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***RESEARCH ARTICLE***

# Targeting Aβ protein in Alzheimer's Disease

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## ABSTRACT:
Alzheimer's disease (AD) is one of the most common causes of dementia in the society. Recent reports suggest that > 4.7 million people of ≥ 65 years of age are living with AD in the USA. AD is predicted to affect one in 85 people globally by 2050. The malfunctioning and gradual death of neurons in the disease results in loss of memory and cognitive functions. The disease is characterized by accelerated accumulation of amyloid β (Aβ) plaque around neurons and hyperphosphorylated microtubule associated tau protein in the form of neurofibrillary tangles within the cells. In this review, we have described and discussed the recent focus on therapeutic interventions targeting at various Aβ-associated pathological mechanisms of AD and experimental strategies focusing on Aβ which aim to decrease the production of the protein, prevent its aggregation or increase the removal of it from the brain. The β secretase is (the enzyme that initiates the generation of Aβ) an attractive drug target for lowering cerebral levels of APP for the treatment of AD. It was noted that that Aβ represents a potent molecular target for pharmacological manipulation to perhaps prevent the onset and progression of Alzheimer's disease.

**KEYWORDS:** Alzheimer's disease, targeting, amyloid β, experimental strategies, and β secretase.

## INTRODUCTION:
Alzheimer's disease (AD) is one of the most common causes of dementia in the society. The malfunctioning and gradual death of neurons in the disease results in loss of memory and cognitive functions. The general overview of AD is illustrated in the Figure 1 below. The disease is characterized by accelerated accumulation of amyloid β (Aβ) plaque around neurons and hyperphosphorylated microtubule associated tau protein in the form of neurofibrillary tangles within the cells. Recent reports suggest that > 4.7 million people of ≥ 65 years of age are living with AD in the USA. AD is predicted to affect one in 85 people globally by 2050.[1]

### Types of AD:
The AD is generally classified into two types: (1) early onset/familial AD (FAD); and (2) sporadic AD (SAD).[1] The FAD is a condition characterized by early onset dementia (age at onset < 65 years) and a positive family history for dementia. The genes: APP (amyloid precursor protein), PS1 (Presenilin 1) and PS2 (Presenilin 2) are responsible for the presence of FAD. To date, 230 mutations in presenilin (PS1, PS2) and APP genes have been identified in FAD.[2]

The APOE gene is responsible for the sporadic form of the disease. Other molecular factors related to the immunological cause (TREM2) of the disease are a disorder of the lipid (ABCA1, ABCA7) or biothiol (MTHFD1) metabolism and of the transport of metabolites (BIN1). Currently, it is believed that APOE is a risk factor for both SAD and late-onset FAD.[3] However, there was no difference in the pattern of distribution of the various pathologic features or in the ratio of neuronal loss or in clinical features, incidence of risk factors for dementia, or MRI or PET features.[4, 5]

**Stages of progress of AD:**

Earliest Alzheimer's - changes may begin 20 years or more before the diagnosis. Impact can be observed on 'learning and memory' and 'thinking and planning' abilities. Mild to moderate Alzheimer's - generally last from 2 - 10 years. Impact can be observed on 'speaking and understanding speech' and on your sense of where your body is in relation to objects around you. Severe Alzheimer's – may last from 1 - 5 years. The patient looses ability to communicate and even, to recognize family and loved ones.

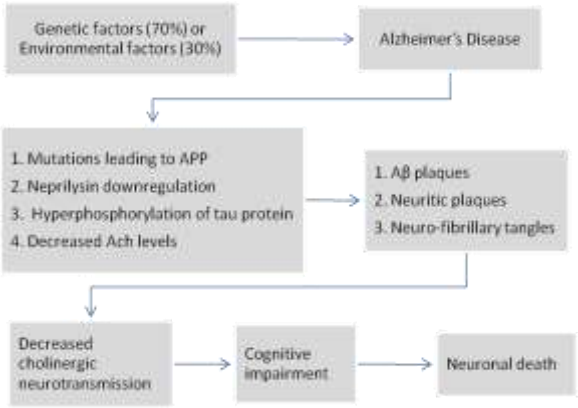


**Figure 1: Alzheimer's disease overview**

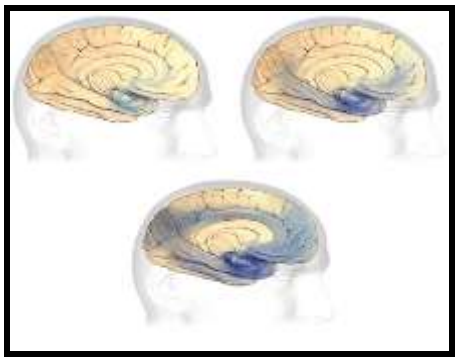

**Figure 2: Stages of Alzheimer's disease**

The progression of AD can also be broken into three basic stages: (1) preclinical (no signs or symptoms); (2) mild cognitive impairment; and (3) dementia.[1] It was noted that Aβ plaques are developed far before an individual starts exhibiting AD symptoms. Shortly after development of plaques, tangles composed of tau protein will also build up in the brain and together, they (plaques and tangles) damage nerve cells in the brain leading to dementia and cognitive decline.[6]

**Overview of targets and drugs for AD:**

There are several drugs available in the market and many are in the clinical pipeline which is summarized in the table below. As this review focuses on Aβ target, we are limiting the general overview of drugs for AD to the Table 1.

**Table 1: Targets and Drugs for Alzheimer's disease**

| Sr. No. | Targets | Marketed Drugs/Molecules in Pipeline |
|---|---|---|
| 1 | Acetylcholinesterase inhibitor (AChE) | Rivastigmine, donepezil, tacrine, galantamine |
| 2 | β-secretase (β-site APP cleaving) | AZD3293, TAK-070, MK-8931 |
| 3 | Activators of specific muscarinic (mAChR: M1 & M4) and nicotinic (nAChR: α7 & α2β4) receptors | 77-LH-28-1, AF102B, AF267B, EVP-6124, VU0364572, VU0364572 |
| 4 | N-methyl D-aspartate (NMDA) antagonist | Abixa, akatinol, axura, ebixa, Memantine, nameda |
| 5 | Inhibition of aggregation of tau | LMTX |

**Overview of Aβ:**

The amyloidogenic hypothesis proposes that Aβ plays a key role in AD. Several pharmacological approaches aim to reduce the formation of Aβ peptides by inhibiting the β-secretase and γ-secretase enzymes. In addition, both passive and active immunotherapies have been developed for the purpose of inhibiting Aβ peptide aggregation. Progress in identifying the molecular basis of AD may provide better models for understanding the causes of this neurodegenerative disease. The lack of efficacy of solanezumab (a humanized monoclonal antibody that promotes Aβ clearance in the brain), demonstrated by 2 recent Phase III clinical trials in patients with mild AD, suggests that the amyloidogenic hypothesis needs to be revised.[7]

In AD, aggregation of Aβ, produced from proteolytic cleavage of amyloid precursor protein, is believed to be implicated in the pathophysiological cascade leading to neuronal death. Most AD drugs currently available can only alleviate symptoms rather than modify the underlying molecular cause of AD. In this review, we have described and discussed the recent focus on therapeutic interventions targeting at various Aβ-associated pathological mechanisms of AD. The described therapeutic strategies include 1) reduction of synthesis of Aβ, 2) inhibition of Aβ aggregation, 3) immunotherapeutic/ enzymatic clearance of Aβ, 4) targeting other amyloidogenic proteins interacting with Aβ and 5) amelioration of Aβ downstream toxic effects.[8]

**What is Aβ?**

Aβ is a small piece of a larger protein called "APP". Although scientists have not yet determined APP's normal function, they know a great deal about how it appears to work. In its complete form, APP extends from the inside of brain cells to the outside by passing through the fatty membrane around the cell. When APP is "activated" to do its normal job, it is cut by other proteins into separate, smaller sections that stay inside and outside cells. There are several different ways APP can be cut; under some circumstances, one of the pieces produced is Aβ.[9]

**Why is Aβ a prime suspect in AD?**
Aβ is chemically "stickier" than other fragments produced when APP is cut. It accumulates in stages into microscopic amyloid plaques that are considered a hallmark of a brain affected by Alzheimer's. The pieces first form small clusters called oligomers, then chains of clusters called fibrils, then "mats" of fibrils called beta-sheets. The final stage is plaques, which contain clumps of beta-sheets and other substances. According to the amyloid hypothesis, these stages of Aβ aggregation disrupt cell-to-cell communication and activate immune cells. These immune cells trigger inflammation. Ultimately, the brain cells are destroyed.[9]

**What evidence implicates Aβ?**
Supporters of the amyloid hypothesis cite three main lines of evidence:

- In a few hundred extended families worldwide, scientists have identified rare genetic mutations that virtually guarantee an individual will develop Alzheimer's. These mutations occur in any of three genes. Each of these genes is involved in biological processes associated with Aβ production or accumulation. Only an estimated 1 percent of people with AD have one of these mutations.
- Scientists have developed mice genetically engineered to carry some of these genetic mutations. The mice develop amyloid plaques, have difficulty remembering their way through mazes and develop other symptoms that mimic human Alzheimer's.
- Individuals with Down syndrome, who have three copies of the chromosome carrying the APP gene instead of the normal two, almost invariably develop amyloid plaques by age 40. Not all people with Down syndrome develop AD, but studies suggest that about 75% of those older than age 65 have Alzheimer's.

Although several clinical trials of anti-Aβ drugs had previously been unsuccessful, a trial published in September 2016 found evidence that an antiamyloid antibody reduced Aβ levels in the brain and slowed the rate of decline in cognitive function in people with mild or preclinical AD. However, not all scientists are convinced that Aβ is the primary cause of Alzheimer's. Researchers worldwide are investigating a variety of other possible triggers for the destructive series of events that eventually kill brain cells.[9]

If Aβ does play an important role, how could treatments block its effects?

Scientists are testing a number of strategies to block the effects of Aβ. Several drugs targeting Aβ have reached human clinical trials. Until the successful aducanumab trial published in 2016, there was no clear indication that these drugs moderated Alzheimer's brain changes or protected brain cells. Aducanumab, an antibody that binds to both insoluble forms of Aβ (amyloid plaques) and soluble forms, reduced levels of Aβ in the brain and slowed the rate of cognitive decline in a group of people who had mild or preclinical AD.

**Experimental strategies focusing on Aβ:**
These aim to decrease the production of the protein, prevent its aggregation or increase the removal of it from the brain:

**1. Decreasing Aβ production:**
To decrease Aβ production, experimental drugs change the behavior of proteins that cut APP into Aβ. Scientists have identified several of these proteins, called secretases, involved in cutting APP into Aβ. Those that have received the most attention are β-secretase (also known as BACE1) and γ-secretase. Changing the behavior of these proteins could prevent or reduce Aβ production. Drugs called "secretase inhibitors" block the clipping action of secretases. Another approach reduces Aβ by changing the way secretases work or encouraging secretases, such as α-secretase, to cut APP into fragments other than Aβ.[9]

Interestingly, over-expression and knockdown of BACE1 increases and decreases the Aβ production respectively. The molecular docking based approach generated two first generation BACE1 inhibitors namely OM99-2 and OM00-3 which mimicked the natural substrate. Synthetic coumarin derivatives were the first reported compounds which were computationally validated to be dual inhibitors of AChE and BACE1. Using docking studies, some dual inhibitors of AChE and BACE1 have been generated using hydroxyethylene (HE), hydroxyethyleneamine (HEA), and hydroxymethylcarbonyl as the scaffolds and two compounds even exhibited excellent activity in cell based assays. In another computational study, flavonols and flavones namely quercetin, kaempferol, myricetin, morin, and apigenin have been validated to be potent BACE 1 inhibitors. The most effective peptidomimetic BACE1 inhibitors have been the statine-based structures with great binding efficacy and IC50 values.[1]

**2. Preventing Aβ Aggregation:**
Because Alzheimer's is characterized by amyloid plaques, scientists have explored drugs that prevent Aβ aggregation as a potential treatment for the disease. Some studies suggest that the toxic effects of Aβ occur before the formation of plaques and oligomers, so researchers are looking for ways to prevent the initial interactions between Aβ and nerve cells that lead to toxicity.[9]

**3. Increasing Aβ removal:**
Methods to increase removal of Aβ from the brain include mobilizing the immune system to produce antibodies to attack Aβ, administering laboratory-produced antibodies to Aβ and administering natural agents with anti-amyloid effects.[9]

**4. Immune system-generated antibodies to Aβ:**
Experimental agents in this category are called "active vaccines." These vaccines incorporate a Aβ fragment that is attached to a carrier protein. When injected, the body should produce antibodies to attack Aβ and reduce levels of Aβ in the brain.[9]

**5. Laboratory-produced antibodies to Aβ:**
Experimental drugs in this category are called "passive vaccines." These vaccines may be safer because they can be given in predetermined doses and do not stay in the body after dosing ends.[9]

**6. Natural agents with anti-amyloid effects:**
Intravenous immunoglobulin (IVIg) contains a broad array of natural antibodies that may reduce Aβ levels. IVIg is obtained from the plasma of human blood donors.[9]

**How Do Aβ Drugs Work?**
Because Aβ was discovered relatively early in Alzheimer's research, it became the focus of the earliest drug discovery efforts in Alzheimer's and, consequently, today those drugs are some of the most developed. The majority of drugs targeting Aβ have taken one of three approaches. The first is to reduce the production of the protein using gamma-secretase or beta-secretase (BACE) inhibitors. Merck's drug verubecestat—expected to complete a large phase 3 trial this summer—is a BACE inhibitor. The second approach is to bind to and remove soluble Aβ proteins (called monomers and oligomers) that are a precursor to plaques. Eli Lilly's failed drug solanezumab tried this and several similar drugs are still in clinical trials, including crenezumab (Genentech) and CT1812 (Cognition Therapeutics). The final approach—used by Biogen's drug aducanumab and Roche's gantenerumab—involves removing Aβ plaques once they have aggregated in the brain.[7]

**Will Any of These Drugs Succeed?**
Findings from early-stage clinical trials provided strong evidence that Biogen's aducanumab can remove plaques, and that Merck's verubecestat can slow the production of Aβ. While these results show that the drugs "worked" as intended, we still don't know whether they have any effect on the clinical progression of Alzheimer's disease. Will these anti-amyloid drugs restore or slow the decline in cognitive function? The results of these Phase 3 clinical trials—Merck is expected to release findings this summer and Biogen in 2018—should provide the answers.[10]

But even if these anti-amyloid drugs fail, the pipeline of potential Alzheimer's treatments in clinical trials is more diverse than it's ever been. Aging is the primary risk factor for Alzheimer's disease. And more and more Alzheimer's drugs are targeting the biological processes involved in aging—such as increased inflammation, epigenetic changes, and neuronal energy failure.[7]

The Alzheimer's Drug Discovery Foundation, was an early funder of Aβ programs. They supported CTS-21166, developed by Dr. Jordan Tang and CoMentis, which was the first BACE inhibitor program to reach clinical trials. But in 2010—as the pharmaceutical industry began to make significant investments in anti-amyloid clinical trials—we strategically chose to stop funding anti-amyloid drugs and focus our investments on other, more innovative areas of drug research. We believe that a combination of drugs focused on the underlying, age-related causes of Alzheimer's (which may ultimately include anti-amyloid therapies) have the potential to slow its progression enough that, in a typical lifespan, most people will never develop the disease. Thanks to our funding, numerous drugs have reached clinical trials and could be available to patients in a little span of five years[7]

**Current status of Aβ related research:**
Currently, several pharmaceutical companies are developing treatments which use antibodies that target amyloid beta protein in the brain. Promising clinical trial results of these antibodies suggest that an anti-amyloid treatment for Alzheimer's disease may be available to patients within a few years.Researchers also are studying whether earlier treatment with anti-amyloid antibodies and other agents may help slow or even halt the progression of Alzheimer's disease before patients become symptomatic.[6]

More than 100 years after the initial description of AD and the identification of Aβ as a key pathologic component, the search for effective anti-Aβ therapies continues. As several pivotal clinical trials in patients with mild-to-moderate AD near completion, studies in patients with mild cognitive impairment/prodromal AD are just beginning, with the hope that targeting Aβ earlier in the disease process will provide better clinical outcomes.[12] Currently, there are 156 studies reported in the clinical trial data base (as either completed, ongoing, recruiting, or yet to start) which are focusing on Aβ protein as target in AD.[10]

Initial experimental and neuro-pathological evidence for clearance of brain Aβ in response to Aβ immunotherapy is associated with structural and functional rescue of neurons, as well as initial signs of clinical stabilization and reduced rates of dementia progression. major challenges in the future improvement of Aβ immunotherapy include the low penetrations rates of IgG molecules through the blood-brain barrier, possible reductions in brain volume, the possibility of autoimmune disease related to unwanted cross-reactivity with endogenous antigens on physiological structures, micro-hemorrhages related to cross-reaction with pre-existing vascular amyloid pathology, possible relocalization of Aβ from Aβ plaques to brain blood vessels resulting in increased amyloid angiopathy, and the lacking activity of Aβ antibodies on pre-existing neurofibrillary tangle pathology, as well as the lacking molecular identification of the forms of Aβ to be therapeutically targeted. The solution to these challenges may lies in the understanding of the pathogenic transition of soluble Aβ into toxic oligomeric aggregation intermediates in the dynamic equilibrium of Aβ fibril assembly. If these issues are addressed in the timely manner, there is a good chance for Aβ immunotherapy to be one of the first disease-modifying therapies of Alzheimer's disease to be introduced into clinical practice.[11]

**Concluding remarks:**

Despite decades of research, there are still no effective disease-modifying drugs available to treat this neurodegenerative disorder. Current FDA-approved medications primarily offer symptomatic relief and are based upon known neurotransmitter deficits. There are, however, many drugs in preclinical and clinical development which target other aspects of AD pathogenesis. Principal among these are drugs which modulate Aβ, a protein that is believed to be central to the cascade which leads to the development of Alzheimer's disease. It was noted that that Aβ represents a potent molecular target for pharmacological manipulation to perhaps prevent the onset and progression of Alzheimer's disease.[13]

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