous trials, this paper also deals with double-blind, placebo-controlled trial in patients with the probability of developing AD. This involves assessment of the changes in cognition subscale and thus changes in the functioning were assessed in such patients. These trials were terminated based on the recommendation by the data and safety monitoring board much before completion of the trials.

These observations indicated that Semagacestat treatment resulted in the loss of more weight and more probability of developing skin cancer and other infections, due to the side effects. It may also lead to reduced levels of lymphocytes, T-cells, immunoglobins, elevation in urine pH etc.

Unlike placebo, Semagacestat did not improve cognition status. Moreover, higher doses of the later resulted in worsening of the functional ability of the patients. Thus, there was no observable benefits seen after the administration of the γ-secretase inhibitor i.e. Semagacestat.

2. Conclusion

It's been more than a decade since the discovery of AD but the scientific community has not been able to find a cure despite several efforts. BACE1 continues to be a promising drug target for the treatment of AD based on computational design approaches. Several drug trials are underway which may lead to a positive outcome sooner than later.

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