IMMUNOTHERAPY FOR ALZHEIMER'S DISEASE

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Immunotherapy for Alzheimer’s disease” Submitted by Sadia Islam Rine (17346062), of spring, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on [10/03/2022].

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Ethics Statement

This is to certify that this project titled “Immunotherapy for the Alzheimer Disease” is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the School of Pharmacy, Brac University constitutes my own work under supervision of Dr. Md. Aminul Haque, Associate Professor, School of Pharmacy, Brac University and I have given appropriate credit where I have used language, ideas or writings of another.

Abstract

Alzheimer's disease is one kind of a brain disorder which has the capability to destroy a person’s memory and thinking ability. To treat this disease various types of immunotherapies are required. This review is basically focused on finding of the immunotherapies that has been currently used for treating the Alzheimer’s disease. Immunotherapy is a kind of treatment for a disease to stimulate the immune system. Thus, immunotherapies have become most prominent to prevent the Alzheimer’s disease. In this literature review it has been shown that in most of the cases active, passive, tau and amyloid-beta immunotherapies are being used to prevent the risk of Alzheimer’s disease. To conclude it can be inferred that, this review might help the reader to know about the immunotherapies of Alzheimer disease however for getting better treatment in AD new treatment strategies need to be established.

Keywords: Alzheimer disease, immunotherapy, tau pathology, amyloid beta, neurodegenerative, dementia.

Dedication:

I want to dedicate this to project work to my beloved family.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

AD	Alzheimer's disease
Aβ	Amyloid Beta
APP	Amyloid Protein Precursor
APOE	Apolipoprotein E
AI	Artificial inteligence
ACH	Amyloid cascade hypothesis
AChEIs	Acetylcholinesterase inhibitor
BBB	Blood Brain Barrier
βA	Beta Amyloid
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EOAD	Early Onset Alzheimer's disease
LOAD	Late onset Alzheimer Disease
MRI	Magnetic Resonance Imaging
NFT	Neurofibrilary Tangles
PET	Positron Emission Tomography
P-Tau	Phosphorylated Tau
T-tau	Total Tau

Chapter 1

Introduction

1.1 Invention of Alzheimer Disease

Alzheimer the name came from a German psychiatrist whose name was Alois Alzheimer (Breijyeh & Karaman, 2020). Alzheimer's disease is actually an early onset disorder, the possible age of infection in this disease is less than 65. The very first person who was infected in this disease, she was associated with neuropathology of AD, however his symptoms appeared in the age of 40. Memory loss, confusion, aggressive, language impairment, paranoid behavior, unpredictable these were her basic symptoms but now these symptoms is associated with the neuropathological markers of AD, extracellular amyloid-positive neuritic plaques and intracellular tau-positive neurofibrillary tangles (NFTs) (Mendez, 2017). Now a days it is one of the leading type of dementia which can be explained as the slow development of the neurodegenerative disease (Breijyeh & Karaman, 2020). Progressive memory loss and cognitive performance deficits, these two are very predominant in case of Alzheimer disease. In statistics it has been shown that this disease is taken place after the age of 65 (Sun et al., 2018). Five neuropathological hallmarks of AD are acetylcholine deficiency, glutamate excitotoxicity, and extracellular deposition of amyloid-β (Aβ plague), formation of intraneuronal neurofibrillary tangles (NTFs), and neuroinflammation, current therapeutic approaches are being influenced by this five neuropathological hallmarks of AD**.** In case of lower concentration of acetylcholine in AD loss of cognitive and behavioral function is very common (Hung & Fu, 2017). There are 12 million people are affected in Alzheimer disease throughout the world. It is not that common in our country however in the USA almost 5.4 million people have been affected by this disease and it has also become a health crisis (Congdon & Sigurdsson, 2018).

1.2 Alzheimer Disease

Alzheimer's disease is also pathologically defined by the extracellular senile plaques that contains amyloid-beta (Aβ) peptide, intracellular neurofibrillary tangles that is composed hyperphosphorylated tau protein, cerebral amyloid angiopathy because of the deposition of Aβ on vessel walls, and loss of neuronal (Sun et al., 2018). Crucial therapeutic strategies for AD is reducing the production and the clearance the deposition of Aβ and hyperphosphorylated tau in the brain. In the past few years many pathogenesis and clinical practice has been gained however the factors that have stimulated the progression of AD, that is still not clear. Some medicine has been given to the AD patients but those are not helpful to minimize the progression of AD. The main purpose is the removal of amyloid beta peptide or to decrease its production but after doing clinical research it still cannot reach its goal. As the way of removing amyloid beta peptide is not successful so now the new strategies tau-targeting is on the top. In the normal condition, tau has the capability to give the microtubules stability and intracellular trafficking regulation. However, for the other conditions like progressive supranuclear palsy (PSP), frontotemporal dementia (FTD), stroke and ischaemia, corticobasal degeneration (CBD), tau does not work properly and that helps to improve the NFT pathology. Though tau is associated with all of these condition; initial location, cell types affected are the some aspects of tau pathology (Congdon & Sigurdsson, 2018). Tau pathology is improving, this process is a complicated multifactorial process (Congdon & Sigurdsson, 2018). On the other hand, some experimental models has been shown that it is possible to prevent the cognitive deficits to apply AβOs in the brain directly by with the help of anti-inflammatory treatment. There are also some clinical tools that depend on immunological activation, those tools are helpful to decrease the deposition of cerebral Aβ. However the amyloid aggregation and control of inflammatory based strategies are not that promising, it has shown a little efficacy (Forloni & Balducci, 2018). All the results of the clinical trials to cure the disease have not been worked. It has become a

necessity to think about the new therapeutic strategies for the AD. Diagnosis at the early stage, early intervention and multi-targeted strategies are being used by the researcher now-a-days (Sun et al., 2018).

Chapter 2

Pathogenesis of Alzheimer Disease

It has already been said that the deposition of the extracellular Aβ in a form of neuritic plaques and congophilic angiopathy also the deposition of the intracellular phosphorylated tau as neurofibrillary tangles (NFTs) are the pathological hallmarks. Basically sporadic and late-onset are the usual form of AD; amyloid cascade hypothesis is the most updated theory and it is also in the dominant position as a cause of AD. This dominant theory has been suggested that the initial pathogenic operator is the deposition of Aβ peptides and that is the most toxic oligomeric form. This results in tau hyperphosphorylation, neuronal loss and NFT formation. To promote the conformational transformation of soluble Aβ and to stabilize pathological oligomeric conformers some proteins perform actively (Wisniewski & Goñi, 2014). In the amyloid cascade hypothesis the Alzheimer disease is characterized by the senile plaques, NFT also the neuronal death so the pathogenesis of AD can be demonstrated depending on the ACH, this is more convenient theory. In this theory it has been said that very first initiative to generate this disorder with the deposition of the Aβ (Kohyama & Matsumoto, 2015). As a neurodegenerative disease AD has been clinically known as progressive memory loss. It has been shown in genetic studies that AD is a heterogeneous type of disease and it contains the early-onset (EOAD) form. All the AD patients are <5%, with onset at <65years and late-onset form LOAD, with onset >65years. Mutation of presenilin 1, presenilin 2 (PS1 and PS2) and the amyloid precursor protein (APP), when it has been attached with the autosomal dominant inheritance that are closely connected with the early-onset AD. In some epidemiological data only 10% of all EOAD cases are found to be autosomal dominant transmission (Kohyama & Matsumoto, 2015).

In case of LOAD, genetics and environment both of the factors are related with the AD and more than 95% patients of AD are afflicted by the LOAD. Inheritance of the apolipoprotein (apo) E4 allele is the prominent genetic risk factor for LOAD and physical activity, diabetes mellitus, hypertension, educational status and head injury are some of the environmental risk factors for LOAD. ApoE functions in AD are quite complicated, however it shows some isotope effects such as aggregation and the removal of Aβ from the brain. A new variant has innovated recently, it is also known as a major risk factor for the LOAD and that also has the similarity with the apoE4. There are many hypotheses that have been claimed to improve the plaques and tangles that may cause synaptic, neuronal loss and loss of memory in case of AD. "Chaperone" proteins help to stabilize the pathological oligomers and it is also useful for the conformational change in the soluble Aβ. Apolipoprotein E (apo E) is the isoform of E4, α1-antichymotrypsin (ACT) and C1q complement factor. Water soluble Aβ fibrils are very much competent and these types of pathological chaperone can be found histologically and biochemically when it is related with the fibrillar Aβ accumulations. However, it cannot be found in the preamyloid aggregates as those are not related to the neuronal loss (Wisniewski & Goñi, 2014).

Moreover, it has been said also from a biochemical perspective, a critical concentration of soluble Aβ and chaperone proteins is achieved when there is a development in the pathologic aggregates. There is an event that is quite important in case of AD and there is a chance for it to reach a concentration of water-soluble Aβ which is critical. It is actually the primary phase of neurodegenerative cascade as this forms the Aβ aggregates. There is some evidence in the FAD (familial Alzheimer's disease) patients also FAD models and those evidence have proved that amyloid cascade hypothesis is actually workable. There are some extensive evidence which actually guides the amyloid cascade hypothesis among the FAD patients also in the FAD model: 1) Inherited AD are basically connected with the APP gene mutation, PRES1 or any

two genes are changeable in the APP process and there is a positive side of that it helps to produce sAβ. 2) An adjunct copy of APP gene is seen because of trisomy 21 that is known as a Down's syndrome. 3) Some other models of amyloid β and tau including transgenic, amyloid β oligomer structure can lead to a pathology which is tau related and the hypothesis related to the NFT structure from Aβ aggregation which can go to downstream. 4) Again in the transgenic mouse models of mutant APP, tau pathology is not related to this. Here the prevention and disposal of Aβ is related with the benefits of cognitive (Wisniewski & Goñi, 2014).

In addition, among the population of the Icelandic the very first report of rare APP mutation has been made and that shows that amyloid cascade is helpful against AD (Wisniewski & Goñi, 2015).

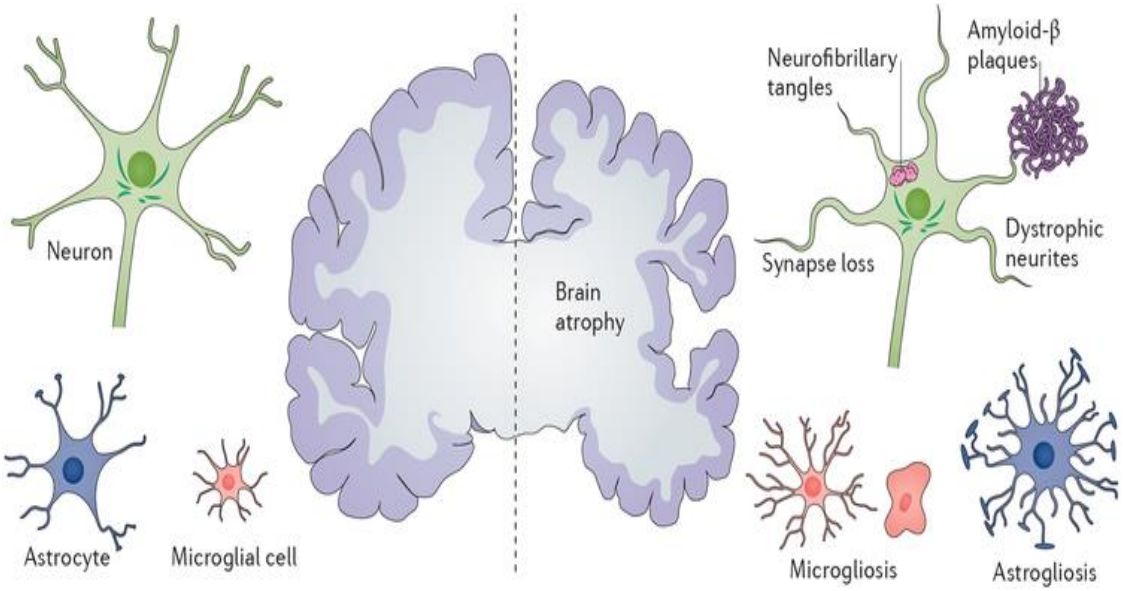

Figure 1: *Pathological hallmarks of Alzheimer disease (Congdon & Sigurdsson, 2018).*

In case of the transgenic mice the mutant APP and tau over-expression both of them are used to help in the (with both amyloid and tau related pathology) prevention of the Aβ pathology that usually leads to amelioration of cognitive deficits and tau related pathology both**.** After that, it has been seen in the providing evidence that Aβ is much known for the late-onset sporadic AD and this is more limited: 1) Correlation that can also be seen biochemically in Aβ

extracted peptides species and it comes from the sporadic AD brains associated with cognitive decline. 2) In the sporadic AD brain there are isolated Aβ peptide oligomers and those are worked for the impaired synaptic structure. 3) When transgenic mice have been injected, amyloid deposits have been induced from the Aβ extracted in the sporadic AD patients. In the primary phase of active vaccination trial in humans from their autopsy data some different evidence to the amyloid cascade hypothesis came and in the nine subjects of the active immunization arm post mortem was accessible. All of these types help to remove plaque and help to decrease Aβ load if it is compared with the non-immunized controls. Though there is evidence, it also can be said that placebo and active immunization group have no differences in case of long term survival effects, dementia in serious level and their effects can be ADAS-Cog, MMSE or DAD. However it is also associated with immunization and has been added as an alternative disease process lately. There are a lot more alternative theories which have been suggested by the various investigators aggregate deposition, Aβ's toxic form and tau both are the pathways which usually goes downstream from a pathogenic default that is upstream. In either of these, the immunotherapy can be addressed as both of these are pathologically efficient in symptomatic AD (Wisniewski & Goñi, 2014).

In AD pathogenesis, hyperphosphorylated may wrap the tau aggregates or tau that may be used to show the toxic effects on the neurons to reduce the tau's affinity for the microtubules. There are many ways, through these pathways Phosphorylation of the tau makes the capability to generate in the various parts of the protein (Panza et al., 2012).

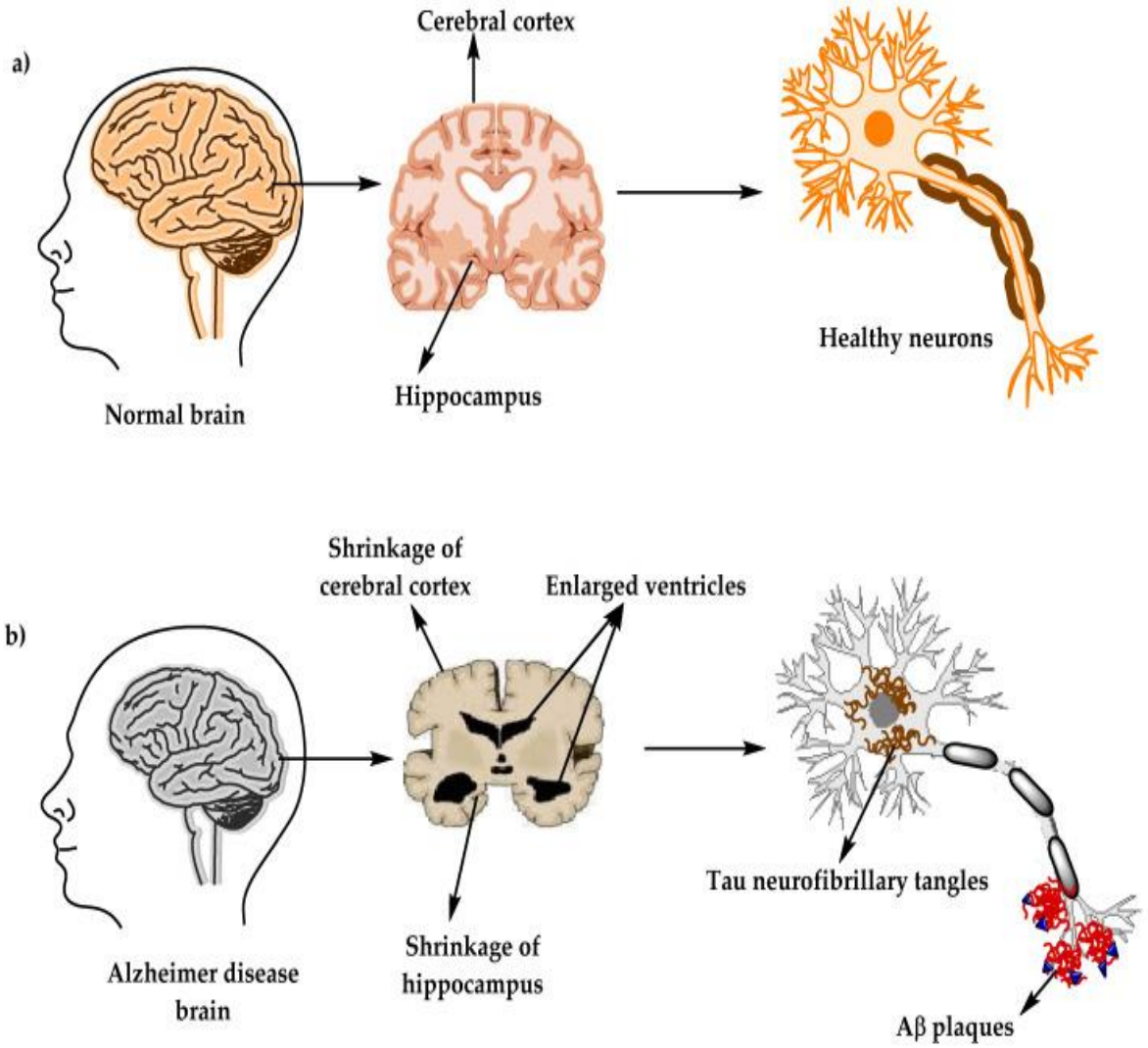

Figure 2: Pathological structure of the a) healthy brain and b) Alzheimer disease brain (Breijyeh & Karaman, 2020)

Chapter 3

Mechanisms of Aβ Immunotherapy

Decreasing the toxic Aβ deposition from the brain is the main function of immunotherapy in Alzheimer's disease. Till now there are three mechanisms have been established those are, (Wang et al., 2012).

3.1 Activation of Microglia

In pathogenesis and immunotherapy of AD, the two most important glial cells that play a vital role are microglia and astrocytes. In case of AD patients these two glial cells number has been increased and both of them get active when they are in touch with Aβ. These activated microglia and astrocytes can be got around the amyloid deposition also in the fragments of the Aβ (Wang et al., 2012).Some of the studies has been said that the histopathology of the Alzheimer disease use to reflect microglia and astrocytes unusual morphology and proliferation. It can be said that two most common characteristics of the neurodegenerative disease are microgliosis and astrogliosis (Hansen, Hanson et al. 2018). When the activation of microglia and astrocytes are blocked then the clearance of Aβ is also hampered. The activated microglia has released chemokines chemokine (C–C motif) ligand 2 (CCL2) and CCL3 which is promoted by the use of astrocytes to amyloid plaques. Therefore, with the help of the glial cells, microglia is very effective for the clearance of Aβ. Activated microglia are used to clean the Aβ with the help of various molecules in various processes. For example, with the help of scavenger receptors (SRs) like CD36, CD47, CD93, integrin-αβ, the class A macrophage SR, and SR class B type I the activated microglia use to promote the phagocytosis of fibrillar and oligomeric Aβ (Wang et al., 2012).

With the help of some receptors heparan sulfate proteoglycans, insulin receptor, and serpin–enzyme complex receptor phagocytosis of soluble Aβ has been triggered by the Activated

microglia. The Activated microglia helps to degrade Aβ by releasing metalloproteases and insulin-degrading enzymes (Wang et al., 2012).

Moreover, it is clearly seen that an Aβ-independent mechanism is helpful to clear the Aβ by activated microglia. The innate immune response has been produced by the lipopolysaccharide (LPS), therefore microglia activate. When LPS has been injected intracranially in an older APP transgenic mice clearance of Aβ has been increased. It has also been seen in the autopsy of a patient who had died due to ischemia, the amount of Aβ deposition is less as the microglia cell was activated there. In the hippocampus the neuronal cell has died because of the LPS- and Aβ-activated microglia. Sometimes it also can see that the activated glial cell could not clear the amyloid pressure in the brain then inflammatory shows the responses and that may cause neurotoxicity, neurodegeneration also the neuronal death. So that the activated glial cells are known as a double-edge as they are doing both of them at the same time (Wang et al., 2012). In addition, much data has been suggested that if microglia functions properly, it will help to prevent AD. However some of the data also suggested that excess activity of the microglia proved to be disadvantageous for the neuron in neurodegenerative disease (Hansen, Hanson et al. 2018). The microglia cell is playing a vital role to make the clearance of Aβ and in immunotherapy one of mechanisms is to make the clearance of Aβ with the help of activation of microglia. Anti-Aβ antibodies were attached with the cells later on that stimulate the activated microglia and monocytes after the immunization with Aβ. *In vitro* experiments it has been showed that with the existence of anti-Aβ antibody maximum amyloid plaques were in the phagocytic vesicles in the exogenous microglia. Basically Aβ deposition of the phagocytosis was being mediated with the help of the fragment crystallizable (Fc) of the antibody. The findings in the above shows that Fc receptor use to mediate the phagocytosis with the help of microglia and that is one of the mechanisms to make the clearance of Aβ by immunization (Wang et al., 2012)

3.2 Inhibition of Aggregation of Aβ

Another proposed mechanism is inhibition of aggregation of Aβ here Aβ antibodies works like a chaperones protein and that helps to make the dissolution of the amyloid fibrils with the help of direct effect on Aβ. In addition, the efficaciousness that has got from the Aβ antibody fully relies on the elements of the Aβ sequence it is connected with. It has been said that when it has been compared, the one which is bound to the main residues of Aβ is less capable than the antibody recognizing Aβ N-terminus. Moreover, Aβ antibody also has the capability to neutralize the synaptic toxicity that has been caused by Aβ oligomers (Wang et al., 2012).

3.3 Peripheral Sink Hypothesis

First two mechanisms need the help from the antibodies to get in the brain. In case of this mechanism antibodies from the brain have the capability to make the net result of the efflux of soluble Aβ into the plasma. When the Aβ antibody has been administered that may help to recover the shortage of memory but it will not decrease the Aβ deposition in the brain. At the same time the antibody–Aβ complex will be developed in the plasma. It is also necessary to know that this is not a mutually exclusive mechanism. For example, the antibodies work as a peripheral sink that cannot get into the brain. To activate the phagocytosis of glial cells or to make the dissolution of amyloid fibrils the antibodies need to bind with the fibrillar Aβ. The isotype and epitope of the antibodies also help in the initial stage of the clearance (Wang et al., 2012).

Chapter 4

Types of Aβ Immunization

Depending on the immunization strategy, there are two types of Aβ Immunization and Aβ immunization targeting has been included the active immunization that is used to introduce a substance that is exogenous and use to activate the immune response (Wang et al., 2012). It has been found that one of the most common active immunization strategies is being used against the bacteria (Spencer & Masliah, 2014). By using the different strategies several active Aβ immunotherapy trials are in the way of development to bring out the anti-Aβ immune response and decrease the adverse effects, however, there is no biomarker data available in these trials (Blennow, Hampel, & Zetterberg, 2014). Passive immunization is a kind of immunotherapeutic approach that has been introduced an antibodies that is exogenous and that has been directed into the human or animal to stimulate the same types of benefit that has in the active vaccination (Wang et al., 2012) Passive immunization has successfully demonstrated that it is possible to treat the disease with the self –antigen (Spencer & Masliah, 2014). It has also been shown that there are many passive Aβ immunotherapy are ongoing depending on the antibodies that has been directed against the epitopes on oligomeric or fibrillar Aβ antibodies. However, there is no data about the biomarker (Blennow, Hampel, & Zetterberg, 2014).

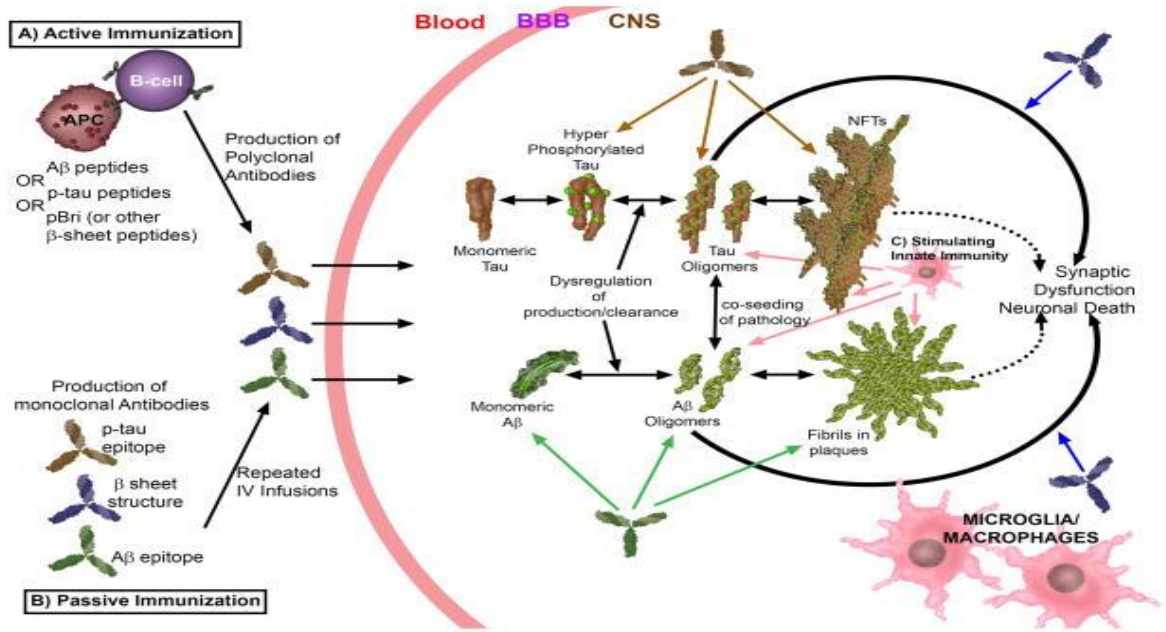

Figure 3: *Various immunotherapeutic Approaches in AD Pathology (Panza et al., 2016)*

4.1 Aβ Active Immunization

Active immunization is basically known as a traditional approach to make the antibody response to the patient by systematically administering a drug (Panza et al., 2012). In the middle of the1990, Beka Solomon's lab has been suggested that for preventing the formation of Aβ fibril and to disaggregate the pre-formed fibrils anti-Aβ could be beneficial. In 1999, it was shown in a pharmaceuticals called ELAN Pharmaceuticals in vivo that active immunization helps to reduce deposition burden in an AD transgenic (Tg) mouse where active immunization is opposed to full-length Aβ. Some studies demonstrated that anti- Aβ antibodies is being generated by the active Aβ immunization that also associated with the human plaques and have B cell epitope not only that these is within Aβ peptide in its first fifteen amino acids however T cell epitopes stay in the middle position also in the C-terminus of Aβ. in both of the case like active and passive Aβ immunotherapy in AD transgenic mice is used to reduce the cerebral Aβ and help to improve the cognition, it mostly works when it is given before the onset of disease pathology (Lemere, 2013).

4.1.1 Preclinical studies of Active Immunization

In 1999 it was tried for the first time in PDAPP mice to immunize with Aβ1–42 peptide. They had found that to decrease the thickness of the amyloid deposition prophylactic and therapeutic immunization was very much beneficial and also resulting in neuritic dystrophy and astrogliosis; those were some neuropathological changes. In various research groups it was replicated in different types of APP transgenic mice. By doing various research on transgenic mice it has been shown a promising result in AN1792 clinical trial. However, at the phase of IIa the clinical trial had been halted as some of the immunized patients had gotten meningoencephalitis. The T-helper (Th) 1 cell epitope was the actual reason behind meningoencephalitis that is produced by the immunization with AN1792 and it is found in the Aβ1–42 peptide. It had also been thought that one of the biggest contributors of meningoencephalitis was Aβ1–42 (Wang et al., 2012).

In addition, a soluble agent polysorbate 80 being used in various commercial products and it was also known as a factors which might cause meningoencephalitis .Recent reports has been shown that severe nonimmunologic anaphylactoid reactions might be caused by the polysorbate 80 (Wang et al., 2012).

It has been recommended that AN1792 vaccination treatment could be more beneficial if it has been begun prior to the AD-related pathologies clinical development. In the cortical areas it has the capability to remove the Aβ with the help of the tau-related pathology. At the early stage of the disease the immunization should have started so that the cognitive benefit could be increased with the Aβ-lowing effects. In the transgenic mouse models of the AD, it has shown that it is possible to prevent the formation of Aβ deposits in case of early vaccination. To protect the formation of the new tangles except causing those have been already formed. (Panza et al., 2012)

Therefore, scientists had started to search for Aβ vaccine that is ideal, that would be helpful to avoid Th1 immune response and that could have the capability to stimulate a Th2 immune response to generate the anti-Aβ antibodies. Immunization with the Aβ1–15 that is used to induce high titers of anti-Aβ antibodies. A vaccine that has two copies of Aβ1–11 and that will not cause any inflammatory response, that type of vaccine can decrease the Aβ deposition. The first used four amino acids are glu-phe-arg-his and these are situated at the position 3–6 of Aβ with the help of the filamentous phage that has been displayed on guinea-pigs and APP [V717I] transgenic mice. The EFRH phage recalled the antibody responses that also help to inhibit the formation of Aβ and the aggregation. AffiRis has developed. AFFITOPE vaccines consist of short peptides mimicking parts of the N-terminus of Aβ which is developed by the AffiRis and which also do not have any types of sequence identity with other types of human proteins. In preclinical study immunization with AFFITOPE vaccines is used to induce the Aβ antibodies and also shows some function (Wang et al., 2012)

4.1.2 Clinical studies of Active Immunization

AN1792, the first clinical trial vaccine of Aβ was AN1792 and in the first stage at least 24 patients had injected QS-21 adjuvant. In the second stage almost 70 patients had injected AN1792 + QS-21 plus polysorbate-80 as a preservative. The results was quite impressive from the two stages where the phase I trial showed the safety and for this reason the clinical trial of phase IIa had been started in September 2001 (Wang et al., 2012)

However, that was stopped in 2002 because of the meningoencephalitis development in 6% of the people, they were all moderate to severe AD patients. The AN1792 vaccine contained the full-length Aβ1-42 peptide, which was also formulated in a strong adjuvant named saporin and polysorbate 80. That has been used in the vaccine to improve the stability and to enhance the solubility of Aβ1-42 peptide. Among all the patients most of them had received 1-3 doses; from

them anti- Aβ antibodies (>1:2,000 titer; “responders”) had been made by 19% of the patients. In the 19% patient they had already got the AD plaques and the vascular amyloid in their brain. For a few responders the deposition of Aβ was decreased focally in a specific region of the brain and over the next few years when that came for the autopsy, most of them were fully demented at the time of their death. However, it also indicated that in the later stage of stage AD pathogenesis withdrawal of the plaque; when the NFTs had already formed and the loss of neurons in specific regions were not advantageous anymore. Interestingly, in neuropil threads and dystrophic neuritis the tau aggregates often related with the plaques and that were also decreased with the help of the AN1792 vaccination. In the case of the neuronal cell bodies there were no changes in tau accumulation. Still the actual reason of the meningoencephalitis in AN1792 vaccine is unknown, however, there are some reasons, and scientists think that might be the possible causes of meningoencephalitis. Those reasons include the full length of Aβ peptide, basically the antigen recognition by Aβ-specific T cells, partial Th1 adjuvant and may be vaccine re-formation with polysorbate 80 (Lemere, 2013).

As a result of the AN1792 trial, a great initiative had been taken here passive immunotherapy had been tested with the help of humanized anti- Aβ monoclonal antibodies (mAb). In addition, several studies had been done to initiate the second-generation active vaccines, there were also many vaccines that had been targeted at an Aβ B cell epitope, at the same time it avoided the T cell epitopes of Aβ. These vaccines were mimotopes vaccines, Aβ-conjugates, DNA, phage, neoepitopes vaccines and adenovirus-associated viral vector vaccines (Lemere, 2013).

The AFFITOPE vaccine is basically composed of 6-amino acid peptides which mimic parts of the native Aβ42. It can easily bring out the immune response as for the human immune system it is the foreign. These vaccines are very much specific. The motive of this vaccine is to target the N-terminus of Aβ where the peptide sequence has the capability to recognize when its N-

terminus is totally free, this is how it is used to protect the cross-reactivity with APP. So this vaccine is known as a highly specific vaccine (Wang et al., 2012)

Some vaccine were being developed by Elan Corporation Inc under clinical trials such as ACC-001 those are NCT00955409, NCT01238991, and NCT00960531 and these vaccine are composed of B-epitope of Aβ (Wang et al., 2012).

DNA vaccines can activate both of the cellular and humoral immune response so it is known as a novel vaccine. After comparing with the other vaccines, it can be said that DNA vaccines are very beneficial. As this vaccine is easy to design, so it is possible to produce this vaccine in a large scale. Their stability is more than the other vaccine and it also gives good safety. Almost one hundred types of DNA vaccines have done the clinical trial and all of them have been reported as safe so far (Wang et al., 2012).

Currently, there are many ongoing clinical studies of the active immunization approaches. Aβ-peptide that has been presented by the antigen, those might have the possibility to show a humoral response and those also have the capability to decrease the potential for the Th1-mediated response. In case of an ideal anti-Aβ vaccine, it should have the capability to stimulate a Th2 immune response by disclosing an anti-Aβ antibody (Panza et al., 2012). There are also some second-generation active Aβ vaccines that have also been tested for the clinical trials. Janssen and Pfizer are doing research where they are monitoring the actual effects of the Aβ short N-terminus peptide-conjugate vaccine which is known as ACC-001(Lemere, 2013).This vaccine is formed in the adjuvant QS-21.Previous year, Novartis Pharmaceuticals had also done some research where they had submitted the data for their active Aβ vaccine which was known as CAD106 (Lemere, 2013).

This vaccine also contains many copies of the Aβ1-6 on QB virus-like particles, sometimes with the adjuvant and sometimes without the adjuvant; however the data analysis is still

pending. Again Affiris AG is testing the mimotopes, molecular mimics for the specific antigen epitopes and they are opposing an unmodified Aβ N-terminus and a pyroglutamate-3-modified Aβ N-terminus. To prevent or protect the plaque deposition is the main motive of all these active vaccines (Lemere, 2013).

4.2 Aβ Passive Immunization

Passive immunization is characterized as the involvement of an antibody administration and that antibody is being generated in a host or model system that is also expanded the efficacy before administering into the patient. In AD, passive immunotherapy may work as an alternative option and that is used to allow the direct control to expand the immune response against Aβ. It is easier to avoid the eliciting of Th1-mediated autoimmunity by the passive transfer of exogenous monoclonal Aβ antibodies (Panza et al., 2012).

4.2.1 Preclinical studies of Passive Immunization

AD transgenic mice that have been treated with the monoclonal Aβ antibodies also demonstrate that the Aβ levels are being reduced in the brain, help to decrease the plaques of the brain pathology and help to develop the cognition. It was possible to observe the beneficial effects because of using different types of antibodies that also showed variety in Aβ-binding properties. After the administration the biological effects were shown within 1 day, as the mechanism of action was simple to remove the plaques of the brain so it takes very short time. It has been recommended that with the help of mechanisms of Aβ binding immunization strategies might function but not be associated with overt SP removal. The plaque formation may be caused by the early soluble oligomeric forms of Aβ and it might be the cause for neuronal death and the development of AD. Thus, for the disease process the elimination of oligomeric species of Aβ might be very much advantageous. In case of the AD animal model systems, the passive immunization demonstrates quite safe (Panza et al., 2012).

A monoclonal antibody is M266 that has been bound to the central domain of amyloid beta peptide. That is used to decrease the Aβ deposition from the brain not only that it also reversed memory loss and broadly learning the PDAPP mice while it has been administered peripherally. Though the M266 antibody binds with the central domain of Aβ, in case of the Aβ deposits in the brain it usually does not bind with them. Plasma Aβ increases are associated with the deposits reduction in the brain and that shows that the Aβ equilibrium between the CNS and the plasma has been changed by administering the M266. To activate the microglia it is possible to reduce the plaques while some of the Aβ antibodies have been bound to the Aβ deposits. However, microhemorrhage can also be seen because of the passive immunization of Aβ antibodies. A modified antibody with less interaction that is also associated with the Fc-γ receptors, some of the complement proteins are being used to decrease the microhemorrhage. However the efficacy is not that high, for decreasing the Aβ deposits (Wang et al., 2012). Pre-clinical studies have shown very beneficial effects in case of the passive immunotherapy against the other Aβ-related targets also (Lemere, 2013).

4.2.2 Clinical trials of Aβ Passive Immunization

In 2000, to begin with illustrated that Bard and colleagues were the first who had shown that Aβ monoclonal antibodies systemic injection were counter for the Aβ N-terminus, 3D6 mAb, into the AD transgenic mice as a result the antibody had been transferred to the brain; bound to the antibody plaques, and the selection of Fc-receptor-mediated microglial phagocytosis of Aβ deposits. It can be said that this antibody is known as the precursor to the humanized N-terminal-specific mAb, Bapineuzumab later on that had been tested in three phases of clinical trials. Whereas Aβ burden in the brain was lower in case Bapineuzumab by in vivo amyloid PET imaging in the second phase of clinical trial for those patients who had AD in mild-to-moderate. However that was not advantageous, there were no clinical benefits found from the phase three clinical trials. There were two possibilities for not having enough clinical efficacy

and the possibility was the appropriate amount of antibody was not enough or the process of treatment was too late for the neurodegenerative changes that may result in memory loss. Previously researched about the Aβ immunotherapy tries their best to clarify these parts. Recently Pfizer and Janssen are doing a research of an Open Label Extension of Phase I clinical trials for the patients who has AD in mild-moderate to know about the safety of the updated version of the Bapineuzumab, AAB-003, to decrease the risk of vasogenic edema and microhemorrhage that was engineered again (Lemere, 2013).

There are three types of anti-Aβ monoclonal antibodies that have been bound to linear epitopes and that is in the Aβ sequence. Antibodies against the N-terminal epitope are from the amino acids 1–10, the central region it is from the amino acids 17–32 and the last one is the C-terminal region of Aβ it is from the amino acids 32–42. Antibodies that are directed to the N-terminal region of Aβ are connected with the Aβ aggregates used to trigger the microglial phagocytic clearance of amyloid plaques with the help of the Fc receptor-mediated mechanism. It is also used to inhibit the aggregation and the neurotoxicity of Aβ. Bapineuzumab demonstrates the monoclonal antibody which is prototypical and directly opposed the N-terminus of Aβ (Panza et al., 2012).

Bapineuzumab is known as a humanized anti-Aβ monoclonal antibody that is against the Aβ1–5. In the Phase II clinical trials it demonstrated that immunization of bapineuzumab is useful to decrease the cerebral Aβ levels, CSF hyperphosphorylated-tau, also the total tau levels if compared with placebo-treated patients. However, the patients who had taken the bapineuzumab did not show any types of clinical benefits. APOEε4 carriers and non-carriers of a *post hoc* analysis recommended that there might be some benefits in the bapineuzumab along with the non-carriers. It can also measure that at least 10% patients among all the patients who had been treated with bapineuzumab had developed the vasogenic cerebral edema. Among this 10%, half of them had shown some transient clinical symptoms like confusion, vomiting,

headache, and disturbance. When the dosages had been reduced these patients had recovered gradually. To monitor the efficacy and safety of the phase three of bapineuzumab in AD patients are ongoing like NCT00667810, NCT00996918, and NCT00998764 (Wang et al., 2012).

Solanezumab is one kind of humanized form of the mouse antibody 266 that is against the Aβ13–28. If compared with the bapineuzumab that is usually associated with the amyloid plaque actively, solanezumab used to be associated with the soluble Aβ and that has sometimes very few or no affinity for its fibrillar form. In the phase II of the clinical trial, the data has been shown that with the help of the dose dependent solanezumab the amount of CSF and Aβ1–42 plasma level were increased. The similarity with the m266, the clearance of Aβ is being promoted by the solanezumab with the help of the peripheral sink mechanism. There is no cognitive improvement observed with in case of the administration of solanezumab in clinical trial phase I and II, no cognitive development has been shown. However, in the phase II clinical trials there were almost 52 patients and none of them had meningoencephalitis and microhemorrhage. In another clinical trial of phase II, there were 33 patients who were from Asia and they all had to face some adverse effects. The phase III clinical trials are ongoing in case of Solanezumab (NCT01127633) (Wang et al., 2012).

Recently, several others Aβ passive immunotherapies research are ongoing. For example, Eisai Inc. is doing research on 800 patients who have early AD and they are conducting large II phase of clinical trials. Their purpose is to know the effects of the Aβ mAb, BAN2401, that is known as the large oligomers and that helps to cure the toxic effects of the neurons. BAN2401 is another one and it has been developed by BioArtic Neuroscience AB and got the licensed to Eisai in 2007. Biogen Idec is conducting the research with the help of Aβ humanized IgG1 mAb, BIIB037 after binding to fibrillar Aβ in plaques however it is not that well to vascular amyloid. The clinical trial for the BIIB307 of the single dose is successful and now they are

moving forward to do the test on the prodromal and mild AD patients for the multiple dose (Lemere, 2013).

IvIg is known as a natural human immunoglobulins that has been obtained from the healthy donor's blood. It has been applied for the treatment for those patients who have immune deficiency disorders but not for the AD, moreover it has got the approval from the United States Food and Drug Administration (Wang et al., 2012).

Chapter 5

Alternative Immunotherapies

5.1 Tau Immunotherapy in Alzheimer Disease

Currently Tau immunotherapies are moving forward to the clinical trials however the field is not properly developed yet, as for the different approaches the safety and efficacy is not well established. After doing so much research by so many scientists the mechanism of action of the amyloid-β (Aβ) antibodies is not properly clear or understandable (Sigurdsson 2016). The microtubule-associated protein's (tau) aggregation and accumulation are the cause of NFTs formation (Wang et al., 2012) In the NFTs there are two aggregated tau species: hyperphosphorylated PHFs of MAP tau (or tau) and straight filaments which are known as the pathological hallmark of AD (Panza et al., 2012).

Moreover, NFTs and other clinical symptoms of AD cannot be detected in the early stage of AD but when the tau oligomer is increased it has been observed in the very early stage of AD (Wang et al., 2012).

Like the Aβ it has been reported that soluble tau aggregates are pathologically known as the tau species and most toxic. Some disadvantages can be seen in tau oligomers, it is used to induce the cell death, memory loss and dysfunction of the mitochondria. There are two reports that have said that removing the intracellular tau vaccine or antibodies could be beneficial. Above all it sounds hard as the soluble tau aggregates stay inside neurons (Wang et al., 2012).

Tau is basically protein 50–75 kDa associated with six various splice variants. For the neurodegenerative disease or injury when the phosphorylated level is being increased in the cerebrospinal fluid (CSF), also known as the biomarker. To form the oligomeric tau, hyperphosphorylated tau needs to bind together from dimers to octamers (Panza et al., 2012).

In the wild-type human tau transgenic mouse observed that hyperphosphorylated tau and the oligomeric tau are being in synaptic loss. To form the detergent-insoluble aggregate the oligomeric tau needs to have at least 40 molecules, structure of the β-sheet and the granular shape formation. This granular shape oligomer tau is related to neuronal loss (Panza et al., 2012). Aβ immunotherapies are not that successful yet as this has to be administered only once so the AD symptoms became apparent. Although scientists are still hoping that it might be beneficial for those who have AD in the early stage. When the symptomatic stage is underway this therapy might be the most sensible course of action (Congdon & Sigurdsson, 2018).

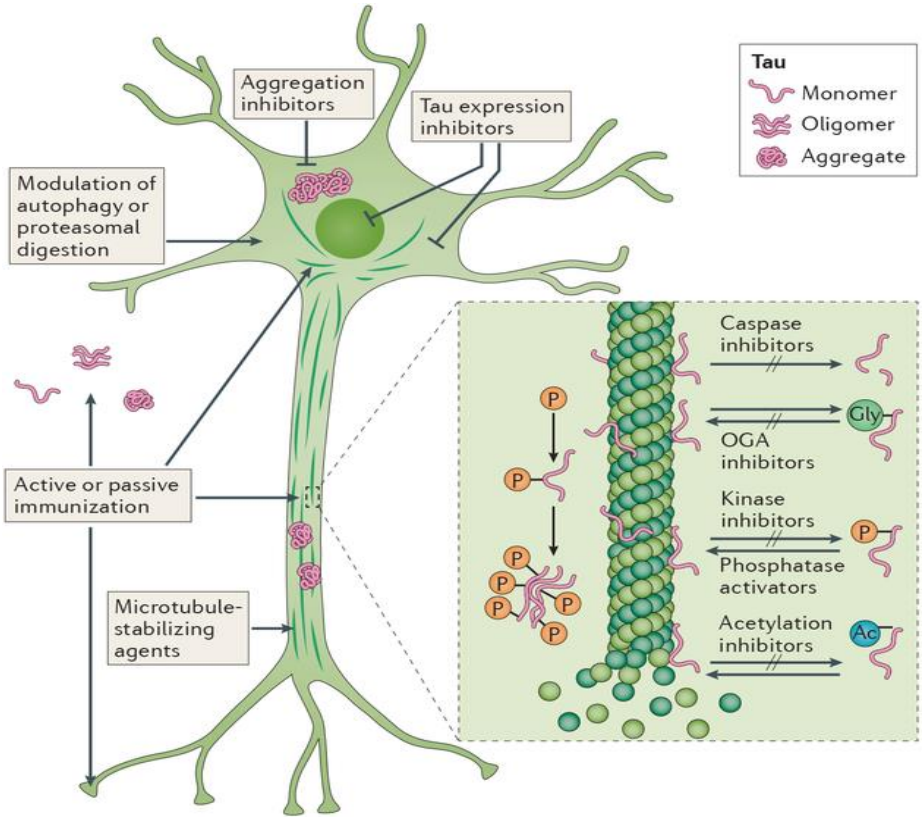

Figure 4: Tau-related therapeutic targets (Congdon & Sigurdsson, 2018)

In the case of the first active immunization, the clinical trial of the AN-1792 vaccine showed some positive effect. Some of the data has indicated that Aβ immunotherapy is remarkable but it shows moderate effects in case of the tau pathology. In the case of the phase II clinical trials

CSF phospho-tau levels have been decreased in AD patients with the help of the AD anti-Aβ antibody bapineuzumab (Congdon & Sigurdsson, 2018).

However in the Phase III clinical trials there were no such effects on the tau pathology. Likewise, tau levels were not altered in case of the solanezumab in any clinical trial. It can be also said that for the clearance of Aβ in the time of active and passive immunization the tau level could not be decreased and targeting the tau directly is the upcoming possible way (Congdon & Sigurdsson, 2018).

Active tau immunization is used to target the one or multiple phospho epitopes, aggregated tau also the amino terminus to decrease the tau pathology. There are some good and bad reports about this reduction of the tau pathology, some of the reports have shown some adverse effects and some of the reports have shown promising immune responses. In case of mice, tau vaccination has not been reposted safely rather than showing the toxicity when it has been taken with the T-helper 1-inducing adjuvants, for this it did not get the approval for the human use. When the same types of tau immunogens have been administered with milder adjuvant it does not show that much adverse effects (Congdon & Sigurdsson, 2018).

Though some concern about the safety has been raised in the active immunization so some potential solution has been offered by the passive immunization. Immunization effects which are transient that are used to decrease the risk of adverse reactions. For targeting the epitope, passive immunization has always shown greater specificity. Throughout the course of the disease the profile of the epitope has been changed after the advance diagnostics, treatment might be different for the different stages of disease for different individuals. Moreover, in vivo the antibody fragments have also been used to decrease the tau level (Congdon & Sigurdsson, 2018).

There are some properties in the antibody like the affinity, charge and isotype but there has been a confusion as to which epitopes should be targeted. In various research it has been shown that phospho-serine 396, 404 are getting the most of the attention as it was also an essential part of the immunogen. Some research has also been done to see the efficacy of the antibodies by binding these antibodies with the several parts of the tau proteins. The observed difference is not because of the epitopes but for other properties of the antibodies like the affinity, charge and isotype (Sigurdsson, 2016).

It is possible for the tau antibodies both extracellular and intracellular to interact with their target. However the extracellular clearance is less efficacious and safer than intraneuronal clearance. A findings from the ADPD 2015 conference has been shown that the life perspective of a tangle mice that has been used intracellularly with a tau antibody fragment is improving if compared with a directed secretory pathway fragment. The pathway has been used depending on the epitopes availability and how the epitope is being targeted with the different properties of the antibodies. The actual property is the charge as this property of antibodies is used to help them to enter into the cell. To make the permeability into the cell the isoelectric point of antibodies has a great influence but some acidic antibodies cell penetrance is very low. With the help of several techniques like the culture and in vivo the tau antibodies can easily enter the neuron cell. Moreover these types of antibodies are near the neutral range. In the tau therapies only the acidic antibodies cannot enter into the cell/ neurons (Sigurdsson, 2016).

In vitro assays phenylthiazol-hydrazides, anthraquinones, polyphenols, benzothiazoles and several other classes of agent help to protect the tau aggregations. There are more compounds that are not used as they do not have the evidence of efficacy. However, methylene blue, a non-neuroleptic phenothiazine, has been used for the treatment purpose of the malaria patient for a long time and it has also shown the progress in clinical trial phase I and II in the AD patients (Panza et al., 2012).

In the tau-based anti-AD drugs some of the microtubule-stabilizing agents have shown that if the tau has been detached from the microtubules then the microtubule stabilizing loss its normal function which may be the cause of the synaptic dysfunction. Paclitaxel or epothilone, this is the antimitotic compound and this compound is being used for the microtubule-stabilizing activity in the tau transgenic animals (Panza et al., 2012).

5.1.1 Application of Tau Immunotherapy

When immunotherapies for tau has been tested clinically it has been seen in case of AD patients it is helpful to slow down the pathology of the brain. Some targeting tau are still on the investigation that has included both of the peptide and the epitopes. There are lot of research that has shown some promising result with the CSF level of tau. The new biologics has been recommended that tau targeting aggregates is useful to improve the brain pathology and some of the clinical symptoms that is related with the AD (Hoskin, Sabbagh et al. 2019).

5.1.2 Clinical trial of Tau Immunotherapy

Active Immunotherapies	**Mode of action**	**Participant characteristics**	**Status**
AADvac-1	It targets the aa 294–305 Possible targets aa 268–283, 330–335, and 362–367.	Mild to moderate AD	Trial I is completed.
ACI-35	It has 16 copies of tau peptides phosphorylated on S396	Mild to moderate AD	Ib trial is still unknown. (Hoskin et al., 2019)

Table 1: Clinical trials of tau immunotherapies in recent time for active immunization

Passive Immunotherapies	Mode of action	Participant characteristics	Status
BIIB092	IgG4 is targeting extracellular, N-terminally fragmented forms of tau	Mild AD	Phase II is still underway.
UCB0107	It binds aa 235–246. Inhibits seeding	Healthy participants	Phase I is completed.
LY3303560	It binds aa 7–9 and 313–322. It neutralizes soluble tau aggregates.	Mild to moderate AD.	Phase II trial.
BIIB076	IgG1 targeting monomeric and fibrillar forms of tau	Healthy participants	Phase I trial
RG7345	Humanized mAB that has targeted the tau phosphoepitope at S422	Healthy men	It has been discontinued in the phase I clinical trial. (Hoskin et al., 2019)

Table 2: Clinical trials of tau immunotherapies in recent time for passive immunization

5.2 Combination Therapy in Alzheimer Disease

Combination trials are not same as the add-on trials, in case of the combination therapy trials, there are two drugs that have been tested alone and also compared with the placebo and this therapy uses 2×2 trial design (Cummings, Tong, & Ballard, 2019).

In case of the add-on therapy, there is a new agent that has been compared with placebo in a patient who has got the treatment with another therapy. Combination treatments are also known as the pharmacodynamic or pharmacokinetic. Pharmacokinetic combinations show the effects of the ADME like the drugs absorption, distribution, metabolism, or elimination

whereas Pharmacodynamic combinations show various types of effects on disease biology. Examples of pharmacokinetic combinations are basically used for Parkinson's disease (Cummings, Tong, & Ballard, 2019).

Pharmacodynamic combinations are used for the treatment purpose of AD which includes some of the symptomatic agents and those agents have been shown the behavioral and cognitive symptoms of AD. However it has not changed the disease-modifying therapies (DMTs) that have the capability to change the disease course with the help of the underlying biology and that may cause nerve cell death. Some of the terms like drug target, delivery timing, and delivery method has been addressed by the combination therapies. There are some benefits in this combination therapy those are that it is possible for this therapy to address two or more targets like tau-targeting and amyloid. It is also possible for this therapy to target one in two different ways (Cummings, Tong, & Ballard, 2019)

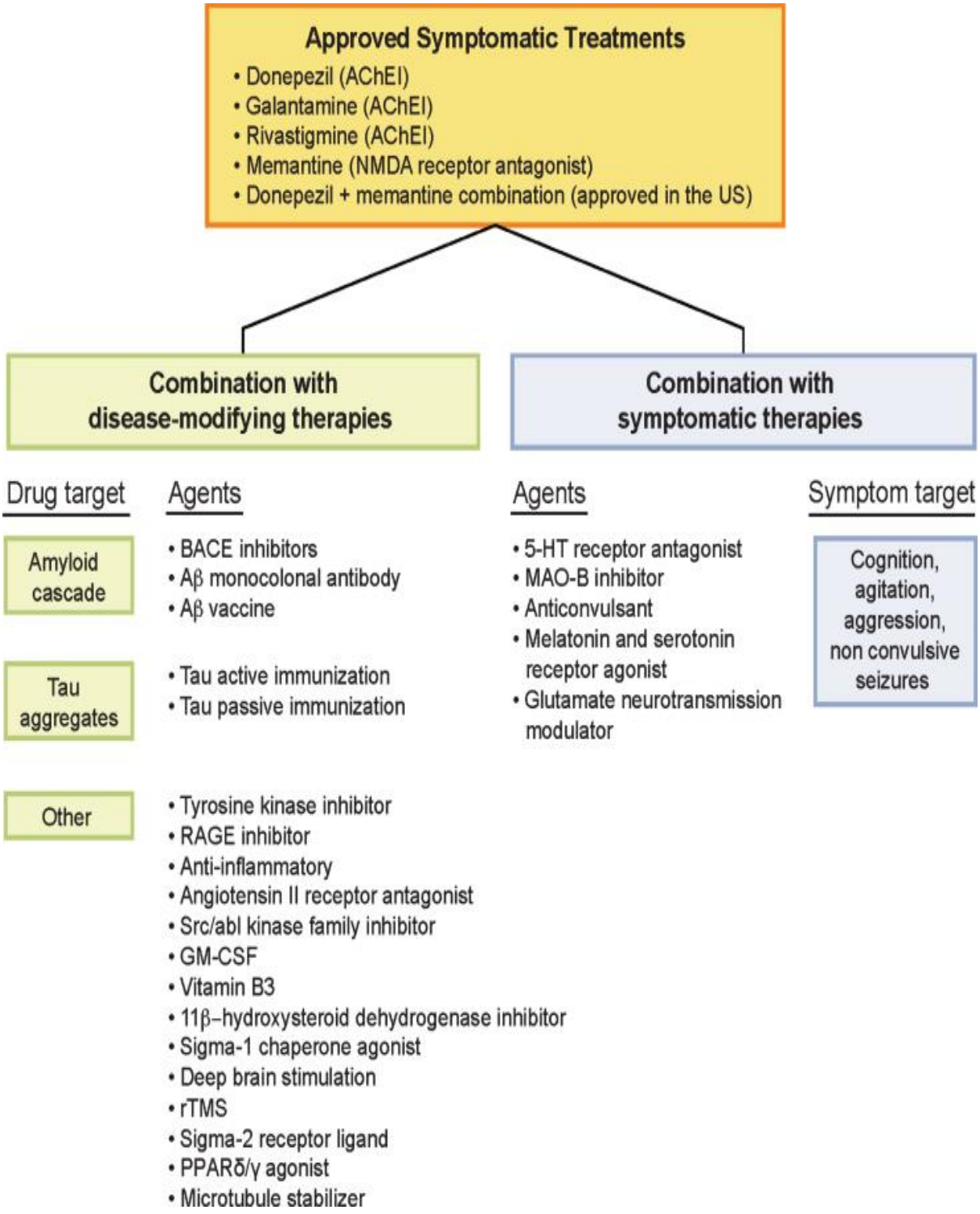

Figure 5: Combination therapies in the Alzheimer Disease drug development pipeline. 5-HT, 5-hydroxytryptamine (serotonin); Aβ, amyloid-β; AChE1, acetylcholinesterase inhibitor; AD, Alzheimer's disease; BACE, aspartyl protease β-site amyloid precursor protein cleaving enzyme; GM-CSF, granulocyte-macrophage colony-stimulating factor; MAO, monoamine oxidase; NMDA, N-methyl-D-aspartate; PPAR, peroxisome-proliferator activated receptor; RAGE, receptor for advanced glycation end-products; rTMS, repetitive transcranial magnetic stimulation. (Cummings, Tong, & Ballard, 2019)

5.2.1 Application of Combination Therapy

Combination therapy is very much advantageous for the drug target, delivery method and the delivery timing. In a development program called AD DMT, the therapeutic agent has the possibility to target the amyloid, tau and some other disease process like inflammation. In the combination therapy it has the capability to address amyloid and tau targeting therapies and it also has the capability to address one target in two methods like two amyloid targeting therapies. Combination therapies is also useful for the oral and intravenous delivery methods (Cummings, Tong, & Ballard, 2019).

5.2.2 Clinical trials of Combination Therapy

Agent	Dose	Type	AD stage	Phase
Gantenerumab	NR		Early	Phase II and III
Crenezumab	NR	Amyloid passive immunization	Early	Phase III
Aducanumab	NR	Amyloid passive immunization	Early	Phase III
Liraglutide	1.8 mg/d	GLP-1 receptor agonist	Mild	Phase II
LY3303560	NR	Tau passive immunization	Early, mild and moderate	
Intepirdine	30 or 60 mg/d	5-HT6 antagonist	Mild, moderate	Phase III

Sertraline	25 to 125 mg/d	Selective serotonin reuptake inhibitor	NR	Phase II and IIII (Cummings, Tong, & Ballard, 2019)

Table 3: Clinical Trials of Combination Treatments for AD

Chapter 6

Risk Factors

It can be said that the amyloid-β (Aβ) deposition and tau proteins are kind of the sign for the AD and that has been aggregated in the brain. However, not only the tau protein and Aβ is not everything in case of AD. There are some other trademarks like the change in the blood brain barrier, it is also known as the marker for the neurodegenerative disease (Ishii & Iadecola, 2020). Cognitive dysfunction degree is associated with the BBB disruption degree that a person can experience, but the actual reason for the breakdown of the BBB breakdown cannot be known yet. In an evidence it has been shown that one of the main genetic risk factors of AD is apolipoprotein E4 and that has been connected with the breakdown of BBB (Ishii & Iadecola, 2020).

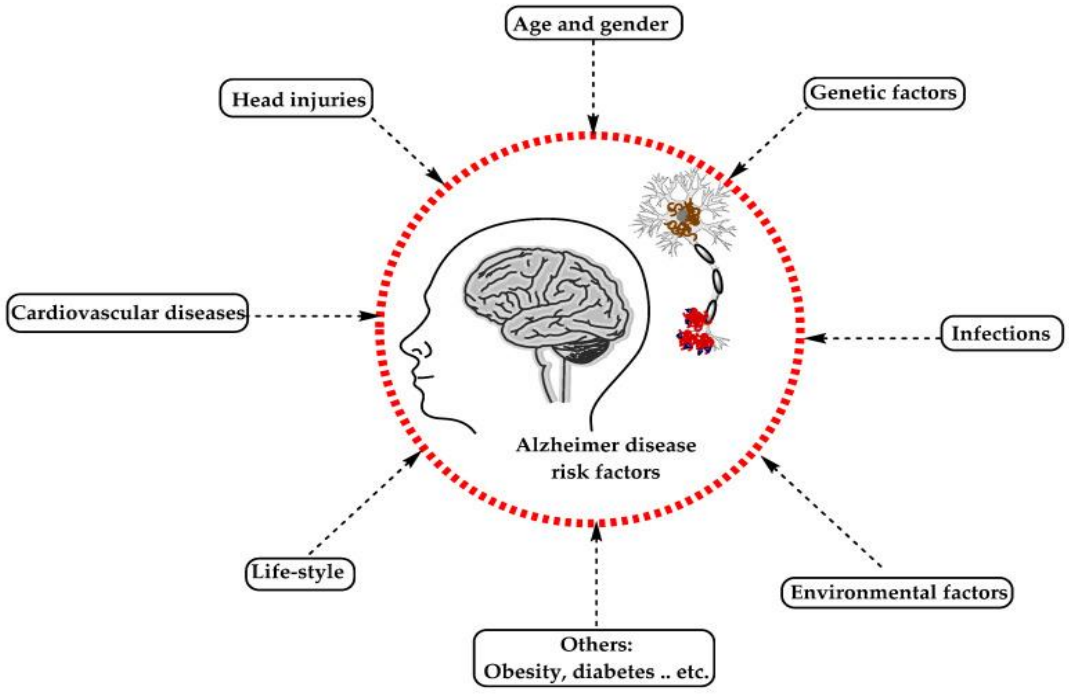

Figure 6: Several risk factors of the Alzheimer's disease (Breijyeh & Karaman, 2020)

6.1 Modifiable Risk Factors

Over the past few years AD has developed a long preclinical stage and those can assess what types of risk factors can be seen in the early or late life in AD patients. After doing several research various modifiable risk factors have been shown in case of AD patients. According to the US National Institutes of Health and based on so much evidence depression, diabetes, smoking, poor diet, physical and mentally instability these are also known as modifiable risk factors. These are the risk factors associated with cognitive decline or AD. According to another study it has been found that hypertension, alcohol consumption, renal dysfunction, depression and high cholesterol level are also known as the modifiable risk factors (Galvin ,2017). After another review this list has been modified and some more risks have been added in this list, those are hypertension and obesity. Recently, an association is working on a project that has shown the variation between the vascular risk factor of the mid-life of AD and amyloid deposition later in life (Crous-Bou, Minguillón, Gramunt, & Molinuevo, 2017).

Not all the risk factors are being authorized by all the research till now there is lots of controversy about these risk factors. Cardiovascular risk factors and lifestyle habits are the most probable risk factors in case of AD. Hypertension, obesity are related with the cardiovascular risk factors and smoking, mental health and diet are related with lifestyle habits. Diabetes has become a major risk for the AD patient as it shows the effects on Aβ accumulation in the brain thus it has increased the risk for the AD patients. To prevent this disease it is essential to remove the modifiable risk factors. Almost a third of the AD patients are being associated with these risk factors. And for the rest of the AD patient's recent therapeutic strategies are urgently needed (Crous-Bou, Minguillón, Gramunt, & Molinuevo, 2017). In one of the study it has recommended that there two influencing factors those are the non-modifiable risk factors, it includes age and genetic risk factors and modifiable risk factors, it includes lifestyle and cardiovascular risk factors (Dubois et al., 2016).

6.2 Genetics Risk Factors

Some genetic disorder like the late-onset AD is much complicated and the estimated heritability is about 60% to 80%.Apolipoprotein E (APOE) genotype is one of the strongest risk factor in case of late-onset of AD and APOE uses to make cholesterol transporter for the brain which has three alleles like ε2 (8.4% estimated allele make the frequency among the population), ε3 (77.9%), and ε4 (13.7%). APOE ε4 has relation with the risk factors of the AD and each of the APOE ε4 allele has the capability of reducing the symptoms of onset of age. Those female has the APOE ε4, they have developed the risk if compared with the male, and particularly the age should be between 65 and 75. Conversely, it can be said the genotypes ε2/ε2 and ε2/ε3 are very much protective. Moreover, there are some mechanisms like the enhanced aggregation and reduced the clearance of amyloid-β (Aβ) polypeptide, that are helping the APOE ε4 to be the risk for the AD. Currently APOE genotyping are not being used for the clinical trials as some risk factors has been seen in case of ε4 allele (Rabinovici, 2019).

6.3 Environmental Risk Factors

Some of the population-based research has been given the confirmation that some environmental factors like the lifestyle choices, comorbidities of the medical can be risk for dementia and cognitive impairment in late-life. Vascular risk factors can be the cause of increasing risk for dementia. In the older age it is possible to disable the risk factors of cognitive impairment by treating those risk factors in the midlife (Rabinovici, 2019).

In case of late-life cognitive decline some types of disturbance in sleep like insomnia is associated. Traumatic brain injury is one kind of curable risk factor for the AD patients and also for the neurodegenerative disease. Sometimes people also suffer from depression in old age and that can be a risk factor for cognitive decline however it’s not confirmed that it is also a risk factor for AD or not. Conversely, to reduce the risk of late-life dementia physical activity

can help a lot, not only that social engagement also plays a vital role in reducing the risk of late-life dementia. Nevertheless, for the healthy brain at aging it is important to do the aggressive treatment for reducing the risk of dementia and healthy lifestyle is also important for all the people (Rabinovici, 2019).

6.4 Unmeasurable Risk Factors

In a study it has been found that there are some types of unmeasurable factors and that has the correlation with various types of mortality risk in any one than another. If a patient had died in incident cancer before he or she had the chance for diagnosing his dementia or AD and it sounds like unmeasured ways from those patients who had incident cancer and had already been diagnosed for the dementia or AD, it could the selective mortality, the association with the incident cancer then dementia or AD. People diagnosed with incident cancers may have had a poor cancer prognosis as evidenced by late stages, tumor grades and the proportion of deaths are worked as evidence for those patients who had already diagnosed for the incident cancer and those patients must have had the low cancer prognosis. Therefore, where there is a chance of the bias because of the information censoring, there must have a biologic rationale to decrease the risk of the AD patients (Bowles et al., 2017). To know the risk factors it is important to compare the association between the age in control group and lipid, with the help of the lipid species and diagnosed the AD clinically, there were 108 lipid species that all has been associated in the identical way along with the p-value that was not correct and the value was less than 0.05 (Lim et al., 2020).

Among these, there were 47 lipid species that were related with AD negatively and with the increasing age those had been decreased. On the other hand another 61 lipid species were related AD positively and with the increasing age it had also been increased. Four lipid species are left, those four lipid species have been related opposingly within the age in control group

and lipid species along with the lipid species and diagnosed the AD clinically. In general, it can be said that with raising age and AD, both of them were associated with the reducing of the ether lipid that contains the polyunsaturated fatty acids, greater level of some kinds of sphingolipid species, developing the odd and branched fatty acids, reducing the ubiquinone and increasing the species of the phosphatidylethanolamine and triacylglycerol (Lim et al., 2020).

Chapter 7

Prevention Strategies of Alzheimer's Disease

Positron emission tomography (PET) of the tracer molecules and cerebrospinal fluid (CSF) protein analysis are now very much useful for diagnosing the AD patient. Phosphorylated tau 181 is beneficial and it is used as a prognostic biomarker for diagnosing the AD patients. To be used as an AD biomarker it has been deposited in the brain and has been secreted in the CSF that also meets the blood brain barrier to get into the blood. Recently, with the help of the PET scan the accuracy in diagnosis has been developed which has been shown the specificity 100% and the sensitivity 96% in the AD patients who are in the milder condition of AD. As a PET ligand Florbetapir, florbetaben are being used for the diagnosis purpose but it is much expensive so it cannot be used widely. Aβ42 and total tau protein is not that expensive in case of the examination of CSF for p-tau. For diagnosing the AD patient this method is beneficial and has got 85-90% accuracy but it takes time to get the result because of the invasive method. However, PET imaging and CSF analysis has been shown the same accuracy and it is also mentioned that there are some tests that have been dependent on the patients, facilities and cost. As a result, for treating the AD patients it is very much helpful to use the biomarkers as it helps to detect AD in the early stage (Khan et al., 2020).

To treat an AD patient it is important to add a care plan with the treatment process like the counselling about patients safety. In 1993, acetylcholinesterase inhibitors (AChEIs) has got the approval as a pharmacologic therapy for treating the AD patients.as it is known that the injury in the cholinergic neurons in the basal forebrain is related with the AD and AChEIs is used to increase the cholinergic transmission by restricting the synaptic cleft hydrolysis of acetylcholine. Donepezil, galantamine, and rivastigmine are three AChEIs, currently used for clinical trials. For treating mild to severe AD dementia, double-blinded randomized controlled

trials have shown some benefits however in the MCI it was not helpful. There are different types of biological effects in different types of AChEIs but for all the agents the efficacy is the same. Although there are some attenuated differences that can be seen in the biological effects of the various AChEIs, the efficacy is similar across agents. The percent of improvement is very much rare so it cannot be appreciated. There are some adverse effects like the loss of appetite, muscle cramps, upset stomach and urinary frequency. However, a study has also shown that 23 mg/d of donepezil can be taken and it will be helpful for treating moderate to severe AD patients, in case of that dose dependent side effects can be seen (Rabinovici, 2019).

7.1 Primary Prevention Strategies

Intervention strategies actually concentrated on the modified risk factors and epidemiological studies have been said that because of controlling the cardiovascular risk factors, the age related dementia has been reduced. As an example, there are so many studies that are ongoing, they are trying to find out the primary prevention of dementia, especially in Europe, just to decrease the incidence. Also, examiners aimed to evaluate the impact of this multidomain intervention on inability, quality of life, depressive indications, the use of health care services and vascular risk factors. Large, long-term and randomized control trial (RTC) has been shown that multidomain intervention has included exercise, diet and checking the vascular risk can be helpful for cognitive functioning for the people whose age is around 60-77. Vascular Dementia can be prevented by the Intensive Care (PreDIVA) trial that's aim is to know whether the cardiovascular risk factors controls are capable of reducing the dementia incidence. PreDIVA has also been tested for the multicomponent intervention that whether it actually can target the vascular risk factors to reduce the new cases of dementia (Crous-Bou, Minguillón, Gramunt, & Molinuevo, 2017).

7.2 Secondary Prevention Strategies

New diagnostic ways for the preclinical AD, alongside the identification of at-risk people through the utilization of biomarkers that are changed some time recently clinical decline, are the key for distinguishing at-risk asymptomatic people who are perfect candidates to take the participation in the secondary prevention trials. One of the essential paths for the AD development could be cerebral Aβ deposition however it is not sufficient. Besides, most of the results from the trials are mostly Aβ-centric approaches, at the dementia phase of AD have been disappointing, recommending that those members have as of now surpassed the ideal therapeutic window for intervention. For getting the therapeutic success the preclinical phase can have the optimal window time and also the opportunity to mediate at the very primary stage of the continuum. Several PPPs for disease prevention are currently ongoing, to set a framework to select someone for the clinical trial of AD are basically concentrated on the preclinical stage of AD. It has an aim that it wants to develop the efficiency to get the clinical signal and improve the sensitive results for discovering the early decline with the new trial designs. The Alzheimer's Prevention Initiative (API) is one kind of an international collaborative initiative that has been made to get some new approach from Alzheimer's research to evaluate the predominant therapies in AD. The API has set up a strong registry where the members use to get all kinds of information including the new trials on a regular basis (Crous-Bou, Minguillón, Gramunt, & Molinuevo, 2017).

7.3 Disease related with Cognitive Decline

Cognitive decline is used to interact with the independent function then patients have the possibility for dementia. Neurocognitive disorder are in the equivalent category and these types of different categories show continuum of the cognitive decline. There are some neurodegenerative disorders, those can also be seen in the late-life cognitive decline, and those are:

- Vascular cognitive impairment
- Dementia
- Primary age-related tauopathy
- Frontotemporal lobar degeneration
- Alzheimer disease
- Argyrophilic grain disease
- Primary age-related tauopathy (Rabinovici, 2019)

Chapter 8

Recent Treatment Strategies

8.1 Gene Therapy in Alzheimer Disease

Gene therapy's aim is basically to repair the faulty gene, DNA or the protein and permit the cell to make the solution for the problem. As various genes are being used in Alzheimer's pathology, it has become a great opportunity for gene therapy where the new genetic materials have been inserted into the living cell with the help of the viruses. It can be seen that gene therapy is developed day by day and the approach of being related with recombinant adeno-associated viruses (rAAVs) is increasing to treat this disease. There is a nervous system growth factor (NGF) that is used to monitor the capability of degenerate neurons in AD patients. Research has been done with ten early AD patients with the help of the NGF gene *ex vivo* or *in vivo* therapy. The results have shown the optimistic response and the neurons have shown the cell hypertrophy and help to activate the functional maker (Khan et al., 2020).

8.2 FDA Approved Medicine

The drugs that have the capability to target the cholinergic or glutamatergic neurotransmission are used for the treatment purpose of AD. These types of drugs can give relief to the patient but there are no such drugs that can actually give the curative effects. For those drugs, several phases of clinical trials are ongoing. Some of the new molecules have been developed that have the capability to target the amyloid and tau proteins. These types of drugs are really useful, they can develop the cognition of a patient and are also helpful to ease the economic and social burden. These types of drugs have shown their effectiveness in treating mild to moderate AD patients (Khan et al., 2020).

For treating the severe AD patient, Memantine got the approval from the FDA in 2003. The NMDA receptor antagonist that helps to decrease the excitotoxicity that has been seen in AD patients, it has been caused because of the excess amount of glutamatergic transmission. There are some more drugs that have been developed to treat the AD patients, because of failing the clinical trial it could get the chance to get in the market (Khan et al., 2020).

Figure 7: Recent treatment strategies and newer strategies in early detection and treatment of Alzheimer's disease (Khan et al., 2020)

Cholinesterase inhibitors	**FDA approval**	**Uses**
Rivastigmine capsules	2000	Mild to moderate AD
Donepezil tablets (or oral solution)	1996	Mild to moderate AD
Rivastigmine transdermal system	2000	Mild to moderate AD
Galantamine extended-release capsules, tablets, or oral solution	2001	Mild to moderate AD. (Hampel et al., 2018).

Table 4: Cholinesterase inhibitors that has been approved by the FDA for Alzheimer's disease are commonly prescribed for the AD patients.

Chapter 9

Clinical Trials

Immunotherapy is one kind of essential research direction which target is to remove the Aβ in AD the patients however, the finding and results has got from the clinical trials is not that much satisfactory (Sun et al., 2018).

Drugs name	Mode of action	Clinical trial	Relieved symptoms
Brexpiprazole	D2 partial agonist	Phase III	Agitation
Pimavanserin	selective 5-HT2A serotonin inverse agonist	Phase II and III	psychotic
Suvorexant	dual antagonists of the orexin receptors	Phase III	Insomnia
Lemborexant	dual antagonists of the orexin receptors	Phase II	Insomnia
Tetrahydrocannabinol	CB1/CB2 partial agonist	Phase II	Agitation
Nabilone	semisynthetic cannabinoid derivative	Small scale of phase III	Agitation (Khan et al., 2020).

Table 5: Some important drugs currently in clinical trials

Drug name	Therapy type	Target	Trial status	Reasons for the discontinuation
Idalopirdine	Small molecule	Serotoninergic (5-HT6 receptor antagonist)	Phase III	There was no clinical efficacy
Encenicline	Small molecules	Acetylcholine response	Phase III	gastrointestinal side effect
Riluzole	Small molecules	Glutaminergic	Phase II	It is not applicable
BI 1181181	Small molecules	BACE inhibitor	Phase I	Low oral bioavailability and low blood-brain barrier penetration
Semagacestat	Small molecules	γ-Secretase inhibitor	Phase III	No clinical efficacy
Epothilone D	Small molecules	Tau stabilization	Phase I	Not applicable
Alzhemed™	Small molecules	Microglial activation inhibitor	Phase III	There is no clinical efficacy
AN-1792	Active immunotherapy	Aβ clearance	Phase III	Meningoencephalitis was seen (Hung & Fu, 2017)

Table 6: Some of the anti-Alzheimer's disease drugs result in the clinical trials in 2017.

Chapter 10

Conclusion

10.1 Conclusion

To conclude it can be said that through the neurovascular and humoral pathways brain and heart are connected. It is also important to give more attention to know the connection between the cardiovascular disease and the pathology of the brain that usually leads to cognitive dysfunction (Cortes-Canteli & Iadecola, 2020) .There are some new therapeutic approaches for AD like the β-amyloid vaccine and this is currently under the development .There is much more to know about AD and researchers are trying their best to find out something new about this disease. Another important thing that has been done by researchers to block the β-amyloid is developing, it does not mean there are no setbacks. For example, β-amyloid is also beneficial for the several other necessary activities like the cleavage of Notch. There are some approaches, especially the antibody approach that has been used to block secretase cleavage to make β-amyloid. Despite this there is some confusion that β-amyloid peptides' normal biological function is still unknown. But several studies in animal models have shown that β-amyloid is useful for the treatment of AD (Gouras, 2009). The successful therapeutic targets helped to make the ultimate formulation of AD. Previously the amyloid cascade hypothesis was one of the dominating hypotheses but researchers are trying their best to look for the new targets. In a study it has found that biological transition metals can easily interact with the proteinopathy and also with the various types of gene products in AD, it also tries to generate them as therapeutic targets. Most importantly, it can be said that brain homeostasis for these types of metals have changed with aging and it is also said that for the AD is a risk for the older patients (Lei, Ayton, & Bush, 2021). Though there is lots of evidence and precision medicine approaches, treatment and therapeutics, the proper way of preventing AD is not invisible. In

particular, there is more chance to develop the studies, to know more about the future risk and the targeted treatment for this type of disease especially for the AD patients. The studies have also given hope about the pathophysiology of the disease that may bring a fresh or new target for the drug development process. As the number of AD patients has been increasing day by day, it is actually necessary to improve some new ways vigorously to reduce the risk of AD (Niculescu et al., 2020).

10.2 Future prediction of Alzheimer Disease

Various active and passive immunization of therapeutic approaches are on the way of its development. The approaches are trying to target the Aβ peptides that might have enough efficacy when it has been begun early for the disease onset before the clinical symptoms. Tau pathology has also given some hope, but toxicity is another case and it is still not clear that the approach can be useful within the preexisting disorder (Wisniewski & Goñi, 2014). In many findings it has been suggested that several pathological pathways are involved with the AD but new research has raised questions that for the AD therapy, Aβ is the actual target or not. In the AN1792 vaccination trials which was pre aggregated with Aβ1–42 the result was almost satisfactory that it has the capability to remove the SPs from AD patient's brain but by removing plaques it is not possible to cure the progressive cognitive. Researchers has found lots of scenario behind it, like why the Aβ immunotherapy is not effective for treating the AD patients. NFTs and their precursors are the target of the AD that is composed by the hyperphosphorylated tau proteins (Panza et al., 2012). Preclinical and clinical studies has been suggested that immunotherapy that works against the tau is one kind of leading approach for the treatment of AD patients as it is known that the tau and Aβ use to regulate each other in case of AD. To decrease tau, immunization against Aβ will play an essential role if it has been administered to the patient at a very early stage (Panza et al., 2016). In addition, some of the studies are saying that to get the new approach for the AD tau immunotherapy research should

be more valid and precise and need to give the details information about the therapy (Panza et al., 2012). Bioscience is also trying to find the treatment of the AD, in some of the studies it has been shown that Artificial intelligence (AI) has the ability to combine the data that has been accumulated and to make some of the valuable assumptions for the therapeutic applications. As it is not possible for a single gene to conclude for a disease as recently the failure in the BACE1 inhibitor for AD has been noticed and it has also been shown the various roles of the multiple gene in the pertinent biological pathways. AI has the capacity to handle an enormous amount of whole-genome information to recognize the most significant pathways, and it also has the capability to discover the best target for the therapy. These targets seem to possibly reply to more crucial questions in future. One of the advantages of AI is the algorithm and it has the capability to be used in the various desperate and associated sets of data (Khan et al., 2020). It will be fundamental for worldwide advocacy activities to create public and private support and funding for this domain of health services results research, with the goal of conducting a broad-scale, multisite study powered to absolutely assess clinical efficacy of the diverse sorts of comparative effectiveness mediation standards. Eventually, the creation of a strong and well-characterized information set (both genotypically and phenotypically) will be able to predict the analytics also including the learning that is traditional statistical and artificial neural systems, to assist the clinical work. Moreover, there is a conflict in the application of the clinical care that there is a possibility for the predictive analytics to fall in the continuum that is fully human-guided or in the patient care that is entirely machine-guided (Isaacson et al., 2018) . In another research it has found that diseases related to cardiovascular and stroke prevention, soon will be able to know whether the risk assessment and the early intervention with the help of the clinical precision medicine approach can decrease the risk or AD or not (Huang, Chao, & Hu, 2020).

10.3 Challenges for Future AD Immunotherapies

In the last few year AD immunotherapies field has been developed in a very great extent however to aware about the clinical safety and efficacy some of the issues are still need to be clear. Firstly the antibodies if it is active or passive immunization that needs to get in the brain very effectively. It can be seen that a very little portion of the antibodies has the capability to pass the blood brain barrier approximately 0.1%. So it is important to find some new method that will be helpful to penetrate the antibodies into the brain. With the help of chaperone proteins it may be possible (Lemere, 2013).

Secondly, reimbursement mechanism is not that well defined so the fiscal challenges may be posed. However in United States the APC providers used to help the severe medical insurance plan and they also use the billing codes. Not only that health care provider, health care system, opportunity cost and clinic these are also expensive as well. For diagnosing AD it may require $7 trillion in the US (Isaacson et al., 2018).

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