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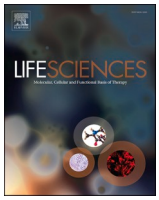

Review article

A review on advances of treatment modalities for Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease which is mainly characterized by progressive impairment in cognition, emotion, language and memory in older population. Considering the impact of AD, formulations of pharmaceutical drugs and cholinesterase inhibitors have been widely propagated, receiving endorsement by FDA as a form of AD treatment. However, these medications were gradually discovered to be ineffective in removing the root of AD pathogenesis but merely targeting the symptoms so as to improve a patient's cognitive outcome. Hence, a search for better disease-modifying alternatives is put into motion. Having a clear understanding of the neuroprotective mechanisms and diverse properties undertaken by specific genes, antibodies and nanoparticles is central towards designing novel therapeutic agents. In this review, we provide a brief introduction on the background of Alzheimer's disease, the biology of blood-brain barrier, along with the potentials and drawbacks associated with current therapeutic treatment avenues pertaining to gene therapy, immunotherapy and nanotherapy for better diagnosis and management of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is acknowledged as the leading cause of dementia, and its prevalence are found to increase substantially among the elder population [1]. The incidence of AD was expected to double every five years after the passing of 65 years, with each year an estimation of 1275 affected individuals per 100,000 people who are above age 65, giving rise to 30–50% of AD patients by the age of 86 [2]. The most recent data from the 2010 U.S. Census and the Chicago Health and Aging Project (CHAP) had reported approximately 5.8 millions of Americans who age 65 and above are currently battling AD [3]. However, this figure is expected to escalate as an estimated 88 millions of individuals will be over age 65 by year 2050 [4]. Statistical figures projected an annual financial of 236 billion dollars being used for AD treatment in the United States alone, signifying that AD itself also poses a tremendous economic burden to the country [5]. United Conventional treatment, which implements cholinesterase inhibitors (ChEIs) have long been considered as the first-line choice for targeting of mild to moderate AD [6]. Sadly, incidence of reports implying the marked increase of hepatotoxicity and gastrointestinal-related outcomes such as diarrhea, nausea and vomiting associated with the consumption of galantamine (Reminyl®), rivastigmine (Exelon®), donepezil (Aricept®) and memantine (Ebixa®) among patients warned the adverse compromising of drug efficacy by dosage rate [7–9]. Additionally, diverse and contradicting hypotheses have made it harder to pinpoint the exact mechanism and physiological functioning of the disease itself alongside the hurdle in drug transportation across the impenetrable blood-brain barrier (BBB) [10] On top of that, current treatments are also reaching bottlenecks due to inability to target a large area of neuronal and synaptic death presented in an AD brain. To address the aforementioned issues, several novel treatment avenues integrating nanomaterials, antibodies, stem cell transplantation and nucleic acids insertion have paved the way towards improvement in disease modification of AD. The approach of combining these fields may very well offer significant insights for advancement of clinical diagnosis and therapeutic strategies related to central nervous system (CNS) disorders later on. In this review, we highlight the recent status and major developments in the field of nanotechnology, immunotherapy, stem cell therapy, gene therapy, with the aim of depicting the versatility of these treatments against AD.

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2. AD background

AD is a neurodegenerative disease characterized clinically by progressive cognitive decline, memory impairment, emotional disturbances, aphasia, language dysfunction and an inability to perform daily life tasks as usual. In the present, it is commonly classified into two categories: early onset AD (EOAD) and late onset AD (LOAD), with the former mostly contributing to our current understanding of AD pathology. EOAD cases are known to be both familial and sporadic with an early onset before 65 years of age, and merely takes up to <5% of the entire AD population. In the context of exploring the genetic and molecular biology behind EOAD emergence, autosomal dominant genetic defects are highly suggestive to be the determining factor, presumably by pathogenic variants occurring in amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) genes located at chromosome 21, 14 and 1 respectively [11–13]. This phenomenon is specifically observed in families with a lineage of EOAD that spanned across multiple generations [14]. *APP*, *PSEN1* and *PSEN2* mutations are strongly associated with abnormal processing of the APP, resulting in genetic heritability of 92–100% [15]. On the other hand, the occurrence of LOAD is more predominant as seen when it corresponds to approximately 90–95% of the total AD cases out there. Clinical symptoms of LOAD usually arise when the patients reach a more advanced age, typically after the age of 65 [16].

Histopathologically, the prominent hallmark of AD can be categorized into two common types: (i) extensive brain atrophy amyloid plaques composed amyloid beta (Aβ) deposition; and (ii) neurofibrillary tangles (NFTs) consisting of hyperphosphorylated microtubule associated tau protein [17]. The trigger for Aβ production is long postulated to be APP, a transmembrane protein highly expressed within the brain neurons [18–20]. Sequential proteolytic processing of APP with the participation of multiple enzymes generates several isoforms of Aβ, the most common ones being $A\beta_{40}$ and $A\beta_{42}$ species, along with other minor forms such as $A\beta_{15}$, $A\beta_{16}$, $A\beta_{17}$, $A\beta_{34}$, $A\beta_{37}$ and $A\beta_{39}$ [21,22]. Importantly, $A\beta_{40}$ and $A\beta_{42}$ are implicated in AD development, with the latter having a higher tendency to form neurotoxic assemblies compared to the former [23]. Under normal conditions, AβPP will be cleaved by α-secretase to produce soluble amyloid precursor protein α (sAPPα) along with an α-carboxyl terminal fragment (αCTF) that lacks the amino-terminal region typical of Aβ domain. Subsequent cleavage by γ-secretase will then give rise to APP intracellular domain (AICD) and p3 peptide, a truncated form of Aβ [24–26]. However, cleavage performed by β-secretase instead results in soluble amyloid precursor protein β (sAPPβ) and β-carboxyl terminal fragment (βCTF). On the consecutive cleavage by γ-secretase, neurotoxic Aβ peptides will be released into the extracellular cerebrospinal fluid and plasma [27,28]. Substantial number of epidemiological studies has stressed the neuropathological consequences of Aβ peptides aggregation into amyloid plaques and NFT as shown in the loss of dendritic spines and synapses which eventually bring about massive neuronal death [29–31]. Moreover, excessive oxidative stress is also observed as the levels of reactive oxygen species (ROS) increase dramatically, in response to the abnormal energy metabolism incurred by imbalance in mitochondrial function [32,33]. The presence of Aβ also drives the migration of inflammatory cytokines and microglia to the affected sites to activate phagocytosis events [34,35]. Sustained activation of immune response indicates greater microglia accumulation, inducing a positive feedback loop which further exacerbates neuroinflammation and AD pathology [36]. All in all, the resultant neurodegeneration contributes to tremendous loss in brain volume and cortical thinning in specific brain regions, instigating cognitive and language impairment [37,38].

3. Early detection of AD

Early detection is vital in most disease especially in AD, as study showed that early intervention has better prognosis [39]. Diagnosis can be complicated due to early symptoms that can only be reported by patients and guardians as AD is not easily detectable and routinely tested in clinical practice. A guideline led by the National Institutes of Health and the Alzheimer's Association states that AD can be divided into three stages, preclinical where there are evidence of amyloid build-up or nerve cell changes but no significant clinical symptoms, Mild cognitive impairment where symptoms of memory loss and other reasoning problems are greater than normal for patient age and education but do not interfere with their independence, and the final stage which is the confirmed Alzheimer's dementia [40]. Diagnostic test is critical as it is able to determine the severity level of AD patients besides acting as a point of indicator to track symptom progression in the future.

If an individual is suspected of AD, health practitioner will usually follow a uniformed guideline to confirm the diagnosis. The first step is to conduct a neuropsychological testing to determine specific type and level of cognitive impairment patient exhibit. Some test will also reveal patient strengths and preserved abilities. This information is vital as it helps to establish a treatment plan in the future. Administer tests were developed through research studying short and long-term memory, attention, concentration, reasoning, and ability to solve problems besides learning new information. The types of neuropsychological assessments used in determining AD include Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog), Behavioral Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD), Cambridge Neuropsychological Test Automated Battery (CANTAB) and Dementia Rating Scale - 2 (DRS-2). Test administered will focus on patient's attention, behavioral symptoms, language, executive functioning and memory skills [41]. A standard blood and urine test will also be carried out to eliminate other potential memory loss or confusion causes that is associated with other medical condition such as thyroid disorder, vitamin B-12 deficiencies or depression. It is commonly understood that the main cause of AD is due to rapid progression of neuron degeneration, hence the best way to monitor is through brain imaging measures. Health practitioners usually recommend performing Magnetic resonance imaging (MRI) for detailed view of the brain, Computerized tomography (CT) for a cross-sectional view of the brain and Positron emission tomography (PET) which will visualize pattern of metabolism changes indicating cell degeneration. In MRI, a sensitive marker of AD which is the atrophy of medial temporal lobe can be observe and may indicate disease progression [42]. There are three types of PET imaging, however, only Fluorodeoxyglucose PET scans which illustrates poorly metabolized glucose regions are routinely used in clinical practice. On the other hand, Amyloid PET and Tau PET Imaging are primarily applied in research so as to measure the burden of amyloid deposits and neurofibrillary tangles respectively [43]. Though genetic testing is also available, it is not routinely recommended for AD evaluation, with the exception of high risk patients known to have a strong family history of EOAD. Additionally, patients are also referred to a genetic counsellor to discuss their options prior to undergoing any kind of genetic test as the final results may very well be life changing. Since there is no effective therapeutic study for AD, it is important to identify the risk factors of AD especially in their preclinical and prodromal stages of the disease with the aim of modelling disease trajectory besides developing novel targets for early disease intervention. Study has shown that before the onset of clinical symptoms, there is a predictable pattern of disease process that can be observed. Early intervention may disrupt this cascade and may be the key in stopping Alzheimer progression.

4. Structure and functions of BBB

The BBB is a complex multicellular structure made up of closely-packed endothelial cells sheathing the cerebral capillaries region, serving as a form of restrictive barrier regulating the movement of ions, proteins and leukocytes into the brain and at the same time exporting pathogens, neurotoxins and inflammatory agents out into the bloodstream [44,45]. The very presence of tight junctions connecting every

endothelial cell equally cast a physical barrier that limits paracellular diffusion of ions and molecules across the cells [46]. Partnered with astrocytes, pericytes and microglia in the CNS, BBB works to reinforce the physical, chemical and functional membrane integrity so as to provide a specific microenvironment for optimal functioning of the brain [47–49]. In an attempt to reduce permeability of substances across the BBB, a wide array of specific enzymes and transporters are put into motion. In general, there exist five types of transport mechanism being involved: (i) receptor-mediated transcytosis (RMT); (ii) adsorptive-mediated transcytosis (AMT); (iii) diffusion; (iv) carrier-mediated transcytosis (CMT); and (v) paracellular diffusion (Fig. 1).

RMT represents a selective permeable vesicular trafficking system that drives influx such as insulin and transferrin molecules *via* specific ligand-receptor recognition [50,51]. Ligands initially bind to the specific surface receptor at the apical plasma membrane of the endothelial cells to initiate invagination process. As a result, intracellular transport vesicles are formed allowing engulfed ligand-receptor complexes to be shuffled towards the opposite basolateral region for release [52]. On the other hand, AMT is dependent upon non-specific interactions which principally trigger electrostatic interaction between polycationic compounds and negatively charged anionic microdomains located on the capillary endothelial membrane [53,54]. For transport of small lipophilic molecules with a molecular weight below 400 Da, diffusion is usually employed in which said substances can easily diffuse across the endothelial cells down the concentration gradient. However, this particular transport machinery is not applicable for large polar molecules. CMT addresses the issue by mediating transport of certain molecules such as glucose and amino acids with the assistance of protein carriers or transporters. Normally, molecules tend to bind to protein carriers that they share the same specificity and affinity, activating conformational change of protein which ultimately discharges the target ligands across the cells [50]. Paracellular diffusion, is instead defined as intercellular transfer of substances between two distinct endothelial or epithelial cell compartments regulated by tight junctions [55]. These features have previously imposed a great strain on CNS drug delivery therapy due to the fact that majority of the drugs are presumed to be hydrophilic and contain high molecular size [56]. Despite these shortcomings, researchers had somehow managed to engineer therapies involving gene, antibodies and nanoparticles in a way that they benefited from the aforementioned characteristics of BBB, evolving into effective targeted drug delivery agents for treatment against CNS-related disorders.

And now, the most intriguing question remains: what sort of mechanistic approaches were employed in the cause of circumventing the formidable barrier for targeted gene or drug delivery? Though not much scientific evidence was published in that particular subject, nevertheless, several reviews have outlined few potential routes commonly undertaken by therapeutics to successfully infiltrate BBB while maintaining barrier integrity at the same time. Interestingly, scientists have made use of the principles behind the transport pathways for delivery of biotherapeutic agents. Over the years, RMT and AMT have been extensively incorporated into the genetically engineered ligands for delivery of biotherapeutic agents into specific brain regions. In particular, immunoglobulin γ (IgG) is a genetically engineered monoclonal antibody (mAb) that works by partaking on the role of molecular Trojan horses (MTH) [57,58]. Under the pretext of binding to the endogenous receptors located on the surface of BBB, IgG can easily internalize the brain endothelial-like cells and shuttle the encapsulated fusion proteins to reach brain parenchyma [59]. Antibody transcytosis is reported to be strongly affected by receptor binding properties such as affinity, epitope and valency. For instance, high affinity binding between transferrin receptor (TfR) and TfR antibodies significantly facilitated trafficking of TfR or drug conjugates into the lysosomal compartment [60]. In the same year, dramatic increase in antibody transcytosis was detected in antibodies with reduced binding affinity against TfR at low endosomal pH, marking the involvement of pH-dependency receptor binding at play [61]. Recently, the type of epitope bin was also proven to impact the overall basigin-receptor-mediated uptake capability of basigin mAb into the brain [62]. Importantly, the same transcytosis pathways are also being taken advantage by nanoparticles so as to transiently penetrate the BBB and ferry therapeutic genes or antibodies alike [63]. Aside from relying on the two physiological mechanisms, studies show that nanoparticles entry can be achieved through implementing ultrasound/microbubbles for disruption of the intracellular tight junctions that exist between the endothelial cells of BBB [64]. Additionally, other factors such as the size, shape and charge of nanoparticles are similarly known to be potential determinants that grant successful BBB crossing [65–67].

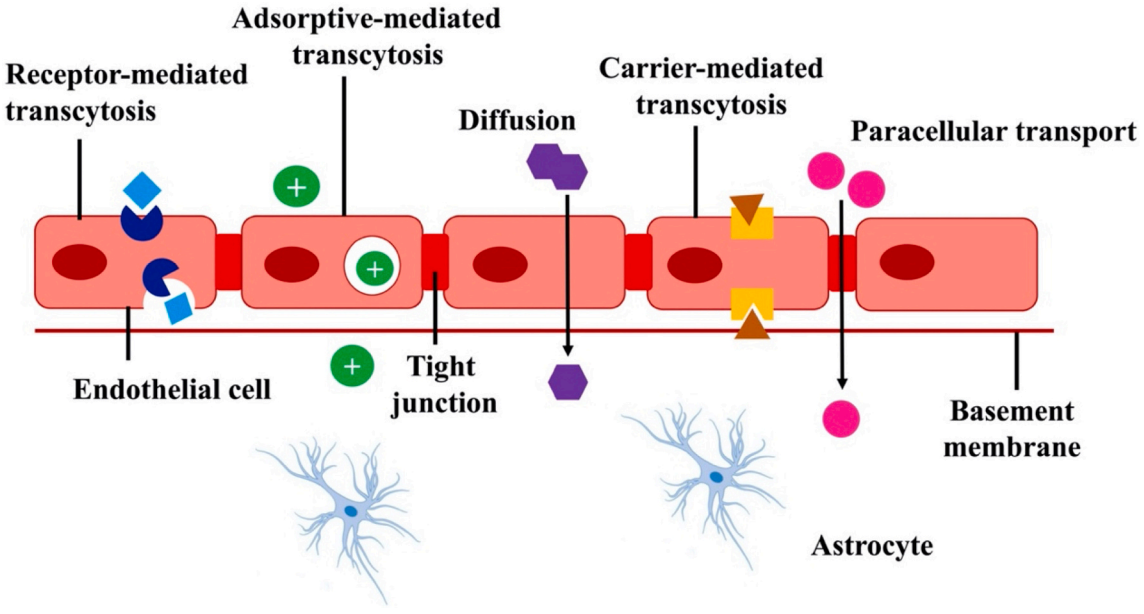

Fig. 1. Schematic representation of the blood-brain barrier and the major transport pathways across the BBB *via* receptor-mediated transcytosis, adsorptive-mediated transcytosis, diffusion, carrier-mediated transcytosis and paracellular transport.

5. Modifiable risk factor

Although we have a deeper understanding of the underlaying mechanism for AD, there is still a high number of sporadic cases with unknown origin. Currently, numerous novel therapeutics targets are being researched and we are hopeful with its initial results and future outcomes but nothing conclusive have been applied clinically. Dementia and AD are multifactorial and heterogenous disorders affected by genetic and environmental factors, many of which are potentially modifiable. Hence it is important to identify these risk factors so preventive measures can be taken as risk reduction is vital at population level to effectively reduce AD prevalence. Two types of studies are mainly conducted to identify modifiable risk for AD, (a) observational prospective studies which will elucidate temporal relationship of potential causal links in large widespread community samples and (b) randomized controlled trials which observe the effect of specific interventions on AD incidence. From the study, modifiable risk factors identified are divided into two categories, treatable medical conditions and lifestyle interventions. Most of the risk factors are connected and interdependent to each other.

In treatable medical conditions, disease such as vascular diseases, Type 2 Diabetes, traumatic brain injury, epilepsy and depression had been studied and literature illustrates a possible causal link. For vascular disease, its mechanism are still not fully understood but neurodegeneration (*via* amyloid deposition) or cerebral alteration (cerebral perfusion) are heavily associated with lowered cognitive performance leading from mild cognitive impairment to eventual AD. Specifically, cardiovascular disease such as hypertension, atrial fibrillation, atherosclerosis induce increased level of Aβ due to lack of brain blood perfusion/oxygenation while cerebral amyloid angiopathy, a condition where Aβ accumulates causes hemorrhages, ischemic lesions and encephalopathies contributes to neurodegeneration [68,69]. Identification of these vascular biomarkers early and disease prevention may contribute to lowering the risk of AD.

For Type 2 Diabetes (T2D) they share a strong epidemiological link to AD (possible physiopathological link) with >65% risk of dementia development compared to non-diabetic patients [70]. Most common theory is due to diabetic vascular disease leading to lowered cerebral circulation and eventual AD manifestation. There are also reports of chronic hyperglycemia, prolonged hypoglycemic episodes and altered amyloid metabolism which may contribute to AD [71]. Recent studies also identified a possible link to hyperinsulinemia due to insulin neurotrophic properties. Insulin-like growth factor (IGF) is a good candidate since its circulating level reduced with age and cognitive decline are observed [72]. There are many types of IGF but no specific study has been conducted to verify this causable link. No mechanistic studies have been done and the cause may be multifactorial. Future studies may also be conducted to confirm weather controlled T2D can control AD.

Traumatic brain injury is a condition where sudden damage occurs to the brain region resulting in mild concussion to severe permanent damage. Majority of cases has been linked to AD, with 24% increased risk seen in individuals with history of traumatic brain injury [73]. This is infer to be due to similarity of molecular mechanism observed in traumatic brain injury and AD such as neurodegeneration process that disrupt memory development, increased Aβ plaques accumulation following brain injury, increased $A\beta_{42}$ in brain and CSF, increased Tau level and also alterations of major protein (kinases, phosphatases, APP, BACE1) [69]. Epilepsy has also been linked to AD as it usually results in cognitive and neuropathological changes and in severe cases, brain atrophy. Multiple studies observed the increased risk for AD in epilepsy patients and is theorized that a parallel link between onset age of seizure correlates with severity of AD [74]. Epilepsy is linked due to presence of increased Aβ, hyperexcitability, elevated Tau levels that can cause neurons deterioration. Depression is also another modifiable risk factor for AD, but there is disagreement in its status since it was known as a symptom of AD. However, a recent study reports that patients with major depression showed an increase of Aβ deposition, enhanced formation of amyloid plaques and elevated cortisol level similar to AD patients. Currently, there is no study done to prove that managing depression *via* antidepressants can be a preventive therapy for AD [69].

Another category of modifiable AD risk is lifestyle interventions which focuses on the physical activity, diet, smoking and alcohol consumption. The causal link between lifestyle interventions and AD are not yet fully understood but generally it involves neurotoxic, inflammatory, vascular, oxidative stress, and psychosocial processes. Physical activity are recommended as it helps to lower stress, metabolic and vascular risk, aide in amyloid clearance, increase brain volume and neurotrophic factor. It also helps to reduce AD symptoms and slows disease progression [69]. Dietary factors has also been linked in maintaining vascular health, lowering inflammation, relief oxidative stress, upregulate neurotrophic factor and also aids in neuronal membrane maintenance. Evidence suggest Mediterranean diet may help reduce risk of dementia or AD [75,76]. Smoking and alcohol consumption have indirect effects on inflammatory, vascular and accelerate neurodegeneration process [77]. Newer findings published has identify the indirect cause of AD (i.e. environmental factors) and since most of the risk factors are modifiable, we should consider a multi-domain life-course intervention strategies to ensure prevention of dementia and AD in early stages before any clinical symptoms are expressed. Alternative strategy that combines both AD mechanism targeted therapy and modifiable risk such as proper disease management for comorbidities and also lifestyle modification should be carry out. More studies in utilizing the potential of these risk factors in disease prevention should be done.

6. Contemporary therapeutic approaches

Before moving in depth into the intricate details of novel therapeutic strategies for AD management, it is good to have a brief picture of the contemporary treatments. Design of several pharmaceutical drugs was long put into motion to combat the deteriorating effects imposed by AD on an individual's memory and cognitive functioning. These drugs were first implemented on the basis of cholinergic and glutamatergic hypothesis. Cholinergic hypothesis is the earliest theory proposed to explain the etiology and mechanisms behind neuropsychiatry symptoms associated to AD, which presumes the loss of acetylcholine (ACh) neurons and enzymatic functions in amygdala, basal forebrain, cortex and hippocampal regions in CNS as determining factors [78,79]. Decline of a7 or a4b2 nicotinic acetylcholine receptors (nAChRs) and M2 muscarinic acetylcholine receptors (mAChRs) in pre- and post-synaptic neurons is also stated to be responsible for AD progression [80,81]. Galantamine, rivastigmine and donepezil are among the ChEIs licensed as standard drug treatment to inhibit the hydrolytic action of acetylcholinesterase (AChE) on acetylcholine (ACh) and augment cholinergic system [82]. This ensures ACh to successfully bind to nAChRs and mAChRs which helps in sustaining ACh level followed by neurotransmission regulation. Memantine, on the other hand, targets *N*-methyl-D-aspartate (NMDA) glutamate receptor to prevent influx of intracellular calcium (Ca^{2+}) and subsequent excitotoxicity which causes neuron degeneration in AD [83]. The efficacy of ChEIs and memantine in enhancing cholinergic functioning have been repeatedly explored in various double-blind, randomized controlled trials and reported on systemic reviews [84,85]. It was shown that AD patients after consumption of ChEIs and memantine make progress in cognitive functioning as indicated by the global assessment tools.

6.1. Galantamine

Galantamine is a competitive and reversible inhibitor that potentially binds to the allosteric site of nAChRs [86,87]. More than one systemic review has postulated the clinical relevance of galantamine against mild-to-moderate AD. As compared to the placebo group, AD patients who were administrated with a dosage of 24 mg and 32 mg

galantamine per day exhibited increased cognitive performance, as indicated in the achieved mean 2.9 points and 3.1 points respectively [88]. Similar data has been replicated in another corresponding study done in United States [89]. Post evaluation of patients' cognitive capacities after 24 mg/day galantamine demonstrated promising and consistent results throughout 6 to 12-month period. Patients subjected to the same flexible dose escalation of galantamine (24–32 mg/day) over 4 weeks, also reported higher proficiency in managing basic daily activities on the basic and instrumental level [90]. Nonetheless, adverse health issues which are predominantly nausea and gastrointestinal are still present in a small proportion of patients, prompting them to relinquish the optimum therapeutic benefits and drop out from the treatment process instead.

6.2. Rivastigmine

Rivastigmine, acts as a type of slow-reversible carbamate inhibitor against both AChE and butyrylcholinesterase (BuChE) that are primarily pronounced in the cerebrospinal fluid of AD brain [91–93]. A 26-week study examined the clinical effects exerted by fixed doses of rivastigmine (3, 6, 9 mg/day) on the brain metabolic activity of 27 mild-to-moderate probable AD individuals [94]. According to the screening results obtained using positron emission tomography using ^{18}F-fluorodeoxyglucose (FDG-PET), there was a statistically significant improvement ($p < 0.01$) in rivastigmine-treated patients, as shown in the increased hippocampal metabolism (32.5%) compared to the non-significant fall in non-responders (6.4%) and placebo patients (4.1%). In their systemic review, Birks and Evans (2015) included a total of 7 unconfounded, multicenter, 24-week double-blinded randomized clinical trials [95]. Recruited subjects were all elderly with a mean age of 75 years and diagnosed with mild-to-moderate AD. Following 26 weeks of rivastigmine treatment, higher overall cognitive assessment scores were observed in patients subjected to an oral administration of rivastigmine (6–12 mg/day) or transdermal patch formulations (9.5 mg/day) compared to those placed in placebo group. Interestingly, some of the trials reported no changes in terms of behavioral or career aspect. Although rivastigmine produced satisfactory cognitive outcomes, patients on rivastigmine reported twice as likely to develop certain side effects such as nausea, dizziness and vomiting, with those on capsules having a slightly higher risk in contrasts to those that go for skin patches.

6.3. Donepezil

Donepezil, also known as donepezil hydrochloride, is a non-competitive and reversible inhibitor of AChE long approved for symptomatic treatment against mild-to-moderate AD back in year 1996 [96]. One Japan study evaluated the effectiveness of 24-week treatment with donepezil (5 mg/day) in 268 patients suffering from mild-to-moderate AD [97]. Significant increase in the total scores of both primary and secondary measures was shown by donepezil-treated patients, suggesting the apparent cognitive improvement presented by this drug. In another 6-month study, 208 patients having probable AD or AD with cerebrovascular disease were selected from 27 nursing homes across the United States to undergo donepezil treatment with the aim of determining the clinical efficacy and safety profile of the drug [98]. Preliminary data indicated donepezil responders experienced stable or improved cognition, as demonstrated by the mean change from baseline Clinical Dementia Rating (Nursing Home Version) – Sum of the Boxes (CDR-SB) and Mini-Mental State Examination (MMSE) at certain time period. Moreover, the study also provided additional merits in such that donepezil usage is noted to be independent of factors including age, comorbid illnesses and high concomitant medication. The benefits of donepezil on treating marked neuropsychiatric symptoms of AD were also being targeted in an open-label, prospective study [99]. Only patients who did not manifest any adverse events or cognitive deterioration were allowed to proceed to another 6-week donepezil treatment at a higher dose (10 mg/day). Continuous donepezil administration revealed a significant decrease in NPI and NPI-Distress scores, as compared to the initial 6-week assessment and placebo group. As a result, further investigations on donepezil are strongly encouraged as this drug exerts good efficacy and tolerability in mild-to-moderate AD patients.

6.4. Memantine

Compared to other ChEIs, memantine received marketing approval from Food and Drug Administration (FDA) in 2003 to mainly target moderate-to-severe AD. Memantine is classified as an uncompetitive NMDA receptor antagonist which elicits antagonistic effects through impeding the activation of NMDA ion channels in a preferential manner, a feat that can be achieved by controlling the dosage intake [100,101]. By reducing the permeability of NMDA ion channels to Ca^{2+}, the release of apoptotic-inducing factors is potentially disrupted resulting in the mediation of normal neuronal activity [102]. Surprisingly, clinical trials using memantine to treat moderate to severe AD failed to meet up to the expectations as the tested drug merely delayed progression of cognitive deterioration [103]. Most research approaches focused on comparing the therapeutic response exhibited by memantine in terms of monotherapy and combination therapy which included other ChEIs. For instance, individual treatment with memantine alone reported clinical benefits represented by the scores obtained from cognitive, behavioral, disturbances, activities of daily living and global function assessments [104]. Another double-blind, 24-week, multinational study similarly indicated the extended administration of memantine (20 mg/day) to induce high efficacy and tolerance profile in moderate-to-severe AD patients [105]. Furthermore, there are also reports concluding combinatorial treatment involving memantine and other ChEIs to potentially slow down or prevent AD occurrence [106,107]. Masitinib, a tyrosine kinase inhibitor administered as add-on treatment into mild-to-moderate AD patients having received memantine and/or ChEIs therapy 6 months prior arrived at the same conclusion [108]. Memantine was reported to associate with amelioration of cognitive deficit in AD and acceptable tolerability. Overall, treatment with memantine projected a hopeful future for moderate-to-severe AD, however much investigations with a larger sample size are encouraged to validate the effectiveness and safety consumption of this drug.

7. Treatment avenues

7.1. Gene therapy

Gene therapy is one of the strategies that is discussed extensively in literature. General treatment involved the introduction of gene that expresses therapeutic enzyme or growth factor. Its main goal is to achieve long-term expression of desired genes with sufficient levels for therapeutic proposes. Treatment differs based on target disease (genetic disease *vs* complex acquired disorder), mode of gene delivery (integrating *vs* nonintegrating) and *in vivo vs ex vivo*. Mainly, it can be achieved *via* gene augmentation, gene suppression and also genome editing [109]. Generally, there are two type of vectors used in gene therapy, either RNA or DNA viral vectors. RNA-based viral vectors are usually derived from retroviruses such as murine leukemia virus (MLV) or lentiviruses such as human immunodeficiency virus (HIV). For DNA vectors, adenoviruses and adeno-associated viruses (AAV) are the most common. Each vector has its own limitations and its used are interchanged depending on the nature of the study and target cell. *Ex vivo* gene therapy is defined as genetically engineered cultured cells that are transformed outside the body before it is introduced back into the host system. *In vivo* gene therapy is introducing the genetically modified vector efficiently into the host system bypassing the BBB. This usually will lead to system integration and depending on the nature of disease may be the ideal or undesired consequences. In neurodegenerative disease specifically, alteration or induction of specific proteins involved in

its pathological pathway induce neuroprotection, neuro-restoration and may essentially correct the underlying pathogenic mechanism. However, gene therapy is a complicated process involving variable factors such as the temporal specificity, spatial specificity, gene regulation and the most common problem faced in correcting neurodegenerative disease, gene delivery mechanism.

7.1.1. NGF

Nerve Growth Factor (NGF) is a family of neurotrophic factors highly involved in the development of peripheral nervous system with high levels observed in hippocampus and cerebral cortex. Imbalance of NGF has been proven to be involved in damage of cholinergic neurons and AD pathology. In AD pathology, the inefficient maturation of NGF cause the declining cholinergic function making it the ideal target for AD treatments [110]. Treatment mainly focuses on maintaining NGF level to support healthy cholinergic neurons growth and increasing activity level to complement the reduced level of Ach [111]. Phase 1 clinical trial using NGF *via ex-vivo* was completed in 2009 (Table 1) and result shows improvement in glucose metabolism, cholinergic growth, cortical nicotine receptor and cognition improvement in patients [112].

The local delivery of NGF managed to overcome the BBB problem with no reported adverse effect. This study is one of the first clinical trial done using NGF and its positive outcome provides a benchwork for subsequent study. In 2012, Eriksdotter-Jonhagen group used encapsulated cell technology which is a catheter like implant for *ex-vivo* gene therapy that secretes NGF secreting cells. This method is proposed due to the limitations and safety concerns of genetic modifications. No adverse effect was observed, and it provides an alternative to direct genetic integration with increased control as treatment can be ceased with simple removal of device [113]. For *in vivo* therapy, a phase 2 clinical study was conducted using stereotactic delivery of AAV2-NGF for long term persistent expression and bioactivity of NGF. Although there was no reported adverse effect, there are no significant clinical outcomes on selected AD biomarkers [114]. Currently, there are no active clinical trial using NGF but in the future we hope to see more *in vitro* studies enter the clinical trial phases.

7.1.2. BDNF

Brain Derived Neurotrophic Factor (BDNF) is highly involved in the nervous system as it helps to develop, maintain and support the growth of healthy nerve cells. Through multitude of research, it can be observed that it is not only involved in AD pathway, but also implicated in other neurological disease such as Huntington's Disease, depression and schizophrenia [115]. The exact mechanism of BDNF impact is still unclear but one theory suggested that there is correlation between Aβ and BDNF levels in AD pathology [116]. This leads to BDNF becoming an obvious viable therapeutic target for AD. An *in vitro* study by Nagahara test the effect of lentiviral vector for BDNF gene delivery in various animal model [117]. There was positive outcome observed such as synapse loss reversal, cell signaling improvements and improved cognitive learning. No adverse effect was reported and this merits usage of BDNF as potential therapeutic target for AD.

7.1.3. APP, PSEN1 and PSEN2

Several genes have been implicated that increases the risk of AD with rapid progression and in an autosomal dominance Mendelian inheritance pattern. *APP*, *PSEN1* and *PSEN2* genes encode protein involved in APP metabolism and Aβ generation, directly contributing to the pathophysiology of AD. It is a rare mutation affecting approximately 5% of AD cases [56]. Overexpression of APP causes deregulation of several signaling pathway causing cellular and molecular alteration seen in AD [118]. Loss of APP has been reported to cause neurodegeneration *via* increase in neuronal plasticity, reduced synaptic signaling activity and increase neurons susceptibility to cellular stress [119]. There are no active clinical trial targeting APP gene currently, but initial pre-clinical results show positive affirmations. Most of the studies targeted the APP secretase but problem arises as APP is involved in other important pathways and disruption may cause adverse effect. Hence, an alternative method *via* direct overexpression of APP was introduced and positive effect can be observed *via* improvement in cell health and growth as it helps in protecting against stress, including growth factor withdrawal, apoptotic stimuli and excitotoxicity [120]. Another *in vitro* study overexpressed APPsα *via* AAV and introduced it bilaterally in hippocampus [121]. The result of the study is promising as it shows long lasting APPsα expression (>5 months) with no major BBB breakdown and increases microglia recruitment, activation and phagocytic function that may elevate Aβ and plaque clearance.

Another interesting take in targeting *APP* is by disrupting its pathway *via* interfering RNA (siRNA). siRNA interfere with gene expression by activation degradation of mRNA molecules with similar sequence homology as the siRNA, effectively inhibiting translation. It is highly selective and is able to downregulate pathogenic genes without affecting wildtype [122]. BACE1 is a transmembrane aspartic protease, directly involved in the cleavage of APP to initiate the production of neurotoxic Aβ [123]. A study done by introduced lentiviral expression of anti-BACE1 siRNA and the experiment shows positive result with 50% reduction of BACE1 expression, reduced amyloid production and significant reduction in neurodegeneration [124]. With its ability to cross BBB, siRNA is a viable option for APP targeted gene therapy but concern still arise as BACE1 is involved in other pathways especially the axonal pathway as observed in adult knockout mice [123]. Nevertheless, siRNA remains a viable option for gene therapy as initial study showed positive results, but further in-depth study is needed especially in identifying viable gene target that will not disrupt normal brain function for AD treatment.

Another study targets BACE1 *via* CRISPR-Cas9 amphiphilic nanocomplexes to suppress Aβ associated pathway [125]. The CRISPR-Cas9 system is made up of single guide RNA (sgRNA) that directs the Cas9 complex to specific DNA sequence which will initiate a double-stranded cleavage of the bound DNA. The study reports promising result as significant reduction of Aβ plaque, decrease Aβ42 level and increased associative learning are observed. Through extensive research, they also confirm successful and efficient delivery of vector with low off target effect, transient delivery and no adverse effect reported. There is still concern especially in applying the treatment in clinical settings which is a problem to progress the research to the next stage. The treatment targets neural circuit dysfunction but it is not widespread enough making it more suitable for disease such as Parkinson or pre-stages of AD. With time and more extensive research, using CRISPR-Cas9 vector may be an option for treatment of neurological disease.

Although APP mutation alone is sufficient to cause AD, γ-secretase mutation, *PSEN1* and *PSEN2* has also been labelled as missense pathogenic variants but its exact role in AD pathogenic pathway is still unclear. These genes along with nicastrin and anterior pharynx-defective 1

Table 1
Available gene therapy clinical trial for Alzheimer disease.

Vector	Phase	Mechanism	Outcome	Immunosuppression	Current status
MLV transduced fibroblast	1	Growth factor	Safe, biologically effective	No	Completed (NCT00017940)
AAV2-CAG-NGF	1	Growth factor	Safe, biologically effective	No	Completed (NCT00087789)
AAVrh.10hAPOE2	1	Gene target	Pending	Unknown	Completed (NCT03634007)

made up the γ-secretase complex [126]. γ-Secretase enzymatic complex to reduce Aβ aggregation, however both enzymes are involved in cleaving substrates in other pathways, with Notch pathway being the most critical and this explains the toxicity effect seen in most failed clinical trials involving suppression of these genes [127]. Hence, targeted gene therapy for both *PSEN1* and *PSEN2* is a good step in modulating Aβ production but further in-depth study is needed to ensure there is efficient introduction to system without adverse effect.

7.1.4. APOE

One of the most extensively studied gene associated in AD is the Apolipoprotein E (*APOE)*. Patients does not only have an increased risk to develop AD but also for other diseases as APOE functions as a transporter of lipids, mainly cholesterol between cells in the CNS [128]. It has three main isoforms, *APOE2, APOE3* and *APOE4* which carries different risk percentage and differs due to presence of cysteine or arginine at amino acids 112 and 158. The difference in amino acid position is reflected in the structure of the isoforms and influences their ability to bind lipids, receptor and Aβ. The three polymorphic alleles, ε2, ε3 and ε4 have a worldwide prevalence of 8.4%, 77.9% and 13.7% respectively. In AD, *APOE4* carrier reports higher levels of amyloid levels while *APOE2* has a lower amyloid burden. Individual with one ε4 allele increases the risk of LOAD 3–4× while individual with two ε4 allele has 9–15× increased risk. However, it is important to note although they do carry an increased risk, disease penetrance is complete and it does vary in individual. Consensus hypothesis is that *APOE4* reduced processing and clearance of beta-amyloid leading to its accumulation which trigger the beta-amyloid pathway. Transgenic mouse studies suggest AD as a toxic gain of function effect, but some studies considered it to be a loss of function mutation due to its pattern of decreasing protein function from APOE2, APOE3 to APOE4 [129,130]. Although APOE has been established as an important role in developing AD, there are very few therapeutic studies being developed targeting it, mainly due to adverse effects encountered during clinical trial. This can be resolved by utilizing gene therapy as we are able to have a more controlled therapeutic target. The main approach is to increase the expression level of *APOE2* due to its reported protective effect *via* decreasing amyloid burden and reduced synapse loss [131]. Early transgenic model study showed that increased *APOE2* expression counter the effect of *APOE4* when introduced in early pathology development *via* AAV gene delivery [132]. A recent study then tested the best route for vector introduction to the CNS by using African Green monkeys and concluded that intracisternal delivery has no adverse effect to host, wide distribution and the least invasive surgical intervention [133]. A clinical study (NCT03634007) was recently completed to test the safety and maximum tolerated dose of intracisternal delivery of AAVrh.10hAPOE2 in 15 test subjects. As the study has only been recently concluded, there is still no official observation released. Another therapeutic method that has been recently gaining traction is the direct conversion of *APOE4* into *APOE3*. This is mainly because *APOE4* pose a higher age-dependent risk as ~65% are estimated to be affected by age 85 in patients with *APOE-ε4* genotype but only ~10% in patients in *APOE-ε3* genotype [134]. A unique case of patient having both *APOE3* genotype and *PSEN1* mutation showed a lower risk factor confirming the protective nature of *APOE3* [135]. Based on the information, a gene rescue study was done using a structure corrector, PH0002 and the study observed a significantly reduced *APOE4* fragments level making it a viable therapeutic option that should be explored further [136]. Regretfully, no study has tested the viability of the treatment *in vivo* yet. If treatment showed positive outcome, direct conversion to *APOE3* may be the future of AD gene therapy.

7.1.5. Other candidate genes

The four major genes mentioned before are known carrier of increased AD-risk due to its direct involvement in Aβ production and clearance. Recently with the concluded genome-wide associate studies (GWAS), research using whole genome sequencing (WGS), exon sequencing and gene expression network analysis has released massive valuable data that are able to identify many susceptibility loci compared to the more traditional gene linkage study done previously. This is especially useful as it helps illustrates other underlying AD pathway that are less known but just as important such as cholesterol metabolism, immune response and endocytosis. So far, a few notable genes were identified to serve as a viable option for therapeutic strategy (Table 2). Most of these genes are still in early functional study phases, with no *in vivo* or *in vitro* treatment testing. However, it provides a future basis that can be further explored and a possibility as viable gene therapy option.

7.2. Amyloid beta (Aβ) immunotherapy

Extensive attention and scientific analysis have been amassed in the domain of immunotherapy to evaluate its potential anti-Aβ therapeutic effects against neurodegenerative diseases, with emphasis posited in the brains of patients diagnosed with Alzheimer's disease [137]. However, not much clinical success was observed as Aβ immunization have repeatedly failed to produce satisfactory results. Having said this, the challenges and drawbacks received during preclinical and clinical testing led to continuous improvement and emergence of various therapeutic avenues such as second-generation Aβ vaccinations and anti-Aβ monoclonal antibodies [138]. According to experts, most of the clinical trials are forced to be discontinued or withdrawn midway due to several factors including, (i) successive optimization of antibody titers and epitopes, (ii) irrelevant immunotherapeutic procedures, (iii) inappropriate clinical trials design, and (iv) inadequate study models [139]. Active immunization and passive immunization dominated Aβ-directed immunotherapy, with substantial studies advancing the depth of interplay between immune system and immunogens in the hope of formulating novel AD vaccines. Active immunization prioritizes the administration of vaccines designed with relevant antigens to activate the body's own immune cells against foreign antigens. Cellular and humoral immune system are the primary routes utilized by active vaccination, in which B and T cells are necessary for production of Aβ-specific antibodies. Although active immunization is generally considered as cost-saving and openly available for the masses with the body's immunological memory at work, it possesses several disadvantages that downplay its corresponding benefits [140]. Since an active vaccine is always consisted of an antigen, it is entirely possible for multiple antibodies to be produced as a result of being in contact with various different overlapping conformational epitopes present on Aβ protein.

Table 2
Summary of other candidate genes involved in LOAD.

Gene	Location	Function	Mechanism of pathogenesis	Reference
ABCA7	19p13.3	Regulate lipid metabolism and involvement in cell phagocytosis process	Cholesterol metabolism	[231,232]
CLU	8p21.1	Involved in apoptosis, complement regulation, lipid transport, membrane protection, and cell-cell interactions	Cholesterol metabolism	[233,234]
ADAM10	15q21.3	Cell adhesion and proteolytic processing of diverse cell surface receptors ectodomains and signaling molecules	Aβ clearance	[235]
CR1	1q32.2	Involved in complement pathway	Immune response	[234,236]
TREM2	6p21.1	Activate phagocytosis and suppress inflammation	Immune response	[234,237]
SORL1	11q24.1	Vesicle trafficking pathway	Endocytosis	[234]

Such phenomenon often gives rise to a polyclonal antibody response, which is not target-specific and technically viewed as "extra work". On top of that, the body is constantly at risk of mounting a deleterious T cell response that can potentially lead to proinflammatory disorders. In contrary, the aforementioned shortcomings can be easily resolved in the case of passive immunization that primes injection of humanized murine monoclonal antibodies or donor-derived polyclonal antibodies into the patient's body without relying on the activation of body immune system. This allows an individual to put a stop to passive immunotherapy should he or she encounters sudden side effects or detrimental reactions. Furthermore, the delivery of a specific antibody can single out other irrelevant conformations and only focuses on the targeted epitopes. That being said, a significant amount of expenses is necessary for large production of monoclonal antibodies and its weekly or monthly infusions which may cause quite a heavy toll on the economy [141].

With the goal of maintaining high specificity in targeted protein and antibody binding, intrabody/nanobody technologies centering on the use of engineered antibody fragments were widely developed as anti-Aβ passive immunotherapy. Nowadays, intrabodies are preferred over conventional antibodies (cAbs) due to the former exhibiting additional advantages over the latter in terms of structural and functional properties. Intrabodies typically consist of either single-chain variable fragment (scFv) or single-domain antibody (sdAb) referred to as V_H, V_L and V_{HH} nanobodies. Interestingly, scFv intrabodies are made up of fusion between V_H and V_L variable domains connected together *via* a polypeptide hinge, while the Fv region is cleaved and genetically engineered to encode specific antigen-binding sites for epitope recognition [142,143]. In line with this notion, localization signals are used by intrabodies to target specific antigen binding and achieve subcellular internalization, including the nucleus, cytoplasm, mitochondria or endoplasmic reticulum (ER) in mammalian cells [144]. Owing to the petite size of scFvs (−30 kDa), they display excellent tissue penetration properties and limited immunogenicity due to the lack of a constant region. As such, successful application of intrabodies against a variety of neurological disorders has been demonstrated both *in vitro* and *in vivo* across several published studies [145]. Anti-Aβ scFv was reported to effectively attenuate Aβ-induced neurotoxicity and fibrillar aggregation in AD [146,147]. Designation of intrabodies specific to the cleavage site of APP resulted in amelioration of Aβ deposition through intra- and extracellular means [148]. Both intracranial and intramuscular administration of AAV expressing anti-Aβ scFvs yielded positive outcomes, as observed in the reduction of amyloid formation in AD mouse models [149,150]. In another study, rAAV1-delivered Aβ-scFv treatment suggested improvement in cognitive functioning and decreased total Aβ and hyperphosphorylated tau levels [151]. The efficacy of scFvs is apparent, however, it is undermined by the micro-environmental conditions of localized subcellular compartments. For example, the reductive conditions within cytoplasm proved to be exceedingly "toxic" for the construct of disulfide bonds within the designated V_H and V_L domains, contributing to misfolding and aggregation in scFvs [152,153,154]. As a result, scFvs are generally known to have limited half-life, along with low stability and solubility which directly downregulates its functionality and expression levels. Adaptations of camelid nanobodies instead present promising alternatives by resolving the issues faced above.

VHH domains are known to be evolved version of cAbs derived from heavy-chain-only antibodies found in alpacas, Ilamas and camels [155]. Possessing one-tenth of the size of normal IgG molecule (75 kDa), nanobodies can easily gain access into narrow tissues in addition to exhibiting enhanced specificity for unique epitopes that are usually inaccessible for cAbs, accounting to the presence of complementary determining region (CDR) loops located at the tip of V domains [156]. VHH domains are further equipped with low immunogenic potential by sharing a high homology with human type 3 VH domains (VH3) [157]. It is equally important to note that prolonged evolution and genetic engineering have played their part by optimizing VHH construct, resulting in the development of resistance towards proteolytic degradation and thermal change, some of the few abilities that give rise to conformational stability and aggregation aversion [158,159]. So far, these attractive features have rendered researchers to repeatedly modify nanobodies into neuroprotective therapeutics for delivery of critical genes or recombinant proteins. VHH is viewed as an ideal substitute of BACE1 inhibitor against AD, as shown in the case of Ilama-immunized with recombinant BACE1 successfully impeding the enzymatic activity of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) *in vitro* [160]. Moreover, the researchers also attempted *in vivo* testing of VHH B3a in double transgenic APPswe/PS1de9 mouse models to measure the BACE1 inhibitory effects. A significant decline in $Aβ_{40}$ and $Aβ_{42}$ blood plasma levels was observed in mice at 24-h after intracisternal injection of VHH B3a [160]. Recently, another study also reported the usage of AAV-encoded VHH B9 to mitigate Aβ burden and effectively inhibits neuronal BACE1 expression in a concentration-dependent manner [161].

Other than antibody engineering modality, alternative novel treatment approaches involving advanced brain delivery methods are postulated to pose immense benefits in facilitating Aβ and phosphorylated tau clearance. Previous report deemed injection of filamentous bacteriophage M13 (M13) sufficient to alleviate Aβ plaque and microglia-induced brain inflammation in *APP*-expressing transgenic mice [162]. M13 is particularly advantageous when it comes to binding and remodeling various forms of misfolded protein aggregates, including Aβ and tau while sparing the monomers [163]. This major feat is revealed to be accomplished by the attached capsid protein g3p on M13 which acts as a general amyloid interaction (GAIM). In support of the clinical efficacy demonstrated by g3p, researchers have proceeded to engineer a novel fusion protein, NPT088 comprised of an active fragment of g3p and human-IgG_1-Fc. Similar effects as the previous study have been reported, as shown when intraperitoneal injection of NPT088 contributed to diminished Aβ load and enhanced cognition in Tg2576 mice; while Tg4510 mice presented decreased phosphorylated tau, brain atrophy and improvement in cognitive and motor functioning [164]. An alternative approach to reduce tau pathology is through the establishment of scanning ultrasound (SUS). In a recent study, the authors suggested tau isoform-specific scFv, RN2N-conjugated SUS to mediate marked improvement in histological and behavioral read-outs [165]. In support of the ability of SUS to trigger a displacement on the endothelial walls of blood vessels *via* acoustic wave effects [166,167], RN2N was found to be capable of travelling intracellularly into targeted neuronal sites where pathological tau accumulates. Moreover, the fluorescently labelled RN2N further acts as a potential biomarker by allowing antibody visualization within cells and dendrites thus cementing its benefits and practicality [168].

7.2.1. Active immunization

In the past, a multitude of targeted Aβ active vaccinations have been launched for clinical testing by various healthcare companies (Table 3). The earliest report of active immunization against full-length $Aβ_{42}$ peptide was demonstrated in an *in vitro* study using PDAPP transgenic mice. Researchers observed a considerable decline in Aβ fibrillar aggregation, neuritic dystrophy and astrogliosis associated with AD neuropathology, marking the efficacy of anti-Aβ immunotherapy that pave the way for future similar therapeutic efforts [169].

The first active vaccine (AN1792) was manufactured by ELAN Pharmaceuticals using a synthetic Aβ bound to a surface-active saponin adjuvant QS-21. Patients diagnosed with mild to moderate AD reported notable removal of amyloid plague and improvement in cognitive performance in the first two phases of clinical trials. However, the trials were put to an end in year 2002 after approximately 6% of the participants developed adverse side effect defined as meningoencephalitis [170]. One credible explanation was presented in regard to this complication, featuring the mounting immune response generated by Aβ-specific T lymphocytes as T cell epitopes was inserted into the 6–28 sequence of Aβ peptide during the designing of AN1792 [171].

Table 3
Current status of anti-Aβ active vaccinations as stated in ClinicalTrials.gov for AD.

Drug agent	Vaccine design	Phase; clinical trial identifier	Targeted AD severity	Key findings/Outcome	Sponsor
AN1792	Full-length synthetic $A\beta_{42}$ peptide bound to adjuvant QS-21	Phase IIa; NCT00021723	Mild to moderate AD	Approximately 6% of the patients developed meningoencephalitis; aborted in year 2002	ELAN Pharmaceuticals
CAD106	Numerous copies of $A\beta_{1-6}$ peptide conjugated to carrier protein containing bacteriophage coat protein	Phase II; NCT00795418, NCT00733863	Mild AD	Up to 81% of patients exhibited strong serological IgG responses, no reported cases of CNS-related inflammatory disorders	Novartis Pharmaceuticals
AD01	6 amino acid peptide fragment mimicking N-terminal region of $A\beta_{42}$, conjugated to Alum	Phase I: NCT00495417	Mild to moderate AD	Completed	Affiris AG
AD02	6 amino acid peptide fragment mimicking N-terminal region of $A\beta_{42}$, conjugated to Alum	Phase II: NCT02008513	Mild to moderate AD	Early termination due to the results of AFF006 study	Affiris AG
AD03	N-terminal-truncated, pyroglutamated of Aβ conjugated to Alum	Phase Ib: NCT01093664	AD	Terminated due to organizational concerns	Affiris AG

Solubilization of amyloid plaques into Aβ peptides occurred after immunization, rendering the translocation of dissociated Aβ components into cerebral arteries and capillaries. Drastic rise in the brain inflammatory level induces a specific microenvironment that triggers cerebral amyloid angiopathy, microhemorrhages and white matter edema [172,173]. In view of the challenges, efforts were redirected towards modifying vaccines that only contain short fragmented Aβ peptides for antibody stimulation and replacement of T-cell epitopes with B-cell epitopes to prevent T-cell response [174]. Among the novel immunization procedures, second-generation, Aβ-based active immunotherapeutic vaccines were considered as prophylactic interventions for AD. CAD106, is one of the popular peptide vaccines carrying Aβ N-terminus representing B cell epitope and peptide conjugated to a bacteriophage QB coat carrier protein. A large body of studies suggested the enhanced tolerability and safety profiles observed after repeated subcutaneous injections of CAD106 in mild AD individuals. Apart from null cases of aseptic meningitis or meningoencephalitis being reported, increased quantification of Aβ-specific antibody levels in the patients' body indicate successful AD vaccination [175–177].

The next vaccine, Affitopes are functionally mimotopes primarily developed and funded by Affiris using small amino acid peptide fragments to mimic the N-terminus region of $A\beta_{42}$ [178]. Unlike AN1792 and CAD106 vaccines, this particular group utilizes alum as a conjugated adjuvant to enhance immunological efficacy. According to ClinicalTrials.gov, phase I clinical trials were performed by administrating subcutaneous injection of AD01/02 alone or adjuvanted AD01/02 into 24 patients diagnosed with mild to moderate AD. The results fulfilled the primary endpoints of evaluating the affitopes' safety and tolerability levels. Since the first phase of AD01/02 trials were completed and yielded desirable outcome, Affiris proceeded to conduct further experimentation with AD02 by implementing booster vaccination within a separate clinical trial. In year 2013, another randomized control phase II study was staged to examine the safety and tolerability levels together with additional assessment of clinical and immunological effects upon two different AD02 formulations and doses. Intriguingly, the study failed to proclaim any treatment efficacy of AD02, even reporting inconsistencies between antibody titers to aggregated Aβ and non-responders across endpoints, contrasting to the results obtained during the first phase clinical trials [179]. However, early AD patients exhibited significant progress in terms of cognition and function given the treatment of the designated control agent IMM-AD04 (2 mg). Of note, the follow-up study to evaluate the safety and clinical activity of continued immunizations with Affitope AD02 within AD patients who took part in AFF006 was "early terminated by the sponsor based on the results of study AFF006" in the year 2015. As stated in ClinicalTrials.gov, Affitope AD03 (MimoVax) that specifically targets the truncated, pyroglutamate Aβ N-terminus was also applied for phase I clinical testing, however, phase 1b study was abolished due to the sponsor's remarks that "the study could not be performed as planned for organizational reasons" [180].

Another alternative of active immunization that gained tremendous reputation is DNA-based epitope vaccines (genetic vaccines), designed through fusion of a short immunodominant $A\beta_{42}$ B cell epitope with a synthetic, universal T cell epitope, pan human leukocyte antigen DR-binding peptide (PADRE) known to stimulate robust humoral responses against a broad array of antigens [181,182]. Analogous to second generation active vaccines, DNA epitope vaccines appeared to be feasible since they are capable of circumventing T cell-mediated autoimmune response, a scenario established by insertion of foreign Th cell epitope which also allows production of stronger anti-Aβ antibodies compared to initial T self-epitope of Aβ [183]. Multiple studies have repeatedly experimented with different amino acid regions on the $A\beta_{42}$ N-terminus for mapping of B cell epitope, such as 1–5, 1–7, 1–8, 1–11, 1–15, 1–16 or 4–10 [184,185]. In BALB/c wild-type mouse models, PADRE-$A\beta_{1-15}$-MAP epitope vaccine was shown to enhance anti-Aβ antibodies titers and induces potent T cell stimulation specific to PADRE in splenocytes, without triggering any Aβ-related Th cell-activation [186]. Based on the positive results obtained, a second-generation epitope vaccine was set to gear portraying the $A\beta_{1-11}$ sequence as B cell antigenic determinant coupled with PADRE to be administered into APP Tg 2576 mice characterized with pre-existing AD-like pathology. Similarly with the previous epitope vaccine, 2$A\beta_{1-11}$-PADRE-MAP induces significant PADRE-specific $CD4^{+}$/IFNγ/IL-4 Th cell response, thereby elevating the expression of anti-$A\beta_{1-11}$ antibodies and reducing insoluble Aβ plaques concentration while leaving the total levels of soluble Aβ in the brain plasma intact [187]. Importantly, a translation study regarding the novel AD-1955 vaccine was conducted on rhesus macaques to identify its relevance for human clinical testing. The vaccine candidate AD-1955 was refined with inclusion of additional Th cell epitopes from other pathogens such as the surface antigen or nuclear capsid protein of hepatitis B (HBsAg/HBVnc), influenza matrix protein (MT) and tetanus toxin (TT): P2, P21, P23, P30 and P32 [188]. This provided added benefits to said vaccine, enabling it to selectively reactivate pre-existing memory Th cells definitive of the incorporated pathogens thus assisting B cells in secretion of Aβ-specific antibodies, successfully inhibiting $A\beta_{42}$ oligomers and fibrillar-mediated neurotoxic effects.

7.2.2. *Passive immunization*

Another favorable prophylactic and therapeutic measures taken to combat Alzheimer's disease is passive immunotherapy (Table 4).

The first humanized mAb against Aβ peripherally administered into AD Tg mouse models, bapineuzumab, specifically targets the N-terminus of $A\beta_{1-5}$ and initiates Fc-receptor-mediated microglial phagocytosis of amyloid plaques [189]. Bapineuzumab (AAB-001) undergone phase I clinical trial and reported favorable safety and tolerability profiles at a single ascending dosage range of 0.15, 0.5, 1.0 and 2.0 mg/kg among Japanese patients suffering from mild to moderate AD, a phenomenon similarly demonstrated by previous studies performed in the US and

Table 4
Current status of passive immunotherapeutic strategies for Alzheimer's disease.

Drug agent	Targeted binding species	Antibody/ epitope	Phase; clinical trial identifier	Targeted AD severity	Key findings	Sponsor
Bapineuzumab	Aβ monomers and fibrils	$A\beta_{1-5}$	Phase III; NCT00996918, NCT00998764	Mild to moderate AD	Terminated starting August 2012 due to the two large phase III trials not showing any clinical benefits	Janssen, Pfizer
Solanezumab	Aβ monomers	$A\beta_{16-24}$	Phase III; NCT00905372	Mild to moderate AD	EXPEDITION 1: No significant improvement in cognitive and functional performance	Eli Lilly and Company
			Phase III; NCT00904683	Mild to moderate AD	EXPEDITION 2: Only patients with mild AD reported significant reduction in cognitive and functional decline	
			Phase III; NCT01900665	Mild AD	EXPEDITION 3: Terminated because solanezumab did not meet the study's primary endpoint in cognitive and functional measurements	
Crenezumab	Aβ oligomers, fibrils and plaques	$A\beta_{1-15}$	Phase II; NCT01998841	Preclinical $PSEN_1$ $E_{28}{}_0A$ mutation carriers	Active, not recruiting, expected to be completed by year 2022	Genentech
			Phase III; NCT02670083	Prodromal to mild AD	Terminated, study reported zero clinical efficacies as control and experimental group displayed similar results on both primary and secondary outcomes	Roche
BAN_{2401}	Aβ oligomers and protofibrils	$A\beta_{42}$ protofibrils	Phase II; NCT01767311	Early AD	Active, not recruiting, study expected to complete by year 2022	Eisai Inc.
			Phase III; NCT03887455	Early AD	Recruiting, study expected to complete by year 2024	
Aducanumab	Aβ monomers, oligomers and fibrils	$A\beta_{3-6}$	Phase I, NCT01397539	AD	Completed, study implied aducanumab to project acceptable safety and tolerability profile	Biogen, Neurimmune
			Phase I, NCT01677572	Prodromal or mild AD	Terminated because of the futility analysis conducted on phase III trials	
			Phase III; NCT02477800, NCT02484547	Early AD	Terminated, study failed to meet up the primary endpoint	
			Phase IIIb; NCT04241068	AD	Enrolling by invitation, study expected to run through September 2024	
ALZ 801	Aβ monomers and oligomers	$A\beta_{42}$	Phase 1; NCT04157712	AD	Completed, study portrayed favorable safety profile	Alzheon Inc.
			Phase 1; NCT04585347	AD	Completed, potent anti-oligomeric effects reported	

European countries [190,191]. Though the exploratory efficacy assessment revealed bapineuzumab-treated individuals to display cognitive improvement compared to the placebo controls, statistically significant relations were not being presented prompting the study to draw an inconclusive outcome [192]. Remarkably, subcutaneous injection of bapineuzumab was found to reduce amyloid burden in the AD brain of a subset of participants *via* positron emission tomography (PET) scanning during phase II trial [193]. Moving on to phase III, two multicenter, double-blind, placebo-controlled clinical trial were conducted, one study engaging mild to moderate AD patients carrying the apolipoprotein E4 (ApoE4) alleles while the other selecting non-carriers of the gene. Test subjects were randomly assigned for intravenous delivery of varying doses of bapineuzumab and placebo, taking a time frame of 78 weeks with an infusion every 13 weeks. Unexpectedly, there exist no significant differences between the bapineuzumab-treated and control groups based on the outcome measures from Alzheimer's Disease Assessment Scale (ADAS-cog) and Disability Assessment for Dementia (DAD), indicating no clinical efficacy in bapineuzumab despite clear reduction of Aβ fibrillar aggregation [194,195]. Moreover, detection of amyloid-related imaging abnormalities (ARIA) in the form of intracerebral microhemorrhages and edema by magnetic resonance imaging (MRI) warned the potential adverse effects associated with the drug [196,197]. These outcomes resulted in the termination of other phase III trials aiming to investigate the long-term safety and tolerability possessed by bapineuzumab in mild to moderate AD patients.

Based on the contradictory results and setbacks observed in bapineuzumab, attention was shifted towards solanezumab, a humanized version of mAb 266 which contains an epitope located at $A\beta_{16-24}$ and selectively binds to monomeric, soluble Aβ species that induce neurotoxicity. Prior precursor mAb 266 immunization not only lowered amyloid load but also raised the expression levels of soluble Aβ in plasma thus ensuring cognitive improvement in Tg mice plagued by AD [198]. Unlike bapineuzumab, results from phase II trials implied increased $A\beta_{40}$ and $A\beta_{42}$ concentrations in both plasma and cerebrospinal fluid (CSF) of mild to moderate AD patients treated with solanezumab in a dose-dependent manner [199]. Patients subjected to 400 mg doses every 4 weeks demonstrated elevated unbound $A\beta_{42}$ in CSF, suggesting solanezumab to influence the equilibrium of $A\beta_{42}$ resulting in dissociation from amyloid plaques to occur. Following this outcome, two more multicenter, randomized, placebo-controlled, double-blind phase III trials, identifier (NCT00905372 and NCT00904683) were performed on 1012 and 1040 participants having mild to moderate AD respectively [200]. For the principal assessment, both studies failed to report any significant cognitive and functional recovery; while secondary analysis noted the therapeutic efficacy of solanezumab to be more prominent in mild AD population [201–203]. The possible reasons behind such negative findings may be associated to the severity of amyloid neuropathology and administration course of the antibody into affected patient. Treatments may have to be started when the course of disease is still in early and mild stage for the clinical efficacies to kick in. Nonetheless, an additional phase III study was conducted to confirm whether solanezumab will truly delay the cognitive decline of mild AD patients (Identifier NCT01900665) only to have it terminated due to the antibody failing to meet their primary endpoints. Another notable passive immunotherapeutic humanized antibody that managed to gain access into clinical testing is crenezumab ($MABT_{5102}$). Crenezumab principally interacts with the Aβ mid-region and demonstrated high affinity towards oligomers, fibrils and plaques binding [204]. Supplemented with an IgG4 isotype, crenezumab limits the prospect of Fcγ receptor-mediated microglial activation and proinflammatory responses, effectively inhibiting cerebral microhemorrhage and vasogenic edema [205,206]. In year 2013, crenezumab was included in the "Alzheimer's Prevention Initiative" (API), a trial that operates on the concept of preventive measures. Genentech, in collaboration with both the Banner Institute

and National Institutes of Health, have recruited 300 participants of Colombian background happened to either harbor autosomal-dominant mutations in presenilin genes ($PSEN_1$ E_28_OA) or are non-carriers themselves [207]. $PSEN_1$ mutation is long found to be a risk factor for early onset AD, owing to its ability to orchestrate aggregation and deposition of $A\beta_{42}$ plaques at a relatively young age [208]. Test subjects were randomly assigned to receive subcutaneous or intravenous administration of crenezumab or placebo in an extended period of 260 weeks. According to the latest update by ClinicalTrials.gov, the estimated date of completion for this phase II clinical trial (identifier, NCT01998841) has been extended from year 2020 to 2022. Additionally, two multicenter, double-blind, parallel-group, placebo-controlled phase III CREAD studies were also put forth for evaluation of the clinical efficacy and safety of higher crenezumab dosage in patients affected by prodromal to mild AD [209]. Unfortunately, Roche officially ended both studies in year 2019 due to the disappointing results obtained, showing no significance differences between placebo and crenezumab-treated groups, thereby speculating crenezumab to be incapable of inhibiting build-up of amyloid plaques occurring at early stage AD.

Another significant anti-Aβ monoclonal antibody that was under investigation is Ban_{2401}, which is developed upon the discovery of an artic mutation in APP. Ban_{2401} is exclusive only to a specific conformational site located on the large Aβ protofibrils and noted to initiate protofibrils clearance and decrease neurotoxicity in Tg-ArcSwe mouse models [210]. Following single and multiple ascending intravenous doses of Ban_{2401} into mild to moderate AD patients during phase I clinical trial, ARIA was detected to be in similar level between placebo and treated-group, indicating Ban_{2401} to exert high tolerability across the tested dosage (minimum dosage, 0.1 mg/kg; maximum dosage, 15 mg/kg). Moreover, Ban_{2401} exposure into the CSF was found to be dose-dependent, with a mean serum half-life accounting up to seven days [211]. These features paved the way for the next phase II trial, in which participants with mild cognitive impairment (MCI) or mild AD dementia were selected to determine the extent of Ban_{2401} safety, tolerability and efficacy based on Bayesian design [212]. As stated in ClinicalTrials.gov, this study (identifier, NCT01767311) is set to run till year 2022. Meanwhile, the recruiting status for phase III study is still ongoing with the purpose of comparing the clinical efficacy of Ban_{2401} to that of placebo in early AD patients all the way to extension phase and is expected to be completed by year 2024. Additionally, aducanumab (BIIB037) which happened to be a human IgG1 mAb was designed to interact with the N-terminal region of Aβ and preferentially bind towards both oligomeric or fibrillar forms. Aducanumab was derived from B lymphocytes of elderly donors that showed no clinical signs of AD-associated cognitive deterioration, with the conception that this particular mAb may somehow offer some level of neuroprotectivity and resistance against AD development. During phase I clinical trial (identifier, NCT01397539), intravenous infusions of aducanumab in a single-ascending-dose titration ranging from minimum 0.3 mg/kg to maximum 60 mg/kg into patients suffering from mild-to-moderate AD yielded positive results. Aducanumab was reported to be generally safe and well-tolerated, with an exhibited linear pharmacokinetics at dose $\leq$ 30 mg/kg [213]. This leads to the launching of a subsequent multi-dose study named PRIME (identifier, NCT01677572) to further evaluate the safety and pharmacokinetics profile of aducanumab in prodromal or mild AD patients. A drastic reduction of amyloid load in a dose- and time- proportional manner was observed upon monthly aducanumab administration using PET scans [214]. Similarly, application of aducanumab also brought about restoration of calcium homeostasis and amyloid plaque clearance as indicated in experimental Tg2576 murines [215]. Since interim data from PRIME suggested rather promising results, Biogen decided to initiate two efficacy phase III clinical trials with the target population now being those suffering from early AD. ENGAGE (identifier, NCT02477800) and EMERGE (identifier, NCT02484547) were initially projected to complete in year 2022, however, a sudden announcement was made by Biogen to discontinue both trials "based on futility analysis done and not based on safety concerns". Notably, the initial data failed to meet primary endpoints upon completion, with sub studies showing common adverse events including ARIA-E and headaches as reported by ClinicalTrials.gov. As a result of the futility analysis obtained from ENGAGE and EMERGE, PRIME was forced to be terminated as well. Nonetheless, extension of ENGAGE and EMERGE to phase IIIb open-label trial was conducted to determine the safety and tolerability outcome measures of aducanumab in patients who had previously participated in aducanumab studies 221AD103, 221AD301, 221AD302 and 221AD205. The study was expected to run through September 2023.

Recently, an oral agent ALZ 801 had received tremendous expectations from the scientific community due to the continual efficacy reflected in the clinical trials. ALZ 801 is recognized as a modified prodrug tramiprosate, formulated through the coupling reaction between tramiprosate and amino acid valine [216]. Tramiprosate, in an earlier phase III study that target mild-to-moderate AD, failed to generate clinical efficacy in *APOE4* carriers. Intriguingly, a pre-specified subgroup analysis later reported significant clinical benefits in patients expressing *APOE4* homozygotes (2 alleles) [217]. As a result, tramiprosate was reduced into a dietary supplement by NeuroChem while ALZ 801 taken over by Alzheon Inc. for subsequent antibody development against AD. A phase I, randomized, placebo-controlled study (identifier, NCT04157712) in 127 elderly participants revealed that single and multiple ascending doses of ALZ 801 give rise to mild gastrointestinal-related complications independent of clinical dose use. Notably, designation of this tablet signals a favorable pharmacokinetics profile as shown in the display of excellent oral bioavailability, consistent plasma concentrations and intestinal absorption which leads to robust brain penetration [216]. Unlike the other passive immunotherapies described above, ALZ 801 is capable of demonstrating selective and specific binding towards Aβ oligomers over insoluble fibrils or plaques in a dose-dependent fashion [218]. 3-sulfopranpanoic acid (3-SPA), an active metabolite of ALZ 801 commonly expressed in human brain, prevents any form of improper folding and aggregation of Aβ monomers into neurotoxic oligomers. Meaningful results are also achieved in this study, with participants receiving 265 mg of ALZ 801 twice daily showing sustained brain exposures equivalent to administration of 150 mg tramiprosate two times a day, in addition to an observed 5-fold difference in brain concentration than normally required for attenuation of oligomer elongation *in vitro* [216,218]. Of note, ALZ 801 offers potential positive improvement in cognitive and functional measurement along with low incidence of ARIA-E [219]. With these constructive outcomes at hand, Alzheon Inc. intends to launch a subsequent phase III trial by engaging early-to-mild AD patients that are *APOE4* homozygous for evaluation of ALZ 801 drug effects on cognitive functioning based on ADS-COG, DAD, CDR-SB and MMSE assessments. ClinicalTrials.gov similarly disclosed that other clinical testing will be conducted, including the measurement of brain hippocampal volume, cortical thickness MRI and targeted Aβ oligomer inhibitory action [220].

7.3. Nanoparticles (NPs)

Nanotechnology has long emerged as the pioneer of drug delivery research, offering promising platforms for various disease diagnosis and treatment as a result of its nanoscale. In recent years, the combinatorial treatment of nanotherapy and pharmaceutical drugs has received immense support from the medical field as they are central to increasing the efficacy of current treatments besides conferring safe guarantees. Other favorable advantages pertaining to these nano drug delivery devices include enhanced drug stability, biodistribution and pharmacokinetics (PK), inherent ability to overcome tumor drug resistance, solubilize hydrophilic and hydrophobic units, in addition to the added ability in responding to different encountered intrinsic and extrinsic stimuli to gain temporal and spatial control over release of therapeutic payloads [221–223]. Considering the therapeutic potential exhibited by nano-based drug delivery devices, multiple studies have experimented

on and diversify a host of materials for utility during drug delivery applications [224,225]. Constant modification is also incorporated into the engineering of nanodrug delivery vehicles. To name a few, liposomes, polymeric micelles, solid lipid nanoparticles, gold nanoparticles, silver nanoparticles, metal-oxide nanoparticles and quantum dots are among the ones achieving popularity nowadays (Fig. 2) [226–229]. A more comprehensive description of each of these clinically approved nanocarriers is discussed below.

7.3.1. Liposomes

The existence of liposomes is first brought to light by Dr. Bangham, a British hematologist along Horne during the negative staining of phospholipids using electron microscope [230]. As decades passed, the concept of liposomes is gradually established as a potential drug carrier in clinical applications and diagnostics [231]. Liposomes are microscopic vesicles composed of an aqueous inner core space surrounded by one or more phospholipid bilayer and are generally 20 nm to 2.5 μm in diameter [226,232]. Importantly, liposomes are capable of encasing hydrophilic (within the aqueous compartment), hydrophobic (in the lipid bilayer) and amphiphilic compounds (lipid aqueous interface), making them an ideal and versatile carrier for drug delivery and nanotherapy [233]. The solubility and therapeutic index of the encapsulated drug are said to highly correspond to the liposomes, as these nanocarriers project several benefits in clinical applications including, (i) protection against chemical and/or enzymatic degradation; (ii) control of drug toxicity and side effects; (iii) stabilization of drug before arriving at target sites; (iv) decrease of interaction between drug and healthy tissues [234].

The most commonly incorporated phospholipids in synthesis of liposomes include, 1,2-Dimyristoyl-sn-glycreo-3-phosphocholine (DMPC), 1,2-Dimyristoyl-sn-glycreo-3-phosphoglycerol (DMPG), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), sphingomyelin, phosphatidylcholine and cholesterol [235]. Phosphatidylcholine or lecithin as it is normally termed, are choline-enclosed phospholipid molecules that exert their dipolar nature at physiological pH, displaying a positive charge on the quaternary ammonium group and a negative charge in the phosphate group [236]. As a result, they are primarily sought for liposomes synthesis. Additionally, sterols or cholesterol

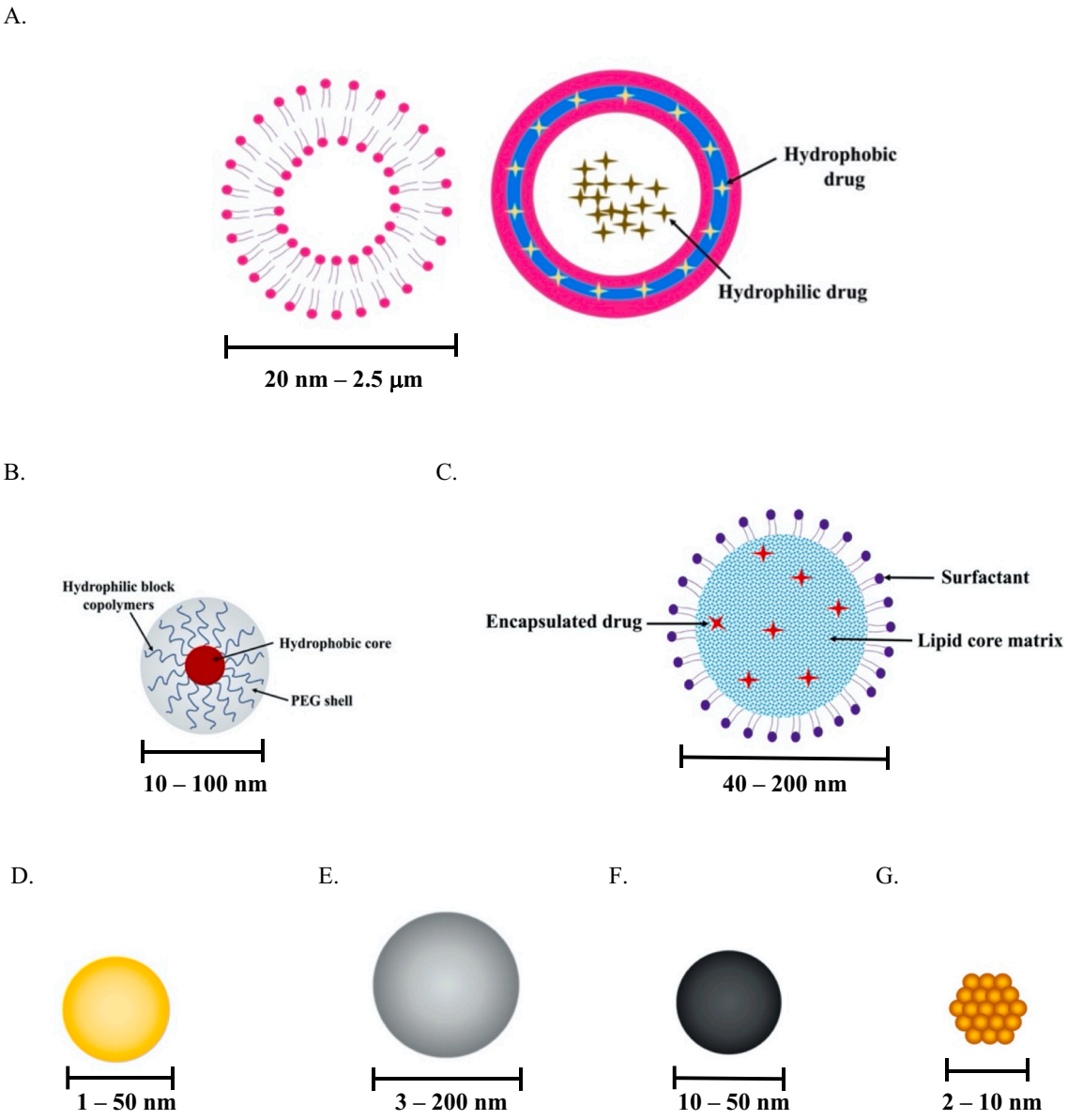

Fig. 2. A schematic representation of the nanoparticles used in Alzheimer's disease treatment. **(A)** liposomes, **(B)** polymeric micelles, **(C)** solid lipid nanoparticles, **(D)** gold nanoparticles, **(E)** silver nanoparticles, **(F)** iron-oxide nanoparticles, **(G)** quantum dots.

components are incorporated into liposomes so as to further regulate membrane fluidity and rigidity in the face of blood or plasma-related components [237]. Such action eliminates or reduces the probability of rapid leakage due to lipid bilayer's instability, thus enhancing the longevity of liposomes and maintaining drug release rates. Nowadays, the apparent bioavailability of liposomes prompted researchers to further modify these particular nanosystems for disease control utilizing various methods. Remarkably, research had reported a major leap in the development of liposomal delivery platform, suggesting that design of polymer-modified thermosensitive liposomes (PTSL) with multi-sensitive characteristic confers accelerated specificity and sensitivity in individual formulations. In a study, the authors internalized copolymer [*N*-isopropylacrylamide-*co*-propylacrylic acid] into liposomes to confer pH and temperature sensitivity into the formulations, thereby instigating greater apoptosis of tumor cells by enhancing the penetration and impeding their growth as compared to the ordinary heat-trigger-based liposomal doxorubicin [239]. In another study, liposomes conjugated with apolipoprotein E (ApoE) was discovered to coordinate the route taken for liposomes to arrive at neural stem and progenitor cells, improving the distribution of small interfering RNA (siRNA) into the brain [240]. Similarly, a research utilizing APP/PS1 transgenic mice as clinical models observed celecoxib-loaded erythrocyte membranes liposomes promoting neurogenesis, biodistribution efficacy and elimination of amyloid beta plaques accumulated in neurons at the same time [241]. Clearly, these findings demonstrated that liposomes inhibit tumor growth and ameliorate cognitive impairment, further strengthening their potential role as promising future drug nanocarriers.

7.3.2. Polymeric micelles

Polymeric micelles are self-assembled core-shell micellar molecules formed in an aqueous amphiphilic environment, constituting of hydrophilic block copolymers as the shell and hydrophobic block copolymers as the main core structure [242,243]. These nano-constructs are characterized by their spherical shape and extremely miniscule size ranging between 10 and 100 nm [244]. Modifications of polymeric micelles with a surface coating of polyethylene glycol (PEG) infuse these nanocarriers with a sturdy barrier which acts as a safety retainer for water insoluble drugs, thus disrupting any possible interactions with blood components and limiting the adsorption of plasma proteins [245]. This in turn imparts drug bioavailability, longevity and stability even if the internal body system is undergoing any dilution process [246–248]. Prolonged duration within the blood circulation is also known to upregulate the enhanced permeability and retention (EPR) effect, thereby increasing the accumulation intervals of polymeric micelles at defective tumor vascular tissues [249]. Perhaps, the brilliance of polymeric micelles lies in their size which provides them evasion against the hepatic mononuclear phagocytic system (MPS) in the liver and spleen but at the same time also shields them from rapid renal clearance [250].

A substantial number of studies revealed that polymeric micelles appear to be suitable candidates for treatment against specifically targeted neurodegenerative disorders. Zhang et al. conducted a research using mirror-image phage display selection and observed that modification of PEGylated polymeric with TGNYKALHPHNG (TNG), a ligand consisting of 12 amino acids and QSHYRHISPAQV (QSH), a D-enantiomeric peptide demonstrated higher permeability and penetration of the BBB [251]. In this regard, TNG binds to the ligand at BBB while QSH targets the $A\beta_{42}$ deposits in the brain lesions within AD mice models. The outcomes implied absence of dual-targeting effects between TGN and QSH, with a 1:3 M ratio of TGN/maleimide and QSH/maleimide as the optimum targeting density. Moreover, the experiment also reported null cytotoxicity, indicating their potential application in early diagnosis and treatment of AD. In another study, Yang et al. developed a mixed-shell polymeric micelle (MSPM) complexed with poly(β-amino ester)-*block*-poly (ε-caprolactone) (PAE-*b*-PCL) and poly(ethylene oxide)-*block*-poly (ε-caprolactone) (PEG-*b*-PCL) to serve as a novel AD therapy [252]. The experiment results suggested that this chaperone exhibits selective binding affinity towards Aβ peptides, leading to formation of MSPM-Aβ complex which significantly promotes Aβ phagocytosis by microglia cells. The authors therefore concluded that MSPM-based nanochaperone restores Aβ homeostasis, attenuates Aβ-mediated cytotoxicity and has the potential to rescue the cognitive deficits associated with the early-onset of AD. Recently, construction of a dual-sensitive polymeric nanomicelle (PM) system was attempted for efficient delivery of 3D6 antibody fragments (3D6-Fab) into targeted sites against $A\beta_{42}$ aggregation [253]. Fabrication of cationic disulfide cross-linked poly(ethylene glycol) (PEG)-poly (L-lysine) block copolymers with charge-converted 3D6-Fab had enhanced the conformational stability of PM, thus enabling the system to self-assemble and preserve the encapsulated antibodies by exerting strong resistance towards acidic and reductive intracellular environments. With the aim of ensuring that the PM also confer brain targeting specificity, the surface of nanomicelle is optimized with glucose molecules for better complexation with recycling glucose transporter (Glut)-1 protein [254]. Overall, this multifunctional PM platform was established as an emerging anti-Aβ therapy with immense potential for directing delivery of therapeutic agents into the brain.

7.3.3. Solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are lipid-derived colloidal nanocarriers constituted of lipids such as steroids, fatty acids, waxes, monoglycerides, diglycerides or triglycerides that are solid at body and room temperature [255,256]. They are lipophilic in nature, a characteristic attributable to the presence of phospholipid monolayer enclosing the hydrophobic lipid core matrix. Nowadays, assimilation of several advantages found within polymeric micelles in conjunction to liposomes rendered SLNs to emerge as a far more superior alternative drug carrier. Supplemented with the stability and biodegradability which ensure their reduced toxicity potential and mass production [257,258], the availability of SNLs for ligand coating further accelerates drug targeting efficiency [259]. Additionally, they can easily squeeze through the restrictive endothelial cells of BBB and bypass the Reticulo Endothelial System (RES) thanks to their submicron particle size ranging from 40 to 200 nm [260–262]. However, SNLs were known to display potential disadvantages as well, indicating low drug loading capacity accompanied with the possible occurrence of drug expulsion under long-term storage conditions [263].

That being said, multiple studies still employed SLNs for brain delivery of a broad array of drugs to treat neurological-based disorders. A study performed showing reaction between avidin-conjugated-SLNs and biotinylated apolipoprotein E (ApoE) reported successful binding of SLNs to low-density lipoprotein (LDL) receptor located on the surface of endothelial cells, thereby promoting BBB permeability and released of encapsulated drug into the brain [264]. Aside from eliciting drug transport into the brain, SLNs can be modified in such a way that they prolong the possible circulation of drug carriers in blood system effectively due to effective total entrapment of the drugs within the core. SLNs conjugated with rapamycin (RP-SLN) and stabilized using polysorbate 80 (PS80) experienced elevated controlled release, suppression of cell proliferation and p70S6K phosphorylation on mammalian target of rapamycin (mTOR) [265]. An intranasal route delivery system illustrating modification of chitosan coated and uncoated SLNs with BACE1-siRNA conjugation reported optimal intracellular nerve transport. The action of embedding siRNA with rabies virus glycoprotein (RVG-9R), induces protection of oligonucleotide and facilitates binding to nicotinic acetylcholine receptors localized in the nasal cavity. Therefore, the authors concluded that loading of BACE1-siRNA in SLNs helps to circumvent the barriers normally encountered during nose-to-brain administration procedures in Alzheimer's disease therapies [266]. In another study, Vakilinezhad and coworkers functionalized SLNs with nicotinamide, a HDAC inhibitor, to evaluate the extent of improved cognitive performance in Alzheimer's disease rat models. The results obtained from behavioral studies and biochemical tests demonstrated a

better spatial reference memory, increased neuronal density and lower tau hyperphosphorylation in rats treated with nicotinamide-conjugated SLNs compared with the negative control group, indicating the efficiency of this nano-system in ameliorating cognitive deficiency associated with Alzheimer's disease [267].

7.3.4. Gold nanoparticles (AuNPs)

To date, gold nanoparticles (AuNPs) have sparked the interest in bioimaging, drug and gene delivery, and vaccine development applications consequential of their myriad benefits that greatly revolutionize the status of nanomedicine [268–270]. In general, AuNPs have low toxic profiles, potential biocompatibility and considerable cell permeability which allow them to cross the BBB [271–273]. Due to their exceptionally strong affinity for amine and thiol functionalities, ligands and functional groups sharing the same functionalities can be grafted onto the surface of AuNPs without much difficulties [274]. Interestingly, AuNPs contain a surface plasmon resonance (SPR) that is responsible for the emission of vibrant colors. The absorption intensity of SPR very much relies on the sizes and structures of gold nanoparticles being synthesized, as shown in the positive correlation between mean diameter of particle to that of ratio of scattering to absorption [275–277]. Another defining optical characteristic of AuNPs is their ability to scatter the absorbed light and quenched nearby fluorescence, heightening the accuracy required for visualization techniques [278–280]. Numerous lines of evidence reported the breakthroughs of AuNPs in curing different types of tumorigenic diseases [281,282,282]. Nonetheless, these particles have gradually been introduced for treatment of neurodegenerative diseases in recent years as well. A study discussed the effectiveness of gold nanoparticles-capped mesoporous silica (MSN-AuNPs) conjugated with metal chelator CQ to not hinder the ability of BBB permeation. Since MSN-CQ-AuNPs were formulated to respond under high H_2O_2 conditions corresponding to copper-ion-induced $A\beta_{40}$ aggregation, unwanted drug release issues can be avoided as the signal release for metal inhibitor CQ will only be triggered upon reaching the affected neurons [283]. Besides, the expression of brain-neurotrophic factor (BDNF), cAMP response element binding protein (CREB) and stromal interaction molecules (STIM) perpetually increased in Aβ-treated rat model after intrahippocampal and intraperitoneal injections of AuNPs [284]. Another novel study similarly suggested the involvement of AuNPs to restore the antioxidant capacity in AD rats in addition to delaying any onset of neuroinflammation and ameliorating cognitive deficits [285].

7.3.5. Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) have progressively made its appearance on varying biomedical applications as accumulating medicinal benefits such as antifungal, antimicrobial, antibacterial and wound healing properties were revealed [286–288]. In suspension medium, disintegration of AgNPs into smaller counterparts (Ag ions, Ag particles) occurs [289]. Unlike other nanoparticles, AgNPs with a size below 100 nm was suggested to activate cytotoxic cell mechanisms as compared to the monovalent Ag ions when introduced into human lymphoma cells [290]. However, several quantitative proteomics analyses proposed the magnitude of such detrimental effects to amplify for AgNPs displaying a size of 20 nm. Evidence reported these particular AgNPs to directly disrupt the mitochondrial dynamics in human colon adenocarcinoma LoVo cells and C3A hepatocytes, resulting in overproduction of reactive oxygen species (ROS) along with cellular and protein metabolic malfunctions [291,292]. Exposure of a comparatively smaller size AgNPs ranging between 3 and 5 nm was equally discovered to stimulate pathogenic changes in Aβ-associated gene expression profiles. This in turn accelerates Aβ plaques deposition prior to massive neuronal death typical of AD [293]. Nevertheless, one recent study refuted the previous results obtained by showing the inhibitory and disaggregation effects on amyloid folding by AgNPs performed on Hen Egg White Lysozyme (HEWL). The authors regarded AgNPs as an ideal nano-chaperone as shown when the cytotoxicity levels of amyloid deposits and time taken for fibrillation phase were diminished [294]. In conclusion, more research and modifications are required to explore and unravel any hidden possibilities in AgNPs as they may deliver exciting alternatives for nanoimaging or nanotherapy in the future.

7.3.6. Metal-oxide nanoparticles

By virtue of an excellent BBB permeability and considerable surface area for ligand binding, metal-oxide nanoparticles play a pivotal role in advancing the sensitivity and specificity of targeted drug delivery system and MRI contrast agents [295]. Particle size, shape, chemical composition, core crystallinity, stability, biodegradability, purity, surface modifiability and encapsulation efficiency are among the factors considered during synthesis of metal oxide-based nanoparticles through addition of oxidizing and/or reducing agents [296,297]. As opposed to other metal oxides, pure iron oxides such as maghemite (γFe_2O_3) and magnetite (Fe_3O_4) nanoparticles are known to be the most fundamental and biocompatible magnetic nanoparticle [298]. Despite their relatively high saturation magnetic forces, iron ions are best regarded as a catalyst in Haber-Weiss and Fenton reactions which lead to secretion of oxidative stress and degradation of cytoskeletal components [299,300]. Consequently, iron-oxide nanoparticles (IONPs) were noted to exert potential *in vitro* and *in vivo* cytotoxicity contributing to neuronal death. Nonetheless, studies revealed that regulatory signals of neurotoxicity are determined by the physicochemical properties of the outer coating layer of IONPs [301].

Ultra-small superparamagnetic iron oxide (USPIO) and superparamagnetic iron oxide (SPIO) nanoparticles, with a diameter ranging between 50 and 150 nm and 10 to 50 nm respectively, are the most commonly documented MRI contrast agents [302–304]. Though multiple research acclaimed IONPs to be toxic and is responsible for inducing a positive feedback loop between iron accumulation and Aβ aggregation [305–307], they are nevertheless been incorporated into the diagnosis and treatment of AD [308]. A study reported that SPIONs coupled to anti-AβPP antibody can circumvent BBB and track deposition of Aβ plaques in $A\beta PP/PS_1$ transgenic mice models, with clear visualization utilizing MRI technique [309]. Similar resolution and detection precision were observed in other research, indicated by binding of Aβ plaques to curcumin-conjugated SPIONs and DDNP-conjugated SPIONs in brain lesions of AD rats and Tg2576 mice models respectively [310,311]. Consistently, conjugation of nerve growth factor (NGF) to SPIO-Au core-shell nanoparticles with a diameter size of 20.8 nm was shown to considerably elongate the length of neurites and cellular differentiation ratio. These findings suggested SPIO-Au NPs to be well-suited for enhancing neuronal growth and differentiation under the influence of dynamic external magnetic field [312].

7.3.7. Quantum dots

Considered as one of the most significant tools used in biomedical applications, Quantum dots (QDs) have paved the path for fascinating developments in the field of drug delivery, sensors, neuroimaging and protein detection [313]. QDs are primarily made up of unreactive zinc sulfide (ZnS) shell surrounding a metalloid crystalline core that consisting cadmium selenium (CdSe), with the outer coating layer of QDs customized in such a way to provide attachment sites for various bioactive molecules such as peptides and antibodies for targeted site delivery [314]. Predominantly, their inorganic framework makes them extremely resistant to photobleaching besides granting phytochemical reactivity which activates selective fluorescent binding of protein ligands to cell surface receptors [315]. With their exceptional chemical and photostability, QDs display broad absorption spectra accompanied with narrow emission spectra, enabling simultaneous measurement of multiple colors of quantum dots during multiplexed bioassays [316]. Fluorescence wavelength emitted by fluorescent semiconductors QDs is easily tunable by altering their nanometer-sized composition and structure (2–10 nm) [317]. Moreover, the extremely high fluorescent

quantum yields and blinking nature of QDs allow one to easily visualize and identify each "shining" nanocrystal dispersed under a fluorescent microscope. Thanks to their optical resolution, a study that researched on QDs suggested them to be able to readily gain access into the specialized neuronal and glial cells, tracking complex intracellular and intercellular molecular dynamics over extended time periods, a feat that is deemed to be unattainable using traditional immunocytochemistry [318].

Back in year 2005, a group of researchers came up with the modification of QDs that help to maintain stable peptide-quantum-dot interactions and allow bypassing of antibodies to occur. It involved genetically encoding the target protein with a 15-amino acid acceptor peptide (AP; GLNDIFEAQKIEVWHE) that will undergo subsequent biotinylation using biotin ligase (BirA), leading to detection by streptavidin-conjugated QD. With this technique, they managed to specifically label AMPA receptors in hippocampal neurons and observe the molecular reactions of the QD-bound protein [319]. Importantly, QDs are shown to be feasible for detection and regulation of $A\beta_{42}$ aggregation related to Alzheimer's disease. *In vivo* studies reported enhanced imaging and quantification of Aβ aggregation with the use of Aβ-conjugated QDs; whereas in another study it was shown that $A\beta_{42}$ conjugated to dihydrolipoic acid (DHLA) capped CdDe/ZnS QDs drastically decrease the fibrillation process [320,321]. Furthermore, formulation of novel nanoprobes consisting of benzotriazole (BTA)-conjugated PEGylated fluorescent QD profiles suggested higher detection sensitivity and specificity of Aβ proteins [322]. A recent study also successfully functionalized QDs with graphene, which assembled with $A\beta_{42}$ upon contact, leading to elevated pH that imparted an electrostatic repulsive force working to dissociate the $A\beta_{42}$/GQDs co-assembled structure. Strong $A\beta_{42}$/assembly quantum dots (GQDs) interaction prevents deposition of the former into neurotoxic aggregative states, providing some sort of alleviation to the disease [323].

8. Conclusion

This review touches upon the commentary and significance of gene therapy, antibody-induced immunotherapy and nanotechnology as a revolutionary platform to combat against AD. By theoretically targeting the root cause of AD, we should not only observe positive memory and cognitive improvement but also reverse neurodegenerative damages in AD patients. Although gene therapy appear to be a promising alternative therapeutics strategy, it still does pose some limitations mainly *in vivo* since there is lack of specificity, low efficiency, and direct host exposure to the non-viral transport vector. Although *ex vivo* gene therapy may reduce the possibility of these issues, it does have its drawbacks mainly on its delivery method and a more invasive procedure. It is also reported that AD clinical trials have very high failure rate of ~99.6% [324] theorized to be due to the multiple pathway mechanism usually involved and poor understanding of the mechanism. Since AD is a complex heterogenous disease, its complex interplay between genetic susceptibility and downstream molecular pathway is the key to developing therapeutics strategy. At this point, the optimal success rate of immunotherapy is also unexpectedly minimal as majority clinical trials were faced with termination due to the inconsistent results obtained during different phases or emergence of adverse side effects. Moreover, most of the active and passive immunotherapy focused on targeting mild-to-moderate AD, with little cognitive improvement in higher severity cases. This aspect implies the limitation of immunotherapy which is incapable of reversing the neuronal loss and cognitive impairment surfaced during the advanced stage of AD. Nano-based therapies, on the other hand, offer a promising resolution due to the high surface-to volume ratio and lipophilic nature that made safe penetration through the blood-brain barrier for drug deliverance possible. Given the positive physicochemical properties demonstrated by nanoengineered systems, lots of research have reported to successfully modified nanoparticles into enveloping highly antioxidant or anti-inflammatory bioactive compounds into targeted brain sites for preventive and control of neurological disorders [325]. Nonetheless, the formulations of nanoparticles are still in need of improvement due to the associated potential toxicity which imparted health concern as demonstrated in several *in vitro* and *in vivo* studies [326,327]. The remarkably high price subjected to nanotherapy rendered it not easily available for majority of the population, leaving them no choice but to go for other cost-effective treatment options instead. It is therefore concluded that extensive research and price control are necessary to further explore and tap on the effectiveness of each therapeutic approach in addition to evaluating the possible efficacies of combinatorial application of all three elements together against AD. Though we may still have a long journey to go judging by the current development status, as time progresses, more studies have been explored to understand the core pathway of AD and we are hopeful that these will help aid in the progress of discovering new therapeutic treatment that should eradicate AD.

8.1. Systemic review

We browsed and searched through PubMed and Scopus, looking for review articles, research articles and studies about clinical trials relevant in the fields of novel treatments against Alzheimer's disease. Our searches involved information of articles ranging from the year 1964 to 2020.

CRediT authorship contribution statement

Se Thoe Ewen, Ayesha Fauzi: Literature searches, Writing-Original draft preparation. **Tang Yin Quan**, **Sunita Chamyuang**: Writing-reviewing and Editing. **Adeline Chia Yoke Yin**: Reviewing and Editing, Funds collection, Project Conceptualization

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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