Current & Emerging Drug Therapies for Alzheimer's Disease; Immunotherarpy about monoclonal antibodies

By

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Declaration

It is hereby declared that

1. The thesis submitted is my/our original work while completing my 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 that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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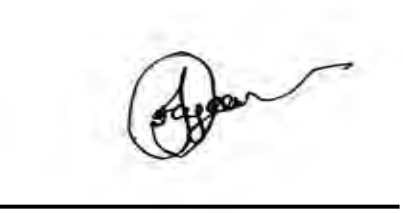

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Approval

The thesis/project titled 'Current & Emerging Drug Therapies for Alzheimer's Disease; Immunotherarpy about monoclonal antibodies' submitted by Raditu Haque Troyee (17346024) of Summer, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The study does not involve any kind of animal trial and human trial.

Abstract

60–70% of dementia cases are caused by Alzheimer's disease (AD). The urgent need to develop effective treatments for Alzheimer's disease has arisen due to the disease's seriousness and steady rise in the number of patients. Currently, the medications used to treat AD, such as cholinesterase inhibitors and an antagonist of the N-methyl-D-aspartate receptor, can only temporarily halt or reverse the progression of the disease. Numerous clinical trials on amyloid clearing therapy have been attempted by numerous global pharmaceutical companies might not be entirely accurate. In 2019, there was a decrease in the number of anti-amyloid trials, which could signal a turning point. For the development of novel pharmacotherapies, an in-depth and comprehensive understanding of the role that amyloid ß and other AD factors play are necessary. The treatment that is currently approved consists of cholinesterase inhibitors and NMDA-receptor antagonists, and their combination therapy only provides brief relief from symptoms. Researchers around the world have made sincere efforts to find new targets, discover novel therapeutic agents, and develop them for the treatment of AD. In addition to a variety of other targets, the upcoming treatments will target one or more targets, such as amyloid ß, neuroinflammation, and amyloids. The purpose of this brief review article is to discuss the most recent developments in drug development as well as upcoming therapeutic agents for AD that target various targets.

Keywords: Alzheimer's disease, N-methyl-D-aspartate receptor, Amyloid ß plaques, Cholinesterase inhibitors, Tau proteins.

Dedication

Dedicated to my parents especially to my ideal father, supportive husband and my supervisor, Kazi Fatema Rahman.

Acknowledgement

Alhamdulillah, all praises to Almighty Allah who bless me with patience, strength, courage, and self-confidence which helped me most to get this project done properly.

First and foremost, I do my most esteemed supervisor (Kazi Fatema Rahman, Lecturer, School of Pharmacy, BRAC University), who has directed me from the idea of the topic to the last line of my project. I would like to express my deepest gratitude and adoration. Working under her supervision enabled me to understand and expand my knowledge. That project would not come to light without her valuable input and monitoring. Her precious feedback, rapid communication, and hard-working for my thesis helped me a lot to finish the project accurately and quickly. I am highly grateful to have had the opportunity to work under her supervision and wish to work with her in the future.

I would also like to express my gratitude and honor to Dr. Eva Rahman Kabir, Professor and Dean, School of Pharmacy, Brac University who is always an ideal to me because of her hard work and passion for her students. She always works very hard to ensure a supportive and creative working environment for each and every student in this department.

Moreover, I would like to show a mark of reverence towards my mother, Mosammat Rupali Parvin Monika; a housewife who taught me how to come up like a shining star in any tough situation. Surely, I would not come this far without her prayers and unconditional love.

Last but not least, I want to thank my husband; Sharat Hossain Shihab, and also my friend; Ashraful Islam for their valuable idea, skill, knowledge, and unconditional support. They inspire me a lot to work hard in life.

Immunotherapy: Current & Emerging Drug Therapies for Alzheimer's Disease.

List of Figures:

List of Acronyms

NFTs = Neurofibrillary Tangles

PET = Positron Emission Tomography

MRI = Magnetic Resonance Imaging (MRI)

CSF = Cerebrospinal Fluid

CNS = Central Nervous System

Aß = Amyloid ß

PHF1 = PHD finger protein 1

PHD = Plant Homeodomain

AD = Alzheimer's Disease

CAA = Cerebral amyloid angiopathy

DM = Double mutant

FAD = Familial Alzheimer's disease

hAPP = Human amyloid precursor protein

JNPL3 = Mutant human tau protein

NFT = Neurofibrillary tangles

PDAPP = PDGF-driven human amyloid precursor protein

PS1= Presenilin 1

APP = Amyloid precursor protein

RCT = Randomized controlled trial

MCI = Mild cognitive impairment

AChE = Acetylcholinesterase

BuChE = Butyrylcholinesterase

Ach = Acetylcholine

BChE = Butyrylcholinesterase

BACE1 = Beta-secretase 1

NfL = Neurofilament Light

APOE4 = Apolipoprotein E4

TOMM40 = Translocase of Outer Mitochondrial Membrane 40

AMD = Age-related Macular Degeneration

VEGF = Vascular Endothelial Growth Factor

ROS = Reactive Oxygen Species

MAP = Mean Arterial Pressure

NMDA-receptor = N-methyl-D-aspartate receptor

MD = Molecular Dynamics

QM = Quantum Mechanics

ONOO- = Peroxynitrite

FTLD = Frontotemporal Lobar Degeneration

DLB = Dementia with Lewy Bodies

LCA = Lithocholic Acid

DCA = Deoxycholic Acid

CDCA = Chenodeoxycholic Acid

Chapter 1

Introduction

1.1 General Information on Alzheimer's Disease

The main reason behind dementia is actually called Alzheimer's disease currently becoming the most prodigal, fatal, and burdening disease of this current century (Scheltens et al., 2021). In this article, we actually focus on its pathogenesis, diagnostics, therapeutics, immunization strategies, currently targeted immunotherapy, and emerging effective therapies in the future (Scheltens et al., 2021). Here, significant developments have taken a journey in understanding the underlying pathology, determining different causative and protective genes, and identifying totally new blood-based and monitoring biomarkers (Scheltens et al., 2021).

1.2 Aims and Objectives

Alzheimer's disease (AD) is a complex, fast growing disorder with a significant impact on global health. Traditional drug therapies for AD have shown limited efficacy, prompting the exploration of innovative treatment options. Immunotherapy, particularly the use of monoclonal antibodies, has emerged as a promising avenue for addressing the underlying pathology of AD. This review aims to provide a comprehensive overview of monoclonal antibodies as a novel treatment option, comparing them with conventional therapies.

- The introduction establishes the context of Alzheimer's disease and the shortcomings of traditional drug therapies. It emphasizes the need for alternative approaches, leading to the exploration of immunotherapy and specifically monoclonal antibodies.
- This section elaborates on the concept of immunotherapy, with a focus on monoclonal antibodies as a groundbreaking treatment option. The rationale behind targeting specific proteins, such as Aβ plaques, is discussed, highlighting the potential advantages over conventional drug therapies.
- The review delves into the origin and biological reactions of Aβ plaques, providing a detailed understanding of their role in Alzheimer's disease pathogenesis. This section sets the stage for explaining how monoclonal antibodies target and interact with these plaques.
- A thorough examination of the varieties of monoclonal antibodies used in AD treatment is provided, along with insights into their mechanisms of action. This includes discussions on how these antibodies engage with Aβ plaques and the potential impact on disease progression.
- The review critically assesses the limitations and challenges associated with monoclonal antibody therapy for AD. Factors such as potential side effects, cost, and variability in patient responses are explored, offering a balanced perspective on the current state of this treatment modality.
- This section highlights recent advancements in AD immunotherapy, focusing on improvements in monoclonal antibody development and administration. Additionally, it outlines ongoing research directions that hold promise for enhancing the efficacy and safety of these treatments.

This comprehensive review aims to contribute to not only existing knowledge but also progressive based on AD treatment, providing valuable insights for researchers, clinicians, and policymakers involved in the ongoing battle against Alzheimer's disease. On that account, this

review concludes by summarizing key findings and emphasizing the potential of monoclonal antibodies as a transformative approach in Alzheimer's disease treatment. It underscores the importance of continued research and development to overcome current limitations and pave the way for increased utilization of immunotherapies in clinical settings.

1.3 Methodology

This scholarly article has been meticulously crafted by drawing upon the latest published research from reputable sources such as PubMed, Google Scholar, Research Gate, Frontiers in Pharmacology, Nature Journal, MDPI, Science Direct, DermNet NZ, Tandfonline, Biologics, and Elsevier. Comprehensive data gathering was conducted, with a focus on the last five years, supplemented by information obtained from the preceding 10-15 years to enhance the depth of the review. The review explores the treatment of Alzheimer's disease with monoclonal antibodies and incorporates terms, definitions, and abbreviations sourced from Google. Access to the full text of selected journals was facilitated through the utilization of the BRACU Athens account and Sci-hub. Some more keywords used to search for relevant papers including Aβ plaques, active and passive immunization, monoclonal antibodies, acetylcholinesterase, astrocytes, aducanumab, bile acid, Cerebrospinal fluid biomarkers, tau protein.

1.4 Cognitive Symptoms

Three cases demonstrate the range of clinical manifestations in Alzheimer's disease (Scheltens et al., 2021). The first case emphasizes Alzheimer's disease with a genetic basis, as indicated by the ongoing global efforts of the Dominantly Inherited Alzheimer Network and Alzheimer Prevention Initiative, which include associated clinical trials (Scheltens et al., 2021). The

second case presents a language-related variant of Alzheimer's disease, typically occurring at a younger age (below 70 years), illustrating the challenge of identifying Alzheimer's disease in individuals whose primary and most prominent symptom is not memory impairment (Scheltens et al., 2021). The final case represents a typical form of memory-related Alzheimer's variant, more frequently observed in individuals over 70 years old, highlighting the increasing population affected by Alzheimer's disease and dementia (Scheltens et al., 2021). This includes older individuals who often live alone and become progressively reliant on others for care (Scheltens et al., 2021).

1.5 Pathogenesis

The realm of research devoted to comprehending the pathogenesis of the disease named Alzheimer (AD) and developing efficacious therapeutic approaches is vast (Tiwari et al., 2019). This disease is highly intricate and progressive in a manner which is neurodegenerative disorder that ranks among the leading causes of dementia cases worldwide (Tiwari et al., 2019). An estimated 5.3 million Americans suffer with Alzheimer's disease (AD), of which 5.1 million are 65 years of age or older and 200,000 have the disease at an earlier age (Tiwari et al., 2019). The presence of internal aggregates known as NFTs and extracellular aggregates known as Aβ plaques are among the histopathological characteristics of AD that have been documented (Tiwari et al., 2019). The hyperphosphorylated microtubule-associated protein τ is the main component of these tangles (Tiwari et al., 2019). Aβ plaques first develop in the cerebral neocortex's basal, temporal, and orbitofrontal regions (Tiwari et al., 2019). Later on, they also impact the neocortex, hippocampal, diencephalon, amygdala, and basal ganglia. In extreme situations, Aβ is found in the cerebellar cortex, lower brainstem, and midbrain. τ-tangles are primarily seen in the brain's entorhinal area, locus coeruleus, and entorhinal area when aβ

concentration is present (Tiwari et al., 2019). Eventually, these tangles extend their impact to the hippocampus and neocortex (Tiwari et al., 2019). The main factors that accelerate the development of Alzheimer's declaration are NFTs and Aβ pillars (Tiwari et al., 2019). The purpose of this review is to examine the causes, pathophysiology, and variables that contribute to Alzheimer's complaint (Tiwari et al., 2019).

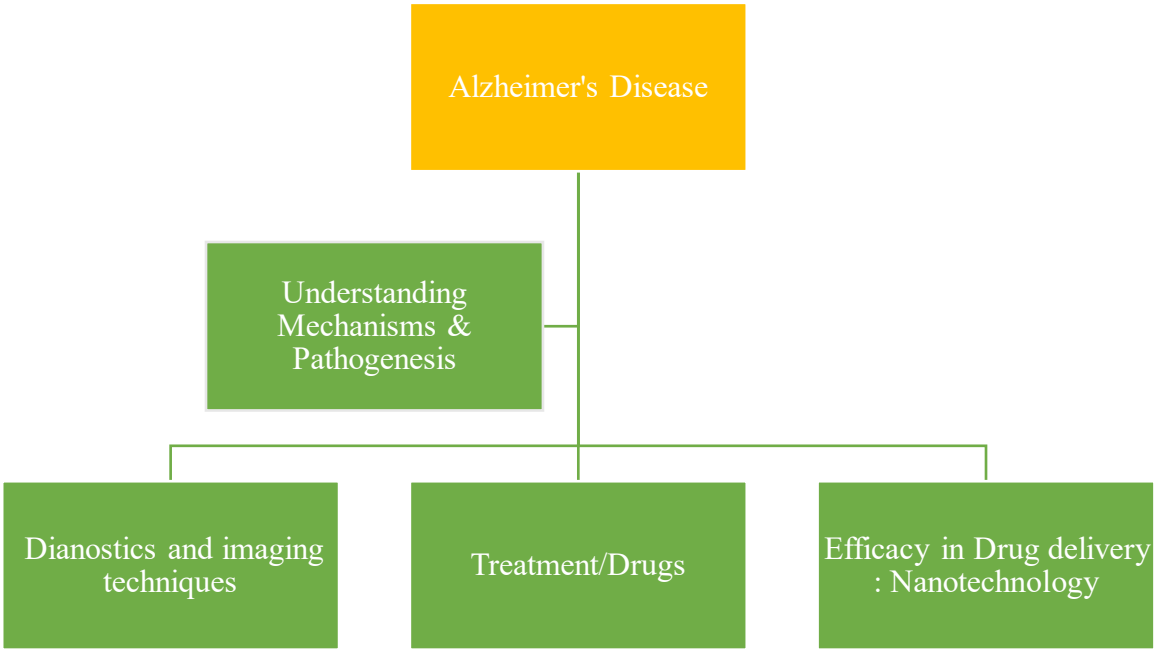

Figure 1: The pathophysiology of AD and devising therapeutic trickeries to combat it.

1.6 Diagnostics

The immediate diagnosis of AD primarily relies on neuropsychological testing (Tiwari et al., 2019). Neuroimaging and monitoring of detected biomarkers, similar as the quantum of Aβ peptides($A\beta_{1-42}$:$A\beta_{1-40}$ rate) and total and hyperphosphorylated τ proteins(Thr181 and Thr231) in cerebrospinal fluid(CSF), will be used in identification to the analysis of uses this common degenerative brain complaint (Tiwari et al., 2019). Amyloid oligomers and shrine accumulation can be observed with imaging ways similar as

positron emigration tomography(PET) using ^{18}F- florbetapir(or alternately Pittsburgh compound B ^{11}C) (Tiwari et al., 2019). Still, enterprises remain regarding the nonlinear correlation between CSF Aβ situations and PET findings (Tiwari et al., 2019). Although CSF collection is invasive and may be difficult in aged grown-ups, it's an important individual tool (Tiwari et al., 2019). In clinical practice, non-invasive imaging ways that give information about brain metabolism, similar to fludeoxyglucose PET, are veritably useful (Tiwari et al., 2019). Different stages of Alzheimer's complaint have been associated with impairment of brain metabolism, characterized by both hypermetabolism and hypometabolism (Tiwari et al., 2019). Another non-invasive system for detecting functional abnormalities is glamorous resonance imaging (MRI), which offers better field strength and resolution (Tiwari et al., 2019). To ameliorate discovery effectiveness in glamorous resonance imaging (MRI), iron oxide nanoparticles (NPs) can be used as discrepancy agents or labeled with γ fluorescent examinations (Tiwari et al., 2019). This is particularly useful for relating and detecting amyloid plaques (Tiwari et al., 2019). Imaging is eased by iron oxide nanoparticles that bind to the N-boundary of Aβ (Tiwari et al., 2019). There are reports of other nanoparticles, similar as B (Tiwari et al., 2019). Poly (*n*-butyl cyanoacrylate) nanoparticles with a polystyrene block that synopsize thioflavin T to attack Aβ (Tiwari et al., 2019). To study the structural way of Aβ tone assembly, gold nanoparticles (NPs) are used as discrepancy agents in MRI images and amount blotches, i.e. semi-nanocrystals and fluorescent operators, are used for labeling (Tiwari et al., 2019). A largely sensitive nanoparticle-grounded biobarcoding system was developed that enables the discovery of answerable forms of Aβ in cerebrospinal fluid (Tiwari et al., 2019). This pattern uses adequate microparticles functionalized with monoclonal/ polyclonal antibodies and gold nanoparticles modified with oligonucleotides (DNA barcoding) to precisely describe answerable oligomers (Tiwari et al., 2019). Electrochemical discovery styles grounded on current invented chemistry were used using gold nanoparticles and finagled

monolayers to interact with Aβ peptides, enabling largely sensitive electrical discovery of $A\beta_{1-42}$ by tunneling microscopy (Tiwari et al., 2019). These recent technological and conceptual advancements have significantly added the diagnosis of AD (Tiwari et al., 2019). Once AD is diagnosed, the therapeutic options currently available are primarily disease-modifying treatments with relatively limited benefits (Tiwari et al., 2019).

1.7 Therapeutics

AD is distinguished by the misfolding several proteins, which disrupts cellular systems and leads to neuronal death (Tiwari et al., 2019). This misfolding can result in the loss or gain of toxic function in the protein (Tiwari et al., 2019). Abnormal protein aggregation is often responsible for this misfolding, causing the protein to deviate from its normal function and evade clearance by the cell, thereby triggering harmful biological reactions (Tiwari et al., 2019). Extensive research on AD focuses on inhibiting the production, aggregation, and spread of misfolded proteins to mitigate their toxicity (Tiwari et al., 2019). Many therapeutic approaches for AD aim to reduce the levels of toxicity of amyloid-β and tau (τ) proteins involved in diverse neurodegenerative procedures throughout first and final stages of this disease (Tiwari et al., 2019). Several drugs targeting these proteins have undergone rigorous analysis and reached different phases of clinical trials (Tiwari et al., 2019). In this summary, we highlight drugs that specifically target amyloid and are currently being studied, with a focus on fundamental and immediate degenerative mechanisms (Tiwari et al., 2019). However, it is important to note that these current therapeutic targets (e.g., rivastigmine, galantamine, and donepezil) are considered subordinary and are disbelieved in terms of adhering to the uplifting of AD (Tiwari et al., 2019). Treatment failures often occur due to unfavorable drug properties such as hydrophobicity, poor absorption through biological membranes, undesirable pharmacokinetic characteristics (e.g., rapid metabolism and clearance), drug instability (e.g., oxidation, hydrolysis, or photolysis), and tissue toxicity (e.g., hepatotoxicity, neurotoxicity, or

kidney toxicity) (Tiwari et al., 2019). Various treatment strategies have been proposed to remove Aβ, including drugs for Aβ degradation (Tiwari et al., 2019). However, many drugs that cultured promise in vivo manner have failed demonstration of the efficacy in human clinical trials, highlighting the urgent need for new therapeutic approaches (Tiwari et al., 2019). Most available drugs have limited effectiveness in crossing the BBB and reaching the neuron (Tiwari et al., 2019). Consequently, there is a growing need to explore neuroprotective strategies specifically designed for the CNS (Tiwari et al., 2019). Nanoparticles (NPs) offer intriguing potential in this regard, as they can be multi functionalized to mimic physiological mechanisms for BBB transport (Tiwari et al., 2019). The BBB acts as a crucial barrier that protects brain from subjectically poisonous substances impending in the flow of bloodstream, but it also restricts the passage of 98% of neuropharmaceuticals, also diagnostic constituents (Tiwari et al., 2019).

Chapter 2

Immunization Strategies

2.1 Active immunization in AD:

2.1.1 Anti-Aß tactics:

The immunization which seems to be an active manner is a conventional method used to administer drugs or molecules with a view to stimulate the desired antibody for patient's body type to repercussion (Panza et al., 2012). For evaluating not only antigenicity but also toxicity during immunization of multiple-dose with the presence of AN1792 among patients get affected with lenient to median AD (Panza et al., 2012). However, the trial was halted prematurely due to acute meningoencephalitis symptoms observed in some vaccinated patients (Panza et al., 2012). Autopsy findings indicated an excessive Th1-mediated response (Panza et al., 2012) Importantly, T cells accumulated in the brain after people entered the Aß vaccine (Panza et al., 2012). The posterior activation of microglia cells by these T cells helped to successfully remove Aß from the brain (Panza etal.,2012). Histopathological studies have verified that the vaccine can increase amyloid concurrence in the mortal brain by demonstrating the concurrence of parenchymal pillars in some individualities (Panza et al., 2012). Histological analysis showed that parenchymal pillars were removed in some cases, attesting the vaccine's capability to accelerate the concurrence of amyloid deposits from the mortal brain (Panza et al., 2012). Roughly 25 of people treated with AN1792 developed an vulnerable

response to Aβ, manifested byanti-Aβ antibodies (Panza et al., 2012). Compared to cases who didn't show an vulnerable response to the medicine, these people endured significant advancements in cognitive function and a slower loss of the capability to perform everyday conditioning (Panza et al., 2012). People with a favorable antibody response performed more on memory tests than people who didn't respond to treatment or entered a placebo (Panza et al., 2012). It has been suggested that starting treatment with AN1792 beforehand in the course of the complaint, before clinically significant changes associated with Alzheimer's complaint do, may ameliorate cognitive benefits (Panza et al., 2012). Early immunization inhibited the conformation of new Aβ deposits and the aggregation of hyperphosphorylated tau proteins without affecting the presence of tau befuddlements in studies using transgenic mice bodiess of AD (Panza et al., 2012). Numerous clinical studies are currently investigating active immunization approaches, with modifications to the antigenic presentation of Aß-peptides aiming to stimulate a humoral response and reduce the likelihood of a Th1-mediated response (Panza et al., 2012). The vulnerable system is stimulated by a variety of chemicals in the coming- generationanti-Aß vaccinations, and other constituents boost their effectiveness (Panza et al., 2012). To ameliorate safety, efficacity, and convenience, these vaccines are given via a variety of styles, including oral, intranasal, and transcutaneous (Panza et al., 2012). Mucosal vaccination in particular has a lot of pledge since it generally activates an vulnerable response involving antibodies and, when combined with particular enhancers, can affect in significant serum situations of IgG titres (Panza et al., 2012). Nasal immunization with Aß, along with a proteosome-based adjuvant, effectively reduced amyloid burden in transgenic mice without triggering a cell-mediated immunological response (Panza et al., 2012).

2.1.2 Tau-based tactics:

The success of active immunotherapy against tau protein led to the conformation of anti-tau antibodies, which were also used to exclude abnormal tau protein conformations and ameliorate neuronal function (Panza et al., 2012). The first studies of active immunotherapy with tau were conducted in 2007 by Asuni and associates on mice with the JNPL3 P301L inheritable mutation, which led to the emergence of the mortal tau protein, known for its shifted parcels (Panza et al., 2012). They used the 30 amino acid tau phosphopeptide, which spans amino acids 379 to 408 and contains specific phospho-Ser remainders at positions 396 and 404, which are frequently associated with neurofibrillary tangles (Panza et al., 2012). An aluminum-containing adjuvant was used in the study (Panza et al., 2012). These JNPL3 P301L mice are used as a model for tau-related conditions because they show the development of added up tau and NFT proteins at 5 months of age as well as significant motor and behavioral detriments (Panza et al., 2012). After administering multiple boluses of the vaccine, experimenters observed a targeted vulnerable response and reduced accumulation of undoable phosphorylated tau protein using immunohistochemical and biochemical ways (Panza et al., 2012). Compared to particular mice that had just entered the adjuvant, immunized mice showed bettered motor performance in behavioral tests similar as the swing bar and bar tests (Panza et al., 2012). Beforehand vaccination of youthful creatures at 2 months of age redounded in a cabalistic retrenchment in tau-based abnormalities in specific brain regions, with reductions of over to 96 at 5 or 8 months of age (Panza et al., 2012). Studies showed that antibodies produced in response to the immunogen were suitable to pass though BBB which attach to tau protein which are phosphorylated (Panza et al., 2012). Although the mice's geste bettered after tau junking, the effect on delaying cognitive decline couldn't be determined because motor poverties bloodied routine literacy and memory testing (Panza et al., 2012).

In a study published in 2006, Rosenmann with his team used full chain immunogen mortal tau protein in genial mice (Panza et al., 2012). This immunogen was combined with pertussis poison and Freund's complete adjuvant (Panza et al., 2012). The results of this study showed that this approach touched off a severe autoimmune response, leading to the development of motor problems, axonal damage, glial cell inflammation, vulnerable cell infiltration in vaccinated mice, and the conformation of NFTs structures (Panza et al., 2012). These results suggest that tau immunization may have neurotoxic side goods (Panza et al., 2012). This examines specifies the implicit shortcomings of using responsible tau protein as an immunogen or unresistant immunization with antibodies that bind to full-length tau epitopes (Panza et al., 2012). It's intriguing to note that in a different study, transgenic mice models expressing a double stranded cross link mutant mortal tau protein(K257T/ P301S) were immunised with an admixture of Tau195- 213(p202/ 205), Tau207- 220(p212/ 214), and Tau224- 238(p231)), which contained phosphorylation spots linked to tauopathies and Alzheimer's complaint (Panza et al., 2012). Over a prolonged observation period, there was no substantiation of encephalitogenic goods, neurological abnormalities, negative goods on brain seditious cells, or damage to axons, performing in a 40 drop in the cargo of NFTs in both the brain and spinal cord (Panza et al., 2012).

Two recent exploration studies have reported favorable issues following immunization with tau phosphopeptides (Panza et al., 2012). The very first read used the standard 30 amino acid tau phosphopeptide as in the case result by Asuni and his growing teams (Panza et al., 2012). Still, hTau/ PS1 mice were used in this work (Panza et al., 2012). These mice also retain a shifted mortal presenilin- 1 gene(M146L) and express all six normal mortal tau isoforms in a knockout mouse tau background (Panza et al., 2012). In this beast model, the cortex and hippocampus were the main spots of tau- related problems (Panza et al., 2012). These mice

were first immunized at 2- 3 months of age, also entered yearly injections the following month and a supporter cure two weeks latterly (Panza et al., 2012). Cognitive assessment began at 7-8 months of age and mice were euthanized at 8-9 months of age (Panza et al., 2012). The study results showed that tau immunotherapy significantly reduced tau protein phosphorylation, bettered motor function, and reduced cognitive decline (Panza et al., 2012).

In the alternate study, a peptide with a shorter tubulin binding sphere, Tau260- 264(pS262), was used (Panza et al., 2012). This peptide was deposited between the first two peptides (Panza et al., 2012). The robust vulnerable response after active immunization of JNPL3 P301L mice reduced phosphorylation of tau(particularly PHF- 1) in the dentate gyrus by 64 (Panza et al., 2012). Likewise, this vaccine bettered motor function, supported tau concurrence, and had favorable issues (Panza et al., 2012).

Tau undergoes a number of variations during hyperphosphorylation, including phosphorylation, truncation, glycosylation, and ubiquitination. Tau changes from a structurally disordered answerable protein to undoable, malformed summations (Panza et al., 2012). Immunization with recombinant misfolded and abbreviated tau delayed the onset of behavioral poverties and averted the development of neurofibrillary befuddlements(NFTs) in a rat model of tau- related complaint discovery (Panza et al., 2012). This procedure reduced the attention of answerable misfolded tau protein by a chance before neuronal damage was detected (Panza et al., 2012). To evoke an antibody response that helps exclude tau, emphasis has been placed on targeting erroneously phosphorylated tau spots or specific pathological conformations of tau (Panza et al., 2012). In several beast models, scientists are presently probing new immunotherapeutic strategies targeting tau, fastening on prefibrillar forms of tau to stop neurodegeneration and the conformation of NFTs (Panza et al., 2012).

Table 1. A summary of key preclinical investigations on active, passive immunization conducting β-amyloid, also tau protein as potential therapies for AD (Panza et al., 2012).				
Originators (year)	**Kind of immunization**	**Immunogens/epitopes**	**Animal simulation**	**Primary Discoveries**
Schenk *et al.* (1999)	Systemic response	Aß1–42	PDAPP mice	Amyloid-beta (Aß) levels were diminished in juvenile mice in cerebral part.
Lemere *et al.* (2000)	Intranasal administration	Aß1–40 and Aß1–42	PDAPP transgenic mice	Amyloid-beta (Aß) levels were diminished in cerebral.
Weiner *et al.* (2000)	Intranasal administration	Aß1–40 and Aß1–42	PDAPP transgenic mice	Amyloid-beta (Aß) levels were diminished in cerebral.
Morgan *et al.* (2000)	Subcutaneous Delivery	Aß1–42	APP/PS1 transgenic mice	Abstaining cognitive decline or retention impairment.
Bard *et al.* (2000)	Systemic immunity	Monoclonal: 10D5 or 21F12; Polyclonal: Aβ1–42	Transgenic PDAPP mouse model	Amyloid-beta (Aß) levels were diminished in Cerebral.
Sigurdsson *et al.* (2001)	Actively affecting the entire system.	Aβ1-42 antibody that has been aggregated.	Transgenic Tg2576 mouse model	Amyloid-beta (Aß) levels were diminished in cerebral.

DeMattos *et al.* (2001)	Passively influencing the entire system.	m266	PDAPP mice	Cerebral amyloid beta (Aß) levels decreased through modulation of Aß clearance in the central nervous system (CNS) and plasma.

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Originators (year)	**Kind of immunization**	**Immunogens/epitopes**	**Animal simulation**	**Primary Discoveries**
Pfeifer *et al.* (2002)	Passively influencing the entire system.	Anti-Aß3–6 antibody	APP23 mice	Cerebral amyloid beta (Aß) levels were decreased in association experiencing microhemorrhage in cerebral.
Dodart *et al.* (2002)	Passively influencing the entire system.	m266	Tg2576 mice	Memory deficits were reversed.
Das *et al.* (2003)	Affecting the entire system through both active and passive means.	Aß1–42 and anti-Aß antibody	Tg2576 x FcRg$^{(-/-)}$ mice	Amyloid-beta (Aß) levels were diminished throughout this process in cerebral.
Racke *et al.* (2005)	Passively influencing the entire system.	m266, 3D6 and 10D5	Transgenic PDAPP mouse model	While the m266 antibody had no similar goods, the 3D6 and 10D5 antibodies increased the extent of cerebral

				amyloid angiopathy(CAA) and microbleeds.
Rosenmann *et al.* (2006)	Actively affecting the entire system.	Complete human tau protein created using recombinant techniques.	Wild-type BL/6 mice	The antibodies caused mononuclear cells, gliosis, axonal damage and the growth of NFT- suchlike structures.

Table 1. A summary of key preclinical investigations on active, passive immunization conducting β-amyloid, also tau protein as potential therapies for AD (Panza et al., 2012).

Originators (year)	**Kind of immunization**	**Immunogens/epitopes**	**Animal simulation**	**Primary Discoveries**
Okura *et al.* (2006)	Actively affecting the entire system.	DNA vaccine that does not involve viruses.	APP23 mice	Aß was decreased in cerebral without any signs of inflammation.
Lee *et al.* (2006)	Passively influencing the entire system.	NAB61	Tg2576 mice	Cerebral Aß levels decreased, leading to improve learning.
Maier *et al.* (2006)	Actively affecting the entire system.	2 × Aß1–15	hAPP (FAD) mice	Cerebral Aß levels decreased, leading to improve learning.
Asuni *et al.* (2007)	Actively affecting the entire system.	Tau379–408 (pS396–pS404)	JNPL3 P301L mice	We observed an increase in motor task performance and a drop in undoable phosphorylated tau clusters.
Petrushina *et al.* (2007)	Actively affecting the entire system.	Aß1–11 fused with the versatile T-cell epitope.	Tg2576 mice	While answerable amyloid β remained unchanged in

				the brain, undoable Aβ dropped.
Movsesyan *et al.* (2008)	Actively affecting the entire system.	pMDC-3Aß1–11–PADRE construct	3 × Tg-AD mice	Behavior improved when levels of amyloid β (Aβ) in the brain decreased without glial cell activation or microbleeds.

Table 1. A summary of key preclinical investigations on active, passive immunization conducting β-amyloid, also tau protein as potential therapies for AD (Panza et al., 2012).

Originators (year)	**Kind of immunization**	**Immunogens/epitopes**	**Animal model**	**Primary Discoveries**
Schroeter *et al.* (2008)	Passively influencing the entire system.	3D6 and m266	PDAPP mice	Cerebral amyloid β levels decreased, but there was an increase in vascular Aβ and Cerebral Amyloid Angiopathy.
Yamada *et al.* (2009)	Passively influencing the entire system.	m266	Tg2576 mice	Cerebral concurrence of amyloid β(Aβ) is braked by the supplemental processing pathway m266.
Khrisnamurthy *et al.* (2009)	Actively affecting the entire system.	Tau260–264 (pS262)	JNPL3 P301L mice	When tau was suppressed and motor performance was bettered, there was a reduction in tau phosphorylation in the dentate gyrus.

Novak *et al.* (2009)	Actively affecting the entire system.	Truncated tau protein	Tau-Tg rat	This approach delayed deportmental problems and averted the development of NFTs.
Wang *et al.* (2010)	Actively affecting the entire system.	SDPM1 peptide, which has an affinity for Aß1–40/1–42-tetramers.	APPswePSEN 1(A246E) mice	Reducing amyloid β (Burro) from the brain without causing inflammation helped ameliorate cognitive function.

Table 1. A summary of key preclinical investigations on active, passive immunization conducting β-amyloid, also tau protein as potential therapies for AD (Panza et al., 2012).

Originators (year)	**Kind of immunization**	**Immunogens/epitopes**	**Animal model**	**Primary Discoveries**
Boimel *et al.* (2010)	Actively affecting the entire system.	Tau195–213[p202–205] + Tau207–220[p212–214] + Tau224–238[p231]	DM-tau-Tg mice	During long-term follow-up, there was no substantiation of encephalitogenic responses, impersonal acoustic poverties, inauspicious goods on brain seditious nucleus or axonal reparation, although a reduction in neurofibrillary distraction(NFT) conformation was observed.

Wiessner *et al.* (2011)	Systemic second generation active.	CAD106 - Anti-Aß1–6 antibody with Qß	APP23/APP24 mice	The attention of β-amyloid (Burro) in the brain decreases, with minimum threat of negative consequences.
Cattepoel *et al.* (2011)	Passively influencing the entire system.	Anti-Aß30–42 scFv antibody	APPswe/PS1dE9 mice	Cerebral amyloid angiopathy(CAA) and cerebral amyloid β (Burro) situations dropped.

Table 1. A summary of key preclinical investigations on active, passive immunization conducting β-amyloid, also tau protein as potential therapies for AD (Panza et al., 2012).

Originators (year)	**Kind of immunization**	**Immunogens/epitopes**	**Animal model**	**Primary Discoveries**
Boutajangout *et al.* (2011)	Passively influencing the entire system.	Tau protein-targeting PHF1 antibody	JNPL3 P301L mice	Functional Poverties and tau abnormalities dropped.

2.2 Passive immunization in AD

2.2.1 Anti-Aß tactics:

The administration of an antibody to a case that is optimized for maximum effectiveness and produced in a host or model system is called unresistant immunization (Panza et al., 2012). According to AD, passive immunotherapy offers a potential approach for directly controlling the immune response against amyloid-β (Aß) plaques (Panza et al., 2012). By administering exogenous monoclonal antibodies (mAbs) targeting Aß, Th1-mediated autoimmunity can be avoided. Studies on AD transgenic mice treated with monoclonal

antibodies targeting Aß have shown a significant reduction in brain Aß levels, improved cognitive function, and a decrease in brain plaque pathology (Panza et al., 2012). Various antibodies with different Aß-binding properties have demonstrated these positive effects, suggesting that the mechanisms of action may involve Aß binding beyond simply removing existing plaques (Panza et al., 2012). The preface of an antibody produced in a host body and improved the efficacity previous to manage a case is known as unresistant immunization (Panza et al., 2012). Therefore, targeting and removing these oligomeric species could be beneficial in treating the disease (Panza et al., 2012). The proposition is that early answerable oligomeric forms of Aβ, which do before the appearance of pillars, contribute to neuronal degeneration and the progression of Alzheimer's complaint(announcement) (Panza et al., 2012). Although some challenges remain, similar as cerebral microbleeds, nonspecific reactivity, and the possibility of loss of antibodies through supplemental shedding, unresistant immunization has proven to be relatively safe in announcement beast models (Panza et al., 2012). Clinical trials are presently underway to probe unresistant immunization with colorful antibodies against Aβ (Panza et al., 2012). The humanized monoclonal antibodies bapineuzumab(AAB-001) and clinical cognitive assessments with bapineuzumab treatment, especially at lower doses, further studies are needed to establish its efficacy (Panza et al., 2012). Phase II studies with multiple boluses of bapineuzumab had no significant goods on cognitive functions and conditioning of diurnal living in the overall patient population (Panza et al., 2012). Still, a post hoc analysis showed implicit benefit in individualities lacking the ApoE- ε4 allele, particularly on tests similar as ADAS- Cog, NTB, MMSE and CDR- SB(banning pater) (Panza et al., 2012). In some cases who entered bapineuzumab in clinical trials, vasogenic cerebral edema redounded in headache, confusion, puking, and difficulty walking as clinical symptoms (Panza et al., 2012). MRI scans detected increased T2 signal intensity, indicating vasogenic edema, at regular intervals throughout the entire study (Panza et al., 2012).

Amyloid β(Aβ) and tau situations in cerebrospinal fluid(CSF) were examined in a lower extension study as part of the first phase II study in 20 cases who entered bapineuzumab and 15 cases who entered placebo (Panza et al., 2012). Compared to birth, bapineuzumab treatment had no significant effect on CSF Aβ situations at week 52 (Panza et al., 2012). There was a clear trend toward lower cerebrospinal fluid(CSF) tau situations in the bapineuzumab group, but this trend wasn't statistically significant (Panza et al., 2012). Bapineuzumab compared to the placebo group (Panza et al., 2012). Depending on the ApoE genotype, the same volumetric MRI reviews of cases produced different results (Panza et al., 2012). In discrepancy to subjects without the ApoE ε4 genotype, treatment with bapineuzumab in subjects with the ApoE ε4 allele was associated with an increase in ventricular size but no change in total brain volume (Panza et al., 2012). Other adverse events related to bapineuzumab did not show a dose-dependent relationship (Panza et al., 2012). Cases of deep tone thrombosis and pulmonary embolism have been reported, but they're rare and clinically important (Panza et al., 2012). Four deaths were reported during the study, all of which passed in the bapineuzumab group (Panza et al., 2012). Still, they were unconnected to cure, ApoE ε4 status, or circumstance of vasogenic edema and were thus considered unconnected to the medicine (Panza et al., 2012). Cerebral microbleeds passed in a case who also suffered from vasogenic edema during the phase I study (Panza et al., 2012). In unresistant immunization studies, the presence of cerebral microbleeds is problematic (Panza et al., 2012). According to some experts, Aβ is important for maintaining the health of the blood- brain hedge and vascular system (Panza et al., 2012). Its pullout can beget serum factors to enter the brain and spark an vulnerable or autoimmune seditious response (Panza et al., 2012). The circumstance ofmini-strokes may be a significant consequence of Aβ elimination (Panza et al., 2012). Vascular amyloid deposits, alsoknown as congophilic amyloid angiopathy, are allowed to be related to cerebral microbleeds (Panza et al., 2012). These

discussing deposits can damage the walls of bloodvessels, which can lead to the destruction of smooth muscle cells (Panza et al., 2012). Nearly all cases with Alzheimer's complaint and about 33 of aged grown-ups with good cognitive function suffer from this type of amyloid angiopathy (Panza et al., 2012). Studies in transgenic mice used as models of Alzheimer's complaint have shown that unresistant intraperitoneal immunization with colorful monoclonal antibodies increases the number of microbleeds (Panza et al., 2012). In studies with transgenic mice, microbleeds were observed after vigorous immunization (Panza et al., 2012). Injection of Aβ antibodies in these beast studies stopped the accumulation of vascular amyloid (Panza et al., 2012). Elimination of vascular amyloid was also observed in necropsy of mortal smarts after studies with the AN1792 vaccine, with multiple cortical hemorrhages recorded in one case (Panza et al., 2012). Analogous results were attained in other neuropathological studies with AN1792 (Panza et al., 2012). Posterior long- term studies, which showed that people with significant antibody responses had analogous brain volumes to those who entered a placebo, verified original findings that AN1792 vaccination could beget brain loss in cases with Alzheimer's complaint due to the elimination of Aβ (Panza et al., 2012). Solanezumab (LY2062430), developed by Eli Lilly in Indianapolis, IN, USA, is the primary competitor to bapineuzumab (Panza et al., 2012). The mechanism of action of solanezumab sets it apart from other passive immunotherapies (Panza et al., 2012).

2.2.2 Tau-based strategies:

Passive immunization ways for immunotherapy against tau oligomers in beast models using antibodies analogous to those developed for Aβ are extremely promising (Panza et al., 2012). Recent scientific studies have discovered naturally being tau antibodies(IgM and IgG) in the bloodstream, although cerebrospinal fluid(CSF) contains lower quantities of these antibodies (Panza et al., 2012). These antibodies, targeting unphosphorylated and phosphorylated tau,

exhibit a decline with age, similar to Aß antibodies (Panza et al., 2012).A unique class of monoclonal antibodies that serve as chaperones have shown pledge in precluding the aggregation and dangerous consequences of misfolded proteins and in guarding certain proteins from abnormal shapes (Panza et al., 2012). In particular, antibodies with chaperone- suchlike parcels can inhibit tau aggregation, grease the folding of proteins into their native forms, and potentially rear abnormal conformations (Panza et al., 2012). By neutralizing the toxicity associated with misfo may play a crucial role (Panza et al., 2012). Unlike the body's natural chaperones, which have large goods on numerous classes of proteins, antibodies have protein folding goods that are specific to the material they target (Panza et al., 2012). Antibodies directed at specific regions, such as the microtubule-binding domain's first or second repeat, effectively inhibit aggregation into paired helical filaments (PHFs) (Panza et al., 2012). However, site-directed antibodies do not affect tau-induced microtubule assembly (Panza et al., 2012). The sequence motif 306-VQIVYK 311 was set up to produce a β- distance conformation that's necessary for the development of tau fibrils (Panza et al., 2012). A high-affinity monoclonal antibody against remainders 300-QPGGGSVQIVYKP-312 was developed, which fully reverses the abnormal arrangement of microtubules caused by tau misfolding (Panza et al., 2012). Further studies are needed to validate this system, although only one in vivo study showed that problems with tau could be solved and mouse function could improve slightly after repeated injections of the tau protein of the anti-tau monoclonal antibody PHF (Panza et al., 2012). Adverse goods observed in unresistant immunization trials with anti-Aβ antibodies, similar as some conditions, similar as cerebral amyloid angiopathy and microbleeds, may not be convinced by tau immunization with antibodies targeting specific conformations because tau doesn't interact with blood vessels (Panza et al., 2012). However, the intracellular localization of tau oligomers may pose challenges for immunotherapy compared to extracellular Aß (Panza et al., 2012). This is

important because antibodies can remove protein clumps from whim-whams cells without entering them (Panza et al., 2012). This shows that reducing the extracellular total pool can remove intracellular summations, altering the balance. In the case of Aβ, synuclein and tau proteins, this pathway has been demonstrated (Panza et al., 2012). In addition, cells can presumably absorb antibodies against tau and Aβ through endocytosis (Panza et al., 2012). Another possible path is the useof anti-tau intrabodies which have been shown to be effective in treating mice (Panza et al., 2012). Also, the exploration on Aβ and other amyloid oligomers will make a substantial donation to the quest for fresh styles and coffers to study tau oligomers (Panza et al., 2012). Lately, a system for creating tau oligomers in the lab as well as a new antibody designed for the conformation of tau oligomers were published (Panza et al., 2012). These ways are similar to those used to produce Aβ oligomers (Panza et al., 2012). These antibodies will provide important material to study role of tau oligomers in Alzheimer's disease and other neurodegenerative diseases and to target them in mouse models through passive immunization (Panza et al., 2012). They will work in confluence with natural antibodies with the same particularity (Panza et al., 2012).

Chapter 3

Current Targeted Alzheimer's Therapy(Mechanism, Opportunities, Limitations)

3.1 Aducanumab:

Aducanumab was created with a rear restatement drug system with tubes from elderly, healthy donors who were thought to be normal (Mukhopadhyay & Banerjee, 2021). This strategy was tested in a randomised controlled trial (RCT) because of the encouraging removal of Aβ pillars seen in the brains of transgenic mice with a similar patch (Mukhopadhyay & Banerjee, 2021). After intravenous administration, the study showed direct pharmacokinetics in the specified cure range, with a mean tube half-life of 21 days (Mukhopadhyay & Banerjee, 2021). After several boluses, Aducanumab has in fact demonstrated positive effects for patients on Aβ tubes. On brain imaging, it was found to preferentially bind to parenchymal Aβ over vascular Aβ (Mukhopadhyay & Banerjee, 2021). Microglia-mediated phagocytosis was proposed as a hypothesis of the Aβ clearance of it's mechanism (Mukhopadhyay & Banerjee, 2021).

In clinical trials, aducanumab has been shown to be one of several anti-amyloid agents that have shown significant effectiveness (Mukhopadhyay & Banerjee, 2021). Aducanumab performed better on clinical parameters and biomarkers than another monoclonal antibody, gantenerumab, which only showed significant benefits on biomarkers (Mukhopadhyay &

Banerjee, 2021). The effectiveness of these antibodies largely depends on their ability to bind neurotoxic Aβ oligomers and target their neurotoxic effects (Mukhopadhyay & Banerjee, 2021). Despite an interim analysis that led to the completion of Phase 3 trials of aducanumab, the final results of the EMERGE trial demonstrated the drug's effectiveness in tau PET imaging and in "surrogate tracers" such as p-tau in liquid cerebrospinal fluid (CSF) (Mukhopadhyay & Banerjee, 2021). BIIB037, sometimes referred to as Aducanumab, was created as a lethal recombinant IgG1 antibody with a particular emphasis on extra Aβ, encompassing ingestible fibrils and pathogenic oligomers selecting a specific list of pathogenic parenchymal Aβ oligomers is thought to be the goal in order to increase their co-occurrence and maybe impact cognitive decline as well as behavioural and neurological symptoms linked to Alzheimer's disease (Mukhopadhyay & Banerjee, 2021).

The creation of monoclonal antibodies to treat Alzheimer's disease complaint(announcement) is fraught with difficulties, such as financial constraints, nonsupervisory complexity, choosing relevant targets and biomarkers to gauge response, debating essential corrective pathways, and side effect operation (Mukhopadhyay & Banerjee, 2021). As a result, rather than focusing on a specific molecule or cascade, therapeutic strategies that target multiple pathways may be of greater clinical benefit (Mukhopadhyay & Banerjee, 2021). Current evidence indicates that announcement pathogenesis comprises concurrent and accretive alterations in numerous signalling pathways (amyloid, tau, neuroinflammation, etc.), which is in contrast to the previously suggested successional waterfall of events (Mukhopadhyay & Banerjee, 2021). Notable is the "treatment versus forestallment" conundrum in the investigation of monoclonal antibodies for announcement (Daoud et al., 2018). Clinical trials often involve subjects with mild cognitive impairment (MCI) or Alzheimer's complaint (announcement) for a period of 18 to 20 times 48 months, which limits the significant benefit of these antibodies during this period, even though anti-Aβ treatment shows promise in preventing primary Alzheimer's

complaint according to the amyloid waterfall thesis (Mukhopadhyay & Banerjee, 2021). Therefore, the question of why we are using these anti-amyloid medications arises: as agents of disease modification in AD as opposed to preventing the development or progression of AD (Mukhopadhyay & Banerjee, 2021). The latter is supported by scant evidence (Mukhopadhyay & Banerjee, 2021). Several strategies have been suggested to solve this problem (Mukhopadhyay & Banerjee, 2021). The subsequent clinical trials must be more closely aligned with the preclinical studies (Mukhopadhyay & Banerjee, 2021). Research into Anti-Aβ antibodies can be conducted in primary and secondary prevention, or longitudinal studies can be initiated to gain a deeper understanding of their involvement in prodromal and mild manifestation(Mukhopadhyay & Banerjee, 2021). Despite the challenges, the approval of aducanumab is undeniably a positive advancement; however, due to these persisting issues, this early approval may appear unconventional (Mukhopadhyay & Banerjee, 2021).

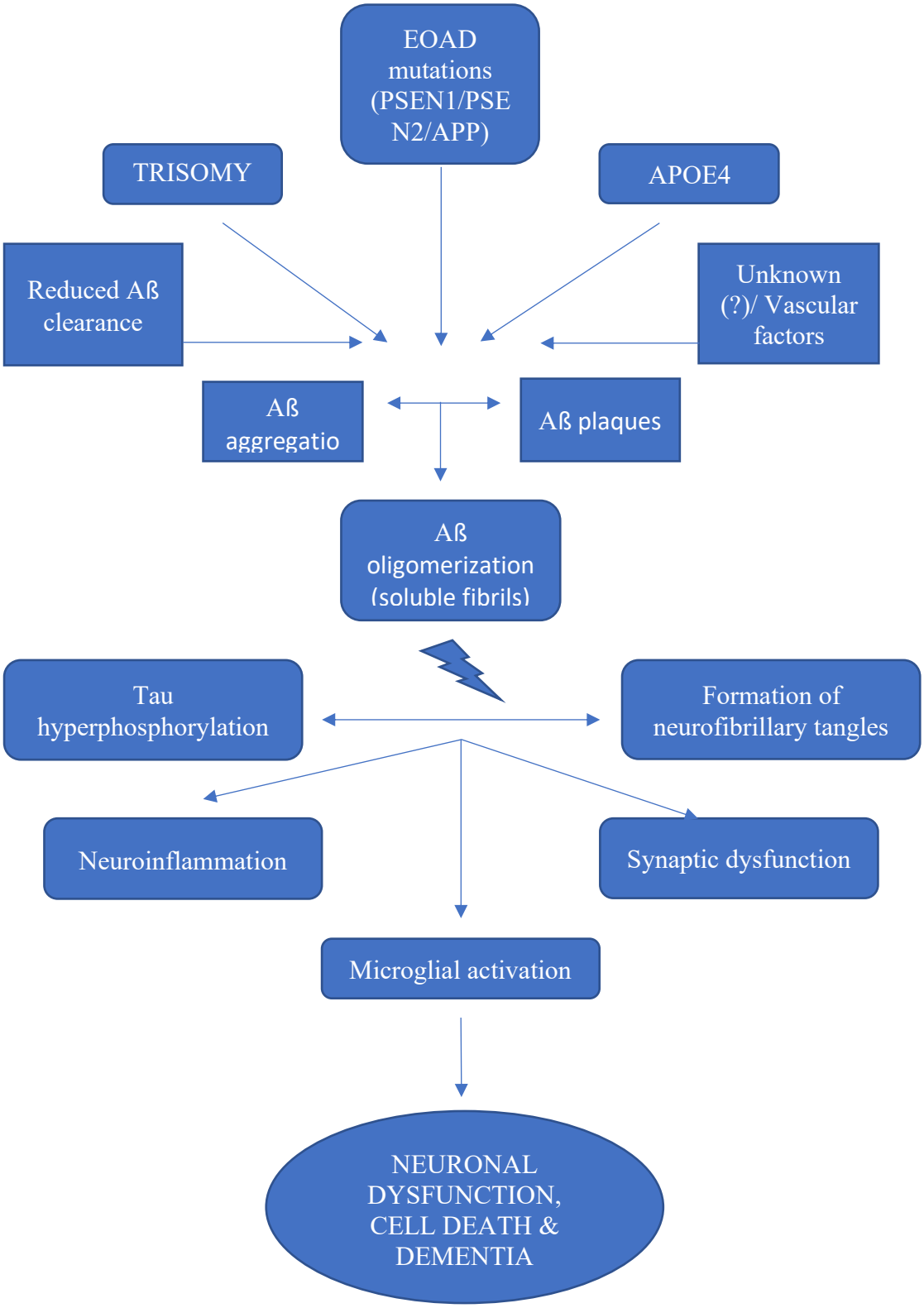
EOAD mutations (PSEN1/PSE N2/APP)
TRISOMY
APOE4
Reduced Aß clearance
Unknown (?)/ Vascular factors
Aß aggregatio
Aß plaques
Aß oligomerization (soluble fibrils)
Tau hyperphosphorylation
Formation of neurofibrillary tangles
Neuroinflammation
Synaptic dysfunction
Microglial activation
NEURONAL DYSFUNCTION, CELL DEATH & DEMENTIA

Fig. 3.1.1. The formation and structure of senile plaques resulting from the amyloid cascade impact APP metabolism and signify both early-onset Alzheimer's disease (depending on genetic risk factors) and late-onset Alzheimer's disease (dependent on the presence of at least one APOE4 allele and other unknown factors). Oligomerization of Aβ initiates a series of events, including tau hyperphosphorylation, the accumulation of neurofibrillary tangles, inflammation, activation of microglia, loss of synapses, and neuronal death (Mukhopadhyay & Banerjee, 2021). This escalating cascade, driven by an increasing amyloid burden, establishes a detrimental cycle that progressively worsens cognitive impairment. This process is particularly pronounced in the medial temporal structures, which encompass the hippocampus and play a crucial role in learning and memory (Mukhopadhyay & Banerjee, 2021).

The initial patch approved for Alzheimer's disease treatment, tacrine, was ultimately withdrawn due to its hepatotoxic effects (Mukhopadhyay & Banerjee, 2021). Instead, acetylcholinesterase inhibitors became the only class of AD medications that are currently approved by the FDA (Mukhopadhyay & Banerjee, 2021). Aducanumab's denial of approval, despite significant advances in our understanding of the amyloid pathway and its treatment, is argued to have stalled subsequent drug development research (Mukhopadhyay & Banerjee, 2021). As a result, this acknowledgement has paved the way for improved AD neurobiology research and drug development (Mukhopadhyay & Banerjee, 2021). The scientific community foresees the imminent development of monoclonal antibodies targeting key components of the tau and amyloid signaling pathways (Mukhopadhyay & Banerjee, 2021). The approval status of aducanumab will be contingent on findings from a post-marketing phase IV study and the arguments presented in its favor, potentially extending the timeline for validating or challenging recent assertions (Mukhopadhyay & Banerjee, 2021).

3.2 Aaptamine

Alzheimer's complaint(announcement) is a neurological complaint that worsens over time and is characterized by cognitive decline and progressive loss of independent cognitive functions in affected individualities. (Daoud et al., 2018). There are two distinct types of cholinesterase that independently hydrolyze acetylcholine (ACh) into acetic acid and choline: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) (Daoud et al., 2018). The activity of acetylcholine (ACh) is diminished in the presence of cholinergic deficiency or the loss of cholinergic neurons associated with cognitive functions (Daoud et al., 2018). In healthy brains, acetylcholinesterase (AChE) predominates, but butyrylcholinesterase (BuChE) is allowed to play a minor role in regulating acetylcholine levels in the brain (Daoud et al., 2018). In a healthy brain, 80% of the ACh is hydrolyzed by AChE. As a result, drugs frequently target it (Daoud et al., 2018).

In this context, a number of researchers propose that the creation of acetylcholinesterase inhibitors may provide novel, efficacious avenues for treating Alzheimer's disease(Daoud et al., 2018). Among the current anti-acetylcholinesterase agents are used to treat AD are donepezil, tacrine, galantamine, and ensaculin (Daoud et al., 2018). Furthermore, a great deal of research has been done to create particular small patch butyrylcholinesterase (BuchE) inhibitors that may efficiently inhibit both acetylcholinesterase (AChe) and BuChE in order to improve clinical problems (Daoud et al., 2018). As strong inhibitors of AChe and BuChE, a novel family of p-hydroxybenzaldehyde derivatives and 4-((diethylamino) methyl) phenoxy-composites were created and evaluated (Daoud et al., 2018).

On the other hand, many people use Molecular Docking simulations to figure out how well a ligand can interact with a target (Daoud et al., 2018). They typically concentrate on a computational method for predicting a ligand's orientation when bound to an enzyme or protein

(Daoud et al., 2018). The most potent ligand can typically be selected from among those with the highest "binding affinity" (Daoud et al., 2018).

Nowadays, docking simulations are combined with additional theoretical approaches like: Molecular dynamics (MD) and quantum mechanics (QM) (Daoud et al., 2018). They try to make use of each of these strategies to improve outcomes (Daoud et al., 2018). Regretfully, the several published workshops only provide basic information verifying the ligand's essenti al residue list by docking computations (Daoud et al., 2018). The enzyme-ligand trade deceived by docking in this script just serves as evidence that the ligand with the highest docking score isn't always a powerful leader (Daoud et al., 2018). Because of this, MD simulations must always be used to validate these kinds of studies (Daoud et al., 2018).

3.3 Cornuside; a reactive astrocytes

We looked at the multidirectional effects of gallotannins and cornuside in a number of assays, with a particular emphasis on cholinesterase inhibition, BACE1 inhibition, and ONOO- intermediated protein tyrosine nitration (Bhakta et al., 2017).

At doses ranging from 1 to 50 µm, cornuside was shown to have protective effects on culture d rat cortical cells exposed to oxygen and glucose deprivation (Bhakta et al., 2017). It was also shown to be able to reduce the production of proinflammatory and adhesio n markers generated by cytokines in human endothelial cells (Bhakta et al., 2017). Moreover, cornuside suppressed lipopolysaccharide-induced inflammatory mediators and prevented acute liver injury caused by carbon tetrachloride at doses of 150 and 300 mg/k g by inhibiting NF-kB activation in RAW264.7 macrophages at concentrations of 3 to 30 µM (Bhakta et al., 2017). In addition, research has shown that polymeric proanthocyanidins can protect humans from cardiovascular disease and cancer (Bhakta et al., 2017). In K562 cells stimulated by butyric acid, tellimagrandin I, at doses of 10–

50 µM, suppressed markers of erythroid development, such as AChe and glycophorin Aβ. At doses between 10 and 50 µM, both tellimagrandin I and II showed evidence of erythroid differentiation, indicating noteworthy anticancer and hepatoprotective actions (Ma et al.) (Bhakta et al., 2017). A study concluded that the antioxidant capacity of gallotannins (10–100 µM) effectively prevented the formation of advanced glycation end products (Bhakta et al., 2017).

In the course of this research, it was determined that cornet and lothanin from the Cornus officinalis plant could entirely inhibit Hurt, BChE, and BACE1 cultures, highlighting the potential therapeutic advantages in addressing production-related issues (Bhakta et al., 2017).

3.4 Cerebrospinal Fluid Sink Therapeutic Strategy

Numerous biomarkers have been studied to see if they can be used in addition to the primary AD biomarkers to help diagnose AD earlier or more accurately (Bjerke & Engelborghs, 2018). Numerous research endeavors have concentrated on diverse facets of the support centre, including aberrant metabolism of amyloid, tau pathology, and degeneration of synapses or neurons, especially concerning cognitive characteristics (Bjerke & Engelborghs, 2018). To improve their clinical value, distinct tau species might, for example, be more specific for FTLD-tau, VαD or AD pathology (Bjerke & Engelborghs, 2018). Expanding upon this idea, an assay was created to quantify unphosphorylated tau at locations T_{175} and T_{181}, exhibiting excellent precision in differentiating between Alzheimer's disease-related Mild Cognitive Impairment (MCI) and healthy controls (Bjerke & Engelborghs, 2018).

Neither the distinction between AD or FTLD and healthy controls provided any additional benefit (Bjerke & Engelborghs, 2018). A lot of work has gone into creating $Aβ_{1-42}$ as an Alzheimer's disease biomarker that can be used in routine clinical settings and operate as an in vivo surrogate marker for brain pathology(Bjerke & Engelborghs, 2018). Its potential is

enhanced by its distinct favourable traits and comparatively inexpensive cost (Bjerke & Engelborghs, 2018). However, there are differences in preanalytical and analytical procedures, as well as in the performance of various assays using diverse calibrators influenced by insulin dysregulation, which are largely responsible for the variations in absolute measurements of $A\beta_{1–42}$ in cerebrospinal fluid (CSF) amongst laboratories (Bjerke & Engelborghs, 2018). This is complicated by the importance of insulin to brain function (Bjerke & Engelborghs, 2018). Because direct measurements vary throughout labs and technicians and compromise dependability, this problem presents a significant obstacle to the development of biomarkers and their use in regular clinical assessment (Bjerke & Engelborghs, 2018). Standard operating procedures for preanalytical sample handling emphasise that cerebral blood flow (CBF) in different brain regions, sample sizes, and storage in different tubes all have a significant impact on CSF biomarker concentrations (Bjerke & Engelborghs, 2018).

Chapter 4

Emerging Alzheimer's Drug Therapy

4.1 Blood-based Biomarkers

The blood measurement of biomarkers for D pathogenesis number of difficulties that necessitate precise and sensitive assays and careful validation work (Zetterberg & Burnham, 2019). The blood-brain barrier restricts the unrestricted movement of molecules between the central nervous system and blood compartments, resulting in generally low concentrations of phosphorylation in the blood (Zetterberg & Burnham, 2019). Relevant information for sample processors related to cerebral pathology is stored in extracerebral appendages, potentially influencing their assessment in blood (Zetterberg & Burnham, 2019). Moreover, the presence of heterophilic antibodies, which are endogenous antibodies interacting with those used in immunochemical tests for biomarker measurement, can lead to inaccurate results, either elevated or reduced. In cerebrospinal fluid, where antibody concentrations are considerably lower, these antibodies pose less risk (Zetterberg & Burnham, 2019). Finally, a variety of plasma proteases may undergo proteolytic degradation of the desired analyte (Zetterberg & Burnham, 2019). We'll talk about the most recent advances in biomarkers for AD's pathological processes below. We employ panels of biomarkers that can mirror tissue responses to similar changes, along with biomarker assays targeting a singular pathological lesion (Zetterberg & Burnham, 2019).

Plasma Aβ

In the Alzheimer's Biomarker database, early results on plasma Aβ showed that neither plasma Aβ40 nor Aβ42 changed consistently in AD (Zetterberg & Burnham, 2019). The observed outcome was probably due to challenges associated with the test; matrix substances (particularly tube Aβ-binding proteins) can influence measurements in tube Aβ, and the initial

assays' analytical sensitivity hindered the elimination of these matrix substances (Zetterberg & Burnham, 2019). In 2011, Simoa, a single-well assay for Aβ42 with high sensitivity, was introduced (Zetterberg & Burnham, 2019). There was a correlation between plasma and CSF Aβ42 and the analytical sensitivity showed that amyloid PET-positive individuals had a lower plasma ratio of Aβ42 to Aβ40 than CSF Aβ42/Aβ40, albeit with less clear separation (Zetterberg & Burnham, 2019).

Plasma tau

The results of highly sensitive testing show that tau levels in blood are greater in the dementia stage of Alzheimer's disease than in cognitively normal controls, but not as significantly as in cerebrospinal fluid—a continuously reproducible conclusion (Zetterberg & Burnham, 2019). The significance of these findings for people with mild cognitive impairment (MCI) are less evident, and their relevance is still up for debate (Zetterberg & Burnham, 2019). However, a recent study by Mielke and colleagues found that in 458 participants in the Mayo Clinic Study of Ageing, greater blood levels of T-tau were associated with a faster progression of clinical symptoms (Zetterberg & Burnham, 2019). The Simoa system was utilised to measure the T-tau levels in the blood (Zetterberg & Burnham, 2019). Based on a replication of the Memento study and data from the Framingham Heart Study, a community-based research conducted in the United States, a prospective cohort analysis found a strong correlation between blood T-tau levels and the prevalence of dementia associated to Alzheimer's disease (Zetterberg & Burnham, 2019). Additionally, Mielke and her colleagues evaluated the phosphorylated tau levels in amino acid group 181 (Zetterberg & Burnham, 2019). The results of this assay showed a link between blood P-tau and PET-tau levels and other characteristics associated with Alzheimer's disease; the blood T-tau test did not show the same degree of association (Zetterberg & Burnham, 2019).

Plasma neurofilament light

An intra-axonal structural protein called neurofilament light (NfL) protein is released into body fluids after axonal injury and is a reliably reproduced biomarker of neurodegeneration in Alzheimer's disease (Zetterberg & Burnham, 2019). Serum or plasma NfL levels (both matrices show high efficacy) are elevated in both familial and sporadic Alzheimer's disease as well as in a number of other neurodegenerative disorders (Zetterberg & Burnham, 2019). These levels substantially correspond with NfL in cerebrospinal fluid (Zetterberg & Burnham, 2019). NfL levels rise in familial Alzheimer's disease about ten times before the disease's expected clinical start (Zetterberg & Burnham, 2019). Age-related changes (NfL levels in cerebrospinal fluid grow by roughly 3 per year in normal ageing) and pathologies unrelated to Alzheimer's disease later in life can also have an impact on changes in NfL levels (Zetterberg & Burnham, 2019). In conclusion, NfL levels in serum or plasma measured from blood may be a useful biomarker for Alzheimer's disease and other neurodegenerative diseases (Zetterberg & Burnham, 2019).

4.2 ApoE4: an emerging therapeutic target

There has been much research done on ApoE's involvement in Alzheimer's disease (Safieh et al., 2019). According to Strittmatter and Roses' early findings, those who have APOE4, one of the three polymorphic variants of APOE, have a higher chance of getting Alzheimer's dise ase (Safieh et al., 2019). Additionally, it has been noted that APOE4 carriers have cognitive alterations earlier in life, which are influenced by the allele's dosage (Safieh et al., 2019). On the other hand, ApoE2 is thought to be "protective" against Alzheimer's disease, whereas Apo E4 is thought to have a "harmful" effect since APOE2 carriers show a protective effect in co mparison to APOE3 and APOE4 carriers (Safieh et al., 2019). This section critically examines

the theory that, in theory, all isoforms can be "protective," with ApoE2 having the strongest effect and ApoE4 having the least (Safieh et al., 2019). All isoforms, however, can possibly have varied degrees of "harmful" consequences (Safieh et al., 2019). Consequently, it is conceivable that ApoE has a variety of consequences, both beneficial and detrimental, with ApoE4 acting as the least effective expression of these effects (Safieh et al., 2019). Comprehending the primary function of ApoE4, be it detrimental or beneficial, will impact treatment approaches for handling ApoE-associated ailments, carrying noteworthy consequences (Safieh et al., 2019). ApoE4 has been linked to several processes, including Aβ crosstalk, and has been shown to affect lipid metabolism and inflammation (Safieh et al., 2019). It is still unclear, though, exactly what functions these variables perform in moderating ApoE4's effects in Alzheimer's disease (Safieh et al., 2019).

Concerns arise from variations in the levels of different haplotypes in serum, cerebrospinal fluid (CSF), and potentially tissue (Safieh et al., 2019). APOE4 carriers exhibit lower ApoE concentrations in serum and the brain compared to carriers of other isoforms (Safieh et al., 2019). It is plausible that the effects of ApoE depend on the quantity of ApoE rather than its quality (Safieh et al., 2019). If we assume the simplest hypothesis that ApoE4 is detrimental to the brain, intervening in its action could potentially slow down or halt the progression of Alzheimer's disease (Safieh et al., 2019). Genetic, biochemical, and immunological approaches provide opportunities to specifically address the effects of ApoE4 (Safieh et al., 2019).

However, a more comprehensive strategy would be to neutralize all effects of ApoE, especially in the adult brain if feasible, under the assumption that all forms of ApoE are detrimental, albeit to varying degrees (Safieh et al., 2019). Nevertheless, this approach would only benefit 40-60% of Alzheimer's cases that carry ApoE4 (Safieh et al., 2019). Additionally, it's crucial to recognize that although ApoE is primarily synthesized in the liver, it is also produced in the brain and serves various roles, some of which may be relevant in Alzheimer's disease (Safieh

et al., 2019). In the brain, ApoE plays a crucial role in lipid transport and cholesterol homeostasis (Safieh et al., 2019). The pathological effects of ApoE4 are linked to lipid metabolism, as it has been observed to be hypolipidated and less effective than ApoE3 in promoting cholesterol efflux (Safieh et al., 2019).

The interactions of ApoE4 with Aβ, tau phosphorylation, mitochondrial dysfunction, and other mechanisms discussed in this review have been extensively studied experimentally in whole organisms and isolated tissues in vitro (Safieh et al., 2019). However, these studies have not yet identified a single function representing the most probable and significant signaling pathway (Safieh et al., 2019). The limitations of these experimental models should be carefully considered, as none of the currently available models fully represent the complexity of Alzheimer's disease (Safieh et al., 2019). Moreover, the nonphysiological conditions of Aβ and tau expression in Alzheimer's models make it challenging to assess the significance of downstream signaling effects (Safieh et al., 2019). Additionally, endogenous rodent molecules may respond differently to human-derived Alzheimer's molecules (Safieh et al., 2019). Genes associated with ApoE4 and Alzheimer's disease, such as TOMM40, located near the APOE gene on chromosome 19 and closely linked to APOE alleles, have not been extensively studied in animal models (Safieh et al., 2019).

In addition to its association with poor outcomes after traumatic brain injury, numerous studies supported by meta-analyses have indicated that APOE4 is a risk factor for other conditions such as cerebral amyloid angiopathy (CAA), Lewy body dementia, tauopathy, cerebrovascular disease, multiple sclerosis, and vascular dementia (Safieh et al., 2019). However, there is less evidence of APOE2 involvement in these conditions, likely due to the low frequency of APOE2 carriers in the population (Safieh et al., 2019).

APOE4 presents a paradoxical defense against age-related macular degeneration (AMD), a condition influenced by APOE genotype (Safieh et al., 2019). AMD is characterized by

abnormal angiogenesis and is typically treated with antibodies targeting vascular endothelial growth factor (VEGF) (Safieh et al., 2019). Despite its protective role in AMD, ApoE4 is associated with accelerated deterioration and compromised synaptic plasticity in other contexts, posing a risk (Safieh et al., 2019).

Studies in cell and animal models have revealed that APOE4 is linked to a reduction in cellular flexibility (Safieh et al., 2019). The detrimental effects of APOE4 may stem from decreased synaptic plasticity in neurons in Alzheimer's disease, while the protective impact in AMD may be attributed to enhanced angiogenesis and vascular flexibility (Safieh et al., 2019).

4.3 Altered bile acid profile

Numerous neurodegenerative and psychiatric conditions are exacerbated by bidirectional biochemical communication between the gut and the brain (MahmoudianDehkordi et al., 2019). A wide variety of small molecules that have an effect on human health are produced jointly by the host and the gut microbiome (MahmoudianDehkordi et al., 2019). Recent research has associated the gut microbiome with motor dysfunction in Parkinson's disease, and various animal models of Alzheimer's disease suggest a potential role of gut bacteria in amyloid pathology (MahmoudianDehkordi et al., 2019). In the transgenic mouse model of Alzheimer's disease (APP), the composition of the gut microbiome differed significantly from that of wild-type mice (MahmoudianDehkordi et al., 2019). Additionally, studies have identified neurotoxic lipopolysaccharides from pro-inflammatory gram-negative bacteria, implicated in cerebral amyloidosis and the systemic inflammation characteristic of Alzheimer's disease (MahmoudianDehkordi et al., 2019). These findings suggest that microbial imbalance or dysbiosis may contribute to the pathogenesis of Alzheimer's disease (MahmoudianDehkordi et al., 2019). The liver is also recognized for its significant role in cholesterol metabolism in Alzheimer's disease, with genome-wide association studies linking several genes associated

with cholesterol metabolism, including BIN1, CLU, PICALM, ABCA7, ABCG1, and SORL1 (MahmoudianDehkordi et al., 2019).

Through the production of bile acids (BAs), cholesterol is eliminated (MahmoudianDehkordi et al., 2019). The primary bile acids, chenodeoxycholic acid (CDCA), and cholic acid (CA) are synthesized in the liver from cholesterol (MahmoudianDehkordi et al., 2019). These acids are conjugated with glycine or taurine, transported to the gallbladder via the bile acid export pump, and then delivered to the intestine, where intestinal bacteria metabolize them (MahmoudianDehkordi et al., 2019). Enteric anaerobic bacteria, through bile acid hydrolases, deconjugate liver-derived bile acids into free bile acids. These anaerobic bacteria also convert primary bile acids into secondary bile acids, with CA transforming into deoxycholic acid (DCA) or lithocholic acid (LCA) after 7α-dehydroxylation (MahmoudianDehkordi et al., 2019).

CDCA undergoes 7β-dehydroxylation to form lithocholic acid (LCA) or ursodeoxycholic acid independently (MahmoudianDehkordi et al., 2019). Bile acids are absorbed by enterocytes in the terminal ileum and colon, released into the portal circulation, and then return to the liver, where they undergo conjugation with amino acids like glycine and taurine (MahmoudianDehkordi et al., 2019). Besides their role in cholesterol metabolism, bile acids contribute to maintaining energy homeostasis by binding to nuclear receptors such as FXR and LXR (MahmoudianDehkordi et al., 2019). BAs appear to be indicators of dysbiosis in the gut because they also alter the microbiome (MahmoudianDehkordi et al., 2019). There is evidence suggesting that both primary and secondary bile acids can cross the blood-brain barrier in the brains of mice and potentially in the human brain as well (MahmoudianDehkordi et al., 2019). Some BAs, li(MahmoudianDehkordi et al., 2019). MCI conversion (MCI-non converter, MCI- converter) and clinical diagnosis at baseline were

categorical response variables (MahmoudianDehkordi et al., 2019). A series of tests were used to assess cognition in the ROS/MAP cohort (details are published) (MahmoudianDehkordi et al., 2019). As previously mentioned, a comprehensive assessment of overall cognitive function was formulated by summing the outcomes of each test. Z-scores were computed based on the mean and standard deviation of the reference group (MahmoudianDehkordi et al., 2019). In the case of a negative z-score, it indicates that an individual's total score is below the mean of the reference sample (MahmoudianDehkordi et al., 2019). Cognitive tests and serum were used in the same cycle, and brain tests were used closest to death (MahmoudianDehkordi et al., 2019).

4.4 Psychosis

Psychotic symptoms are linked to worse disease outcomes and frequently cause distress for the individual as well as their caregivers (Ballard et al., 2020). Stern et al.'s landmark study found that reported that faster cognitive decline was linked to psychosis (Ballard et al., 2020). According to an analysis of 55 studies, 20 out of 30 cross-sectional studies on Alzheimer's disease (AD) with psychosis identified reduced cognitive function compared to AD without psychosis (Ballard et al., 2020). The initial observation of a more pronounced cognitive decline in the presence of psychosis has been confirmed by nine studies investigating the link between psychosis and cognitive deterioration (Ballard et al., 2020). Recent research has upheld this association and found a similar impact with MCI (Ballard et al., 2020). Notably, various factors such as age, age at onset of AD, duration of AD, gender, race, education, and family history do not seem to influence the connection between AD psychosis and cognitive decline (Ballard et al., 2020). The presence of psychosis indicates a more severe AD phenotype, and this consistent evidence suggests that these characteristics may be associated with other independent biological and/or genetic predispositions (Ballard et al., 2020). Outstandingly, a few investigations have recommended that among individuals who create psychosis, there is a more

keen direction of decline even preceding the beginning of candid maniacal side effects, with one investigation discovering that more quick mental degradation was available a year prior to the improvement of psychosis (Ballard et al., 2020). Psychosis is also linked to higher mortality rates, increased hospitalizations, early initiation of treatment, and a more rapid progression of functional decline (Ballard et al., 2020).

Regardless of cognitive decline, the presence of psychosis is also linked to heightened future dependence (Ballard et al., 2020). Other neuropsychiatric symptoms such as agitation, aggression, and depression often precede or accompany psychotic symptoms, exacerbating the impact on the individual and those around them (Ballard et al., 2020).

4.5 Brain insulin resistance

Dysregulation of insulin levels is proposed to be associated with the development of AD and cerebrovascular conditions, as insulin is considered crucial for brain health (Kellar & Craft, 2020). This study introduces a potential therapeutic avenue where improving insulin sensitivity or addressing its deficiency in the central nervous system CNS may delay or alleviate Alzheimer's disease and related conditions (Kellar & Craft, 2020). The discussion begins with the consideration of intranasal insulin, a method directly acting on the brain, followed by other approaches aiming to enhance insulin sensitivity in central and peripheral areas (Kellar & Craft, 2020). One key strategy involves interventions to boost peripheral insulin sensitivity, thus lowering insulin levels (Kellar & Craft, 2020). While there is limited evidence regarding their effects on the human brain, various insulin-sensitizing compounds, with metformin being one of the most extensively studied classes, have been explored to restore insulin sensitivity in the central nervous system (Kellar & Craft, 2020). In mouse models of AD, metformin was found to enhance memory, decrease Aβ-concentrations, hyperphosphorylated tau, and activated microglia (Kellar & Craft, 2020). Furthermore, there was an improvement in insulin signaling

in the brain (Kellar & Craft, 2020). To investigate its potential benefits in individuals without diabetes and with mild cognitive impairment, a placebo-controlled, randomized trial was conducted involving 80 participants who were administered metformin or a placebo daily for a year (Kellar & Craft, 2020). In a study, although the metformin group showed promising results in the selective recall test, no significant differences were observed in ADAS-Cog12 scores, CSF Aβ42 levels, and brain glucose metabolism measured by FDG-PET compared to the control group (Kellar & Craft, 2020). Currently, a phase 2 trial (NCT04098666) is underway to further explore the effects of metformin on patients with AD and in cognitive has mild impairment, building upon these initial findings (Kellar & Craft, 2020).

Research with various insulin-sensitizing agents, such as PPAR agonists, has yielded conflicting results (Kellar & Craft, 2020). In a pilot study involving 30 individuals with Alzheimer's disease and mild cognitive impairment, improved memory and stable CSF Aβ42 levels were observed after six months of rosiglitazone treatment compared to placebo (Kellar & Craft, 2020). However, only APOE-4 noncarriers exhibited enhanced cognitive function (measured by ADAS-Cog12) after 24 weeks of rosiglitazone or placebo treatment, and APOE genotype had no impact on cognitive function in a phase III trial (Kellar & Craft, 2020). A Phase 3 placebo-controlled trial of pioglitazone, aiming to delay the onset of mild cognitive impairment in 3,500 cognitively healthy adults based on their APOE and TOMM40 genotypes, was prematurely halted due to a lack of efficacy (NCT01931566) and remained unpublished (Kellar & Craft, 2020). In conclusion, there is limited evidence supporting the notion that insulin sensitizers can enhance the brain's sensitivity to insulin or serve as effective treatments for Alzheimer's disease (Kellar & Craft, 2020).

4.6 Emerging Microglia Biology

The advent of single-cell RNA sequencing (scRNA-seq) and mononuclear RNA sequencing (snRNA-seq) technologies has significantly advanced microglial biology by revealing diverse cellular states and underlying molecular pathways in the central nervous system (Lewcock et al., 2020). Currently, transcriptional profiling is used to describe a subpopulation of microglia by identifying specific gene sets with differential expression (Lewcock et al., 2020). These technologies have highlighted the dynamic and complex nature of microglial cell populations, with limited overlap observed between well-characterized homeostatic and responsive states in mouse and human microglia (Lewcock et al., 2020). Despite the valuable insights provided by sc/snRNA-seq datasets into the diversity of microglial subpopulations, translating this information into understanding how these subpopulations positively or negatively impact AD is crucial (Lewcock et al., 2020). Challenges arise from the lack of cross-species sequence and functional homology in mouse models, emphasizing the need to study microglial biology directly in human cells (Lewcock et al., 2020). In vitro models, such as isolating induced pluripotent stem cells into microglia (iMG) and employing molecular genetic techniques like CRISPR for gene manipulation, are becoming increasingly popular (Lewcock et al., 2020). However, these models face limitations, including rapid changes in gene expression when microglia are removed from the central nervous system medium, which doesn't entirely replicate in vivo biology (Lewcock et al., 2020). Another approach involves generating humanized mice through xenografting, where human hematopoietic iPSC precursors are introduced into the brains of immunodeficient mouse models, differentiating into mature microglia (Lewcock et al., 2020). While these models represent progress, they are costly, time-consuming, and may not fully capture interactions between neuroimmune cells and other blood-borne cells (Lewcock et al., 2020).

Nonetheless, these models provide a unique opportunity to explore whether the replication of human microglial cell states depends on pathology or the brain microenvironment, and whether microglial diversity observed in humans can be reproduced in mouse models of AD (Lewcock et al., 2020). These models also offer a platform for predicting pharmacological effectiveness and testing human-specific therapeutic mechanisms of action (Lewcock et al., 2020).

The next crucial step involves interpreting the sc/snRNA-seq profiling data to gain a more functional understanding of subpopulations within the central nervous system (Lewcock et al., 2020). Deciding how sub-populaces answer microglia-focusing on treatments will give an undeniably more far-reaching evaluation of their remedial potential (Lewcock et al., 2020). Recent research suggests that the term "microglial activation" is overly broad, encompassing diverse microglial states that may have either beneficial or detrimental effects on the disease (Lewcock et al., 2020). Focusing on the emerging biology in this field, the following sections concentrate on potential therapeutic approaches aimed at inhibiting or promoting specific microglial functions (Lewcock et al., 2020). Scientists have devised strategies for microglial depletion, where remaining microglia, brain-resident precursor cells, or infiltrating cells such as monocytes are repopulated after a period of microglial depletion (Lewcock et al., 2020). This approach was developed under the hypothesis that microglial activation might negatively impact disease progression (Lewcock et al., 2020). Blocking CSF1R, a crucial microglial cell surface receptor for signaling in the brain, has demonstrated therapeutic benefits in mouse models of AD (Lewcock et al., 2020). CSF1R inhibitors reduced overall neuroinflammation and neural plaque formation, preserved dendritic spine loss, and improved cognitive function (Lewcock et al., 2020). In a related study, mice received an alternative CSF1R inhibitor, which prevented synaptic degeneration in the APP/PS1 mouse model by inhibiting microglial proliferation rather than eliminating microglia (Lewcock et al., 2020). However, several studies have failed to show a reduction in amyloid pathology, consistent with previous results from

models involving the crossbreeding of a CD11b-HSVTK mouse model with two different AD mouse models (Lewcock et al., 2020).

Interestingly, the impact of microglial depletion appears to vary depending on the disease stage, suggesting that microglial depletion may be effective at different phases of the disease (Lewcock et al., 2020). In two studies, ApoE burden in amyloid plaques decreased with microglial depletion, providing further evidence for the potential therapeutic benefits of microglial ablation in mouse models of AD (Lewcock et al., 2020). Despite these findings, questions persist regarding whether microglial depletion and subsequent repopulation represent a promising strategy for Alzheimer's disease treatment (Lewcock et al., 2020). In this regard, it is also noteworthy that patients with homozygous CSF1R mutations suffer from severe brain and bone pathology due to the absence of microglia (Lewcock et al., 2020). Depletion of particular subpopulations of microglia could reveal their function and impact on the central nervous system in the future (Lewcock et al., 2020).

Chapter 5

Discussion for preventing Alzheimer's Disease

Every year, noncommunicable diseases claim the lives of approximately 5 million people around the world who are inactive in their daily life (De la Rosa et al., 2020). The understanding of the precise mechanisms and pathways involved in Alzheimer's disease remains incomplete, leading to various ineffective therapeutic strategies (Athar et al., 2021). Current symptomatic treatments aiming to slow the progression of Alzheimer's disease focus on three main approaches: a) cholinesterase inhibitors, b) N-methyl-D-aspartate (NMDA) receptor antagonists, and c) combination therapy (Athar et al., 2021). In normal circumstances, the NMDA receptor allows the entry of calcium ions for effective neurotransmission (Athar et al., 2021). The primary factor in Alzheimer's disease is the deficiency of the neurotransmitter acetylcholine (ACh) (Athar et al., 2021). Cholinesterase inhibitors such as rivastigmine, donepezil, tacrine, and galantamine work to elevate ACh levels by inhibiting its breakdown, preventing a decline in ACh concentration in the brain (Athar et al., 2021). However, in Alzheimer's disease, the receptor becomes excessively active, leading to excessive Ca^{2+} influx, excitotoxicity, and cell death (Athar et al., 2021). Memantine, an anti-Alzheimer's drug, acts as a non-competitive antagonist by binding to the open state of the NMDA receptor, regulating its hyperexcitability (Athar et al., 2021). Combination therapy involves administering a combination of donepezil and memantine (at doses of 28 mg and 10 mg, respectively, once daily) (Athar et al., 2021). This approach has demonstrated positive outcomes in addressing cognitive, language, and behavioral issues in individuals with moderate to severe Alzheimer's disease (Athar et al., 2021). Results were significantly better than those observed with a placebo or memantine alone (Athar et al., 2021). However, this combination therapy was found to be ineffective in cases with mild to moderate disease (Athar et al., 2021).

Unfortunately, currently approved medications only provide temporary relief from the symptoms of this complex disease (Athar et al., 2021). Consequently, efforts are underway to discover and develop new agents for the treatment of Alzheimer's disease (Athar et al., 2021). An increase in cerebral blood flow (CBF) in a number of cortical and subcortical areas and a decrease in Aβ formation and tau protein phosphorylation are two of these mechanisms (De la Rosa et al., 2020).

Chapter 6

Future Perspectives

The development of neurofibrillary tangles (NFTs) is an important sign of AD pathogenesis (Zetterberg & Burnham, 2019). As a result, it is now essential for treatment therapies to also target tau proteins (Zetterberg & Burnham, 2019). Together with tau immunotherapy, strategies to date have focused on tau deposition and phosphorylation (Zetterberg & Burnham, 2019). Given the well-established and comprehensive methods for standardizing therapy and sample analysis, coupled with the increased sensitivity of devices measuring blood biomarkers, the field of investigating blood biomarkers for Alzheimer's disease has advanced (Zetterberg & Burnham, 2019). Single-analyte assays, which were explored a decade to 15 years ago, seem to have overtaken multiplex assays, which were around five times as prevalent, in recent literature (Zetterberg & Burnham, 2019). The most promising analytes for Alzheimer's disease include the Aβ42/Aβ40 ratio, Tau, and NfL, while promising data on novel panels may also indicate systemic responses to Alzheimer's disease-related conditions (Athar et al., 2021). Current strategies aim to address tau deposition and phosphorylation, as well as explore the potential of tau immunotherapy (Athar et al., 2021).

Chapter 7

Conclusion

Millions of people around the world are affected by AD, a complicated neurodegenerative disease (Athar et al., 2021). Various biochemical and pharmacological examinations directed in the recent many years have attempted to loosen up the intricacies of this supportive of agressive sickness (Athar et al., 2021). Researchers have identified and developed new therapeutic strategies for the treatment of Alzheimer's disease, leveraging an enhanced understanding of the molecular basis of the condition (Athar et al., 2021). Between 2010 and 2020, several medications designed to target specific molecules associated with Alzheimer's disease were developed, with many currently in clinical trials (Athar et al., 2021). However, data indicates that the majority of drugs that reached phase 2 did not demonstrate a significant difference from placebos and thus did not advance to phase 3 (Athar et al., 2021). Only one drug has successfully completed a phase 3 clinical trial since 2003, while more than fifty molecules have progressed to the subsequent phase (Athar et al., 2021).

Tacrine, the first medicine approved to treat Alzheimer's complaint(announcement), was no longer effective due to its hepatotoxicity, which damaged the liver. (Mukhopadhyay & Banerjee, 2021). The challenges faced by these agents, characterized by toxicity and ineffectiveness, led to the discontinuation of many trials as they failed to meet primary and secondary endpoints (Mukhopadhyay & Banerjee, 2021). Nevertheless, this situation paved the way for the development of acetylcholinesterase inhibitors, currently the only class of drugs approved by the FDA for Alzheimer's disease treatment (Mukhopadhyay & Banerjee, 2021). There is an argument that denying approval for drugs like Aducanumab, despite significant progress in understanding the amyloid β pathway, could have hindered future research and development of drugs in a similar direction (Mukhopadhyay &

Banerjee, 2021). However, it is unknown how many of these will advance to clinical practice (Athar et al., 2021)

In light of these developments, it is suggested that new therapeutic approaches in the design, discovery, and development of anti-Alzheimer's agents should focus on several key strategies: (i) Repurposing existing anti-Alzheimer's drugs. (ii) Developing multi-targeting ligands that can act on multiple crucial molecular targets in the pathogenesis of Alzheimer's disease. (iii) Reducing the toxicity of drugs that failed in Phase 3. (iv) Improving the pharmacokinetic profile of promising candidates through structural modifications. (v) Exploring natural products to identify potential Alzheimer's disease treatments. (vi) Investigating stem cell therapy. (vii) Identifying new molecular targets, including the development of immunotherapy.

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