Review

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Research Progress on Alzheimer's Disease Biomarkers Based on the ATX(N) Framework

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Early diagnosis and treatment of AD are of paramount importance, with the concept of biomarkers being intrinsically linked to diagnosis and therapy. Biomarkers are indices that can be objectively measured to indicate normal biological processes, pathological conditions, or responses to therapeutic interventions. In 2023, the National Institute on Aging and Alzheimer's Association released updated clinical diagnostic guidelines, refining the 2018 research framework. These guidelines categorize AD biomarkers into three types: core AD biomarkers, non-specific biomarkers of tissue response related to AD pathophysiology, and biomarkers for non-AD comorbidities, thus enhancing the amyloid/tau/x/neurodegeneration (ATX(N)) framework. This article aimed to provide a comprehensive overview of the advancements within the ATX(N) framework and the progress in the study of various biomarkers under this framework. It analyzes how biomarkers can facilitate early disease diagnosis, discusses the challenges of translating biomarkers into effective treatments, and explores their therapeutic prospects.

Keywords: Alzheimer's disease; ATX(N); cerebrospinal fluid; blood; positron emission tomography; biomarkers

Introduction

Alzheimer's disease (AD) is an irreversible, progressively worsening neurodegenerative disorder primarily affecting older adults. Currently, around 36 million individuals globally are affected by AD, and this figure is anticipated to soar to 115 million by 2050 [1]. The main pathological features of AD include the formation of intracellular neurofibrillary tangles (NFTs) and extracellular amyloid plaques (APs). These pathological changes lead to neuronal damage and death, resulting in the loss of brain function [2,3]. AD not only severely impacts the patients themselves but also imposes a significant economic and psychological burden on families and society. Consequently, early diagnosis and intervention are of paramount importance in mitigating the disease's impact. In recent years, with the advancement of biomedical technology, research on AD has made remarkable progress, particularly in the exploration of biomarkers. In 2011, the National Institute on Aging and the Alzheimer's Association (NIA-AA) introduced specific diagnostic criteria for the symptomatic stages of AD, such as mild cognitive impairment (MCI) and dementia. Additionally, they provided guidelines for the AD stage in individuals who do not yet show symptoms, known as "preclinical AD" [4]. In 2018, the NIA-AA proposed the amyloid/tau/neurodegeneration (AT(N)) framework to categorize biomarkers and stratify patients with AD based on biomarker characteristics [5]. Cerebrospinal fluid (CSF) and imaging biomarkers were included and classified into three categories: "A" represents amyloid-beta (Aβ) deposition, including amyloid positron emission tomography (PET) ligand binding and low CSF amyloid-beta42 (Aβ42) or low CSF amyloid-beta42/40 (Aβ42/40) ratio; "T" represents pathological tau, including elevated CSF phosphorylated tau (p-tau) and tau PET ligand binding; "N" represents neurodegeneration, including total tau (t-tau) in CSF, decreased metabolism on fluorodeoxyglucose PET, and medial temporal lobe atrophy on magnetic resonance imaging (MRI).

In 2018, the framework recognized the necessity to revise the AT(N) biomarker classification scheme to incorporate newly developed biomarkers into the existing AT(N) categories. Conceptually, it may be beneficial to consider amyloid/tau/x/neurodegeneration (ATX(N)), where "X" represents a series of biomarkers for a specific pathological process, anticipated to be attainable in the future, and (N) denotes the cumulative brain injury caused by all etiologies. It is important to note that the 2018 research framework is not applicable to routine clinical practice. It is designated as a "research framework" because it requires thorough evaluation and potential modification before it can be integrated into everyday clinical practice [5]. As research progressed, the clinical diagnostic framework was updated in 2023 with additional blood-based

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biomarkers (BBBMs), categorizing AD biomarkers into three groups: core AD biomarkers, non-specific biomarkers of tissue response related to AD pathophysiology, and biomarkers for non-AD comorbidities. This update led to the ATX(N) framework, which is more favorable for early diagnosis and differential diagnosis. "X" includes emerging biomarker categories beyond A, T, or N, representing potential and promising AD biomarkers. The new framework's "X" has three categories: "I" indicates astrocytic activation, including glial fibrillary acidic protein (GFAP); "V" indicates vascular brain injury including white matter hyperintensity (WMH), anatomical brain infarcts, and widespread perivascular space dilation; "S" indicates synucleinopathy, including CSF α-synuclein seed amplification assay (αSyn-SAA). The biomarkers mentioned above are broadly recommended for clinical diagnosis, whereas others are currently suitable for AD research and may be implemented in future clinical practice. Under the framework of ATX(N)-based biomarkers, disease-modifying therapies (DMT) for AD have emerged as a focal point of contemporary research. Therefore, this review aims to elucidate the research advancements of various biomarkers within the ATX(N) framework and their significance for the diagnosis and treatment of AD.

In 2011, the NIA-AA first issued diagnostic criteria for AD, highlighting the importance of clinical symptoms and signs in disease diagnosis [4]. However, with advancements in science and technology, particularly the application of biomarkers in AD research, the 2018 NIA-AA research framework was further updated to emphasize the central role of biomarkers such as Aβ, tau protein, and neurodegeneration in the diagnosis of AD [5]. However, the framework inherently assumed that CSF and imaging biomarkers were equivalent in detecting these pathological processes. This assumption has sparked controversy in clinical practice because the detection methods and sensitivities of different biomarkers can vary significantly [6]. In these updated standards, we have eliminated the assumption that imaging and biofluid biomarkers are equivalent within any given biomarker category. Within the framework of the new paradigm, a distinction is drawn between imaging and fluid biomarkers. Imaging biomarkers are characterized by their ability to provide detailed visuals of brain structure and function, capturing the topography of AD lesions and mapping them onto known neuropathological structures, thereby aiding in pinpointing the exact location and extent of the pathology. Fluid biomarkers, on the other hand, reflect the pathological processes of AD by detecting specific molecules in bodily fluids. They are non-invasive, easy to handle, and useful for early screening and monitoring disease progression. Notably, BBBMs have been incorporated into this new framework. The regulatory approval of targeted therapies for AD represents a significant milestone. The potential incorporation of these treatments into clinical practice necessitates a thorough reassessment by the industry, academia, and clinicians concerning biomarker classification, AD diagnosis, and biologically-based staging of AD. Consequently, an essential new direction is to enhance the 2018 framework, shifting from a purely research-oriented focus to providing guidelines applicable to research and clinical practice [7].

A notable modification in the recent framework is the categorization of core biomarkers into two distinct groups [7]. Core-1 biomarkers outline the early phases of detectable AD *in vivo* and can detect AD in symptomatic and asymptomatic individuals. Core-2 biomarkers become abnormal during the later stages of AD progression and are more closely related to the emergence of symptoms compared to Core-1 biomarkers. When combined with Core-1, Core-2 biomarkers can be used to stage the biological severity of the disease.

Core AD Biomarkers

Aβ Pathology

Aβ accumulation is one of the most significant pathological features of AD and plays a decisive role in its pathogenesis. Aβ is generated through the enzymatic cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase. APP, a glycoprotein composed of roughly 770 amino acids, is located in the cell membranes of various cells, including neurons [8]. The abnormal aggregation of APP into APs is a hallmark pathological feature of the AD brain. APP is processed differently in the non-amyloidogenic and amyloidogenic pathways, with the initial cleavage step being the key distinguishing factor [9]. In the amyloidogenic pathway, APP is initially cleaved by β-site APP cleaving enzyme 1 (BACE1) at the β-site. BACE1, a β-secretase, plays a crucial role in APP metabolism and has been well-characterized [10]. This enzymatic action results in the production of a C-terminal fragment known as C99 within lysosomes and an N-terminal fragment termed soluble amyloid precursor protein beta (sAPPβ) which is secreted extracellularly.

In contrast, the non-amyloidogenic pathway involves α-secretase cleaving the Aβ sequence in the middle, producing the soluble N-terminal fragment soluble amyloid precursor protein alpha (sAPPα) and leaving the C-terminal fragment C83 on the cell membrane. Following this, C99 is further processed by γ-secretase at the γ-site, releasing Aβ and the APP intracellular domain. Aβ exists in various peptide lengths, with amyloid-beta40 (Aβ40) and Aβ42 being the most common. Aβ42 has a lower water solubility than Aβ40, which may contribute to its higher aggregation tendency. Aβ monomers can self-aggregate into oligomers, further forming fibrils and mature fibers, ultimately leading to AP formation in the brain, a characteristic hallmark of AD pathology [11].

Measurement of Aβ plays a critical role in AD diagnosis. Aβ peptide levels are typically assessed through CSF

samples, providing a direct reflection of central nervous system Aβ concentrations. PET is also used to visually observe AP deposition *in vivo*. Additionally, plasma Aβ detection has been included in the latest diagnostic framework.

Aβ-PET

With the development of Aβ-PET, the amyloid hypothesis has gained supportive evidence. Aβ-PET, using specific tracers (Table 1, Ref. [12–17]) can visually display the deposition of Aβ *in vivo*. These tracers bind to APs in the brain, and PET scans visualize this binding, thus aiding physicians in assessing the presence and extent of AD [18]. Systematic reviews and meta-analyses have shown that Aβ-PET has high sensitivity and specificity in distinguishing patients with AD from healthy controls (HC) and predicting the progression of MCI to AD dementia. A study indicates that Aβ-PET has a sensitivity ranging from 89 to 97% and specificity between 63 and 93% in identifying differences between patients with AD and HCs [19], demonstrating that Aβ-PET is an effective tool for identifying AD biomarkers at the preclinical stage, thereby enabling early diagnosis and treatment.

The introduction of the probe [11C]-PIB marked a pivotal advancement in the investigation of Aβ PET imaging agents [12]. [11C]-PIB exhibits a high affinity for APs and has been extensively employed in clinical trials. However, the short half-life of [11C] restricts its widespread clinical utility. To address this challenge, researchers have developed [18F]-labeled probes [20], including [18F]-Florbetapir ([18F]-AV45, AmyvidTM), [18F]-Florbetaben (NeuraceqTM), and [18F]-Flutemetamol (VizamylTM). These probes have longer half-lives, making them more suitable for broader clinical applications. Notably, [18F]-AV45 distinguishes itself as an Aβ-PET probe by offering the substantial benefit of specifically targeting APs in the brain, thereby enabling a non-invasive approach for the early diagnosis of AD.

Despite the significant promise of [18F]-AV45 in diagnosing AD, it encounters several hurdles. One of its primary advantages lies in the production of high-quality images and its strong affinity for APs, which allows for the visual detection of Aβ accumulation in the brain through PET. This capability is instrumental in facilitating early diagnosis and ongoing disease monitoring [21]. Moreover, its relatively short half-life of around 110 min offers a degree of operational flexibility in clinical settings, permitting rapid imaging and minimizing patient wait times.

However, [18F]-AV45 has its limitations. Although it binds specifically to APs, its ability to differentiate AD from other neurodegenerative diseases is limited, because these diseases might also exhibit Aβ accumulation, potentially leading to misdiagnosis [22]. Moreover, interpreting [18F]-AV45 PET images requires a high level of expertise and experience, with image quality influenced by various factors such as equipment performance, operational accuracy, and professional judgment of the interpreter.

Discrepancies often arise during standardized uptake value ratio (SUVr) measurement with [18F]-labeled tracers owing to differences in binding characteristics and kinetics among the tracers, affecting cross-tracer and cross-center comparability. To address this, the Centiloid method was developed, standardizing SUVr values across different tracers on a unified scale, enhancing comparability [23]. The Centiloid method uses conversion equations to linearly transform SUVr values to a Centiloid scale anchored by average SUVr values from young controls and patients with AD [24]. It also accounts for variability in PET scanner performance and image reconstruction parameters, standardizing image and data processing to improve measurement consistency and repeatability [25].

The Centiloid method has been employed to assess the consistency of various analysis techniques in amyloid PET imaging. Despite minor variations in absolute Centiloid scores, the overall consistency suggests that these methods can be used interchangeably [26]. This research indicates that the Centiloid method effectively improves the comparability of measurements across different tracers and imaging centers, thereby promoting global standardization and comparability of Aβ-PET results.

Researchers are persistently investigating novel probe molecules to enhance sensitivity and specificity in diagnostic imaging. One such example is [18F]-FMAPO, which exhibits a high affinity for APs with superior imaging properties in preclinical trials. Furthermore, probes such as [124/125I]-IBETA and [64Cu]-YW-7 have shown significant potential in applications, aiding not only in the detection of APs but also in providing essential data for early diagnosis and treatment monitoring [27].

Future developments might focus on optimizing the chemical structures of probes to increase their binding affinity for APs, thereby enhancing imaging clarity and accuracy. The selection of radioisotopes is also a pivotal factor. Although [11C] and [18F] are frequently utilized in clinical settings, their limited half-lives restrict the range and duration of probe applications. Considering isotopes with longer half-lives, such as [64Cu] and [124I], could broaden the utility of these probes, enabling long-distance transportation and prolonged imaging sessions. It is crucial to ensure the safety and minimal toxicity of these probes through extensive preclinical and clinical evaluations to safeguard patient health prior to clinical implementation.

CSF Aβ

Low CSF Aβ42 concentration is used as a biomarker to detect AD at all clinical stages, including preclinical, prodromal, and dementia phases. A study indicates that this biomarker exhibits high sensitivity for detecting cortical Aβ deposition, with an average sensitivity exceeding 90%. Research shows that patients with AD have reduced

Table 1. Classification and key characteristics of Aβ tracers.

Tracer name	Research stage	Main characteristics
[18F]-Florbetapir	preclinical and clinical	high binding affinity to APs, better clearance rates, less *in vivo* defluorination and longer half-lives [13]
[18F]-Florbetaben	clinical	high cortical uptake in AD patients, less nonspecific bindings in myelin-rich regions [14]
[11C]-PIB	clinical	reliable at detecting fibrillar Aβ pathology, weaker connection with true AD pathology [12]
[18F]-THK-5351	clinical	correlates more closely to clinical symptoms and neurodegenerative processes than Aβ pathology [15]
[18F]-92	first-in-human	low white matter uptake binds to Aβ pathology, distinguishes AD from healthy controls [16]
[18F]-91	clinical	dimethylamino-modified tracer, higher standardized uptake value ratios in AD patients [14]
[18F]-BIBD-124	preclinical	better clearance rates, less *in vivo* defluorination, and higher imaging contrast [13]
[18F]-BIBD-127	preclinical	similar binding sites to [18F]-AV45, better clearance rates, and less *in vivo* defluorination [13]
[18F]-RO948	clinical	high accuracy in the differential diagnosis of dementia patients [17]
[18F]-MK6240	clinical	high accuracy in the differential diagnosis of dementia patients [17]
[18F]-Flortaucipir	clinical	high accuracy in the differential diagnosis of dementia patients [17]
[18F]-Florbetazine	clinical	higher cortical uptake in AD patients, less nonspecific bindings in myelin-rich regions [14]

AD, Alzheimer's disease; Aβ, amyloid-beta; APs, amyloid plaques.

CSF Aβ42 levels, whereas Aβ40 levels remain relatively stable. Therefore, the lowered CSF Aβ42/Aβ40 ratio is associated with AD pathology [28].

Plasma Aβ

BBBMs are highly esteemed for their affordability, convenience, and non-invasive nature. These markers enable swift detection without the need for costly or intricate equipment, making them integral to multi-step diagnostic processes. Recent diagnostic frameworks have underscored the significance of BBBMs, especially in the context of AD diagnosis. Previous research findings indicate that changes in plasma Aβ may be detected earlier than the typical detection thresholds of Aβ PET imaging [29,30]. One pivotal method involves measuring the Aβ42/Aβ40 ratio in the blood. Several advanced techniques have been developed for this purpose. For instance, single-molecule array technology significantly improves the sensitivity of detecting low-abundance proteins, which is crucial for identifying early disease markers.

Additionally, immunoprecipitation coupled with mass spectrometry and liquid chromatography-mass spectrometry allow for the precise analysis of proteins and peptides, facilitating comprehensive biomarker exploration in blood samples [31]. Immunomagnetic reduction technology utilizes magnetic nanoparticles to label specific proteins, enabling rapid detection without the need for elaborate equipment. Lastly, stable isotope labeling kinetics offers methodologies to quantify protein metabolism dynamics, providing insights into biomarker fluctuations under disease conditions.

These technological advancements significantly promote the application of BBBMs in disease diagnosis, particularly in early AD diagnosis and monitoring. However, plasma Aβ is associated with challenges, especially in distinguishing Aβ-positive from Aβ-negative individuals. The minor concentration variations in plasma Aβ can make differentiation difficult [32]. The low stability of the plasma Aβ42/Aβ40 ratio poses significant challenges for using it as a clinical AD biomarker, as slight assay performance shifts or pre-analytical changes may lead to misclassification [33].

The plasma Aβ42/Aβ40 ratio might reflect Aβ production in peripheral tissues, unrelated to cerebral Aβ pathology, hence being less robust than CSF Aβ42/Aβ40 as a biomarker of cerebral Aβ pathology [34]. Enhancing the robustness and reproducibility of plasma biomarker assays and minimizing technical variation errors are key to improving clinical application success rates. Strict pre-analytical and analytical protocols can help mitigate robustness issues.

Advances in Aβ-Based Immunotherapy

In recent years, the scientific community has developed various drugs targeting Aβ for the treatment of AD. These drugs primarily aim to reduce Aβ production, inhibit Aβ aggregation, and promote Aβ clearance. Despite these efforts, many Aβ-targeted therapies have failed to show the expected efficacy in clinical trials, leading to the re-evaluation of Aβ as a therapeutic target [35].

Methods to promote Aβ clearance, such as immunotherapy, aim to activate the immune system to clear Aβ from the brain. Aβ immunotherapy primarily includes two types: vaccines (active immunotherapy) and exogenous antibodies (passive immunotherapy). Active immunotherapy stimulates the body's immune system to produce antibodies against Aβ, whereas passive immunotherapy involves directly administering specific anti-Aβ an-

tibodies to the patient for rapid therapeutic effect. Each method has its advantages and limitations, and the choice depends on the patient's specific condition and treatment needs.

Active Immunization. AN1792 was the first experimental vaccine targeting Aβ. Unfortunately, its application led to severe side effects. Approximately 6% of vaccinated patients developed T-cell-mediated meningoencephalitis, a severe inflammatory response possibly related to T-cell epitopes in the vaccine, leading to the termination of clinical trials [36]. Subsequent research on amilomotide (CAD106) demonstrated good safety and acceptable antibody response in Phase I trials. Further Phase II trials, including IIa and IIb, indicated a balanced antibody response and tolerance. However, CAD106 led to unpredictable changes in cognitive function, brain volume, and body weight in Phase II/III trials, causing premature termination of the study.

Passive Immunization. Two monoclonal antibodies, aducanumab (BIIB037; Aduhelm™; Biogen, Cambridge, MA, USA) and lecanemab (BAN2401; Leqembi®; Biogen, Cambridge, MA, USA), have received accelerated approval from the U.S. Food and Drug Administration (FDA) for treating early patients with AD having confirmed Aβ. Aducanumab is used in DMT for AD and is a humanized monoclonal antibody. In June 2021, aducanumab received FDA accelerated approval, becoming the first monoclonal antibody targeting Aβ and the first approved AD DMT. The drug is intended for patients with early stages of AD, particularly those with MCI or mild dementia, who show clear evidence of Aβ deposition on PET or CSF analysis. The EMERGE and ENGAGE Phase III global, randomized, double-blind, placebo-controlled studies showed aducanumab's potential in treating early AD. The EMERGE study demonstrated a 22% improvement in the Clinical Dementia Rating-Sum of Boxes (CDR-SB) score change with high-dose aducanumab compared to that with a placebo [37]. Despite showing positive effects in some studies, aducanumab's safety profile raised concerns. The most common adverse event was amyloid-related imaging abnormalities (ARIA), including cerebral edema and cerebral hemorrhage, particularly notable in the high-dose treatment group [38]. In the EMERGE and ENGAGE studies, about 40% of participants experienced ARIA, with around a quarter showing related symptoms such as headache, confusion, dizziness, and nausea.

Lecanemab received FDA accelerated approval in January 2023, followed by full approval in July 2023. In an 18-month Phase III clinical trial involving 1795 early patients with AD, participants were randomly assigned to receive lecanemab or a placebo. The results showed significantly lower CDR-SB score changes in the lecanemab group compared to that in the placebo group (difference of –0.45; 95% confidence interval [CI], –0.67 to –0.23; $p < 0.001$), indicating potential efficacy in slowing disease progression. The reduction in brain Aβ burden in the lecanemab group was also confirmed. In an associated substudy with 698 participants, lecanemab showed a significant reduction in brain Aβ burden compared to placebo (difference of –59.1; 95% CI, –62.6 to –55.6) [30]. This further validated lecanemab's potential therapeutic effect on AD at the biomarker level. In terms of cognitive function, lecanemab also outperformed placebo, with more pronounced improvements in the AD assessment scale-cognitive subscale-14 score changes (difference of –1.44; 95% CI, –2.27 to –0.61; $p < 0.001$), indicating its ability to improve patient cognitive function. However, safety concerns remain, with reported adverse events including infusion-related reactions and ARIA. Among lecanemab-treated patients, approximately 26.4% experienced infusion-related reactions, and 12.6% experienced ARIA, necessitating strict monitoring and management during clinical application.

Other treatments, such as gantenerumab (RO4909832; R1450; Roche, Basel, Switzerland), failed to meet primary endpoints in Phase III trials, resulting in trial termination and project discontinuation. Donanemab (LY3002813; N3pG; Eli Lilly and Company, Indianapolis, IN, USA) completed a Phase III study for early AD treatment and is currently under FDA review for clinical data supporting standard approval. Emerging anti-Aβ immunotherapies are ongoing, with ACU193, a monoclonal antibody, undergoing multicenter Phase I trials for MCI and mild AD [39]. Patients are being recruited for a randomized, double-blind, placebo-controlled Phase III study with remternetug (LY3372993; Eli Lilly and Company, Indianapolis, IN, USA), another monoclonal antibody.

Challenges and Therapeutic Prospects of Aβ-Based Immunotherapy. Despite most trial compounds showing strong target engagement, as indicated by reductions in brain Aβ through PET imaging and CSF biomarkers, all failed anti-Aβ trials share a common characteristic: they did not significantly enhance cognitive function in AD patients. This discrepancy implies that these biomarkers are not reliable surrogate indicators of therapeutic effectiveness in clinical trials. More importantly, this raises the question of whether targeting Aβ accumulation is suitable for modifying the disease after clinical symptoms have appeared.

Although clinical trials with Aβ-targeted therapies have made some progress, their effectiveness in enhancing cognitive function has been disappointing. This gap between biomarker changes and cognitive improvement could be owing to various factors. First, eliminating APs does not necessarily result in an immediate cognitive improvement. The accumulation of APs is a long-term process, whereas cognitive decline may involve multiple pathological mechanisms such as neuroinflammation, oxidative stress, and neuronal damage [40]. Therefore, removing APs might not

be enough to reverse the already established neural damage and functional impairments. Second, the timing of Aβ-targeted therapy is crucial. Many clinical trials start only after patients show significant cognitive deficits, by which time irreversible brain damage may have already occurred. Early intervention might be more effective, but current diagnostic technologies struggle to accurately identify AD patients at an asymptomatic stage. Additionally, the side effects of Aβ-targeted therapies may impact their clinical efficacy. For example, some Aβ antibody treatments might cause cerebral edema and microhemorrhages, which could not only negate potential cognitive benefits but also worsen the condition [41]. Such side effects complicate the balance between the risks and benefits of the treatment. Last, individual differences are also significant. Patients may respond differently to Aβ-targeted therapies owing to genetic background, disease stage, and other comorbidities [42]. These individual variations complicate the interpretation of clinical trial results and add to the uncertainty of therapeutic outcomes.

AD Tauopathy

CSF-tau

Recent research indicates that different forms of p-tau in CSF, such as p-tau181, p-tau217, and p-tau231, exhibit varying diagnostic performance and associations with AD pathological features. A study explored the patterns of these new CSF p-tau biomarkers (p-tau181, p-tau217, and p-tau231) during the early stages of AD when only mild Aβ pathology is detected. The findings indicated a significant rise in these p-tau biomarkers during the preclinical phase of AD, effectively differentiating between cognitively unimpaired individuals who are Aβ-positive and those who are Aβ-negative. This evidence reinforces the hypothesis that early alterations in tau metabolism, prompted by Aβ exposure in preclinical AD, can be detected using these advanced p-tau assays [43].

CSF p-tau181 is highly specific for AD, aiding in differential diagnosis. However, a study indicates that the increase in CSF p-tau217 during AD progression is significantly greater than that in CSF p-tau181, suggesting that CSF p-tau217 may outperform in diagnosing early and late-stage AD [44]. Barthélemy *et al.* [45] extended monitoring of multiple p-tau sites in CSF, observing changes in p-tau217, p-tau181, and p-tau205 beginning 21, 19, and 13 years before expected symptom onset in patients with dominantly inherited AD. These data indicate that changes in p-tau217 are closely related to early AD pathology. A study from the Swedish BioFINDER cohort demonstrated that p-tau217 in CSF strongly correlates with tau PET tracer [18F]-flortaucipir, accurately identifying individuals with elevated [18F]-flortaucipir retention. This suggests that CSF p-tau217 may be more useful than traditional CSF p-tau181 in diagnosing AD [46]. Additionally, p-tau217 shows a better correlation with the new cortical Aβ burden measured by PET, more accurately distinguishing AD dementia from non-AD neurodegenerative diseases. Current research indicates that CSF p-tau217 outperforms CSF p-tau181 in diagnosing AD pathology [47].

Novel CSF p-tau biomarkers are emerging, such as CSF p-tau235, a specific AD biomarker and a promising early progression biomarker. p-tau235 levels increase in CSF during early AD. Changes in p-tau235 and p-tau231 levels during preclinical AD align with sequential phosphorylation evidence in the AD brain. Consequently, CSF p-tau235 serves as a reliable marker for tracking disease progression and enhancing clinical trial recruitment [48]. Additionally, recent advancements have introduced the first high-throughput CSF p-tau205 immunoassay. This assay identifies CSF p-tau205 as a biomarker for AD tau pathology, facilitating *in vivo* quantification of tau pathology. Moreover, it represents a potentially cost-effective alternative to tau PET imaging in clinical settings and trials [49]. Another study emphasized that CSF p-tau217 and p-tau205 are improved biomarkers for detecting Aβ and tau pathology in AD compared to currently available tests [50]. These findings highlight the significance of CSF p-tau205 in diagnosing and monitoring AD. In the latest framework, p-tau205 is considered a T2 fluid biomarker, with a higher correlation to tau PET than Aβ-PET. p-tau368, a tangle-rich fragment, reflects tangle pathology in the CSF p-tau368/tau ratio, making it a promising biomarker for improving AD diagnosis and aiding drug development targeting tau pathology [51].

The utilization of t-tau in tracking the progression of AD has its constraints. Primarily, increased t-tau levels are not exclusive to AD; they are also present in other neurodegenerative disorders, complicating the differentiation of AD from these conditions based solely on t-tau measurements. Moreover, the concentration of t-tau in CSF is influenced by various factors, such as age, sex, and other medical conditions, which can undermine its accuracy and reliability in monitoring AD. Additionally, the method of detecting tau in CSF requires a lumbar puncture, an invasive procedure. Consequently, there is a growing interest in blood-based p-tau as an alternative biomarker.

Plasma Tau

Research on plasma p-tau subtypes has only recently started to surface, and the literature in this area is growing quickly. Much like the findings in CSF, elevated levels of p-tau subtypes appear early in the progression of AD and continue to rise as the disease advances along the AD continuum [52–54]. Plasma p-tau181 exhibits significant diagnostic efficacy in differentiating AD from frontotemporal lobar degeneration (FTLD), particularly in single-center clinical cohorts [55]. However, although plasma p-tau181 levels are associated with AD progression, they fail to serve as an independent predictor of disease trajectory [56]. Research further indicates that among non-demented

older adults, apolipoprotein E ε4 (APOEε4) carriers have p-tau181 levels that correlate with cognitive performance. This suggests a link between p-tau181 levels, the transition from MCI to AD, and subsequent cognitive decline [57]. Additionally, plasma p-tau217 levels vary significantly across different stages of AD and are closely associated with cerebral Aβ accumulation [58].

Plasma p-tau217 demonstrates high accuracy in distinguishing AD from other neuropathological disorders such as FTLD. Research indicates that plasma p-tau217 provides more accurate diagnostic information compared to plasma p-tau181, neurofilament light (NfL), and MRI measurements. Additionally, plasma p-tau217 levels show high consistency with CSF p-tau and tau PET results, highlighting its potential utility in diagnosis that is nearly comparable to these traditional measurement methods [59]. Higher baseline plasma p-tau217 levels independently predict disease progression, including brain atrophy, tau deposition, and cognitive decline. This emphasizes plasma p-tau217's role in initial diagnosis, disease monitoring, and progression assessment. Despite all plasma p-tau assays demonstrating a high diagnostic accuracy, plasma p-tau217 markedly distinguishes between AD and non-AD conditions, aligning more closely with the progression of AD [60]. This indicates that plasma p-tau217 serves as a more precise biomarker for identifying AD and its progression stages. Research indicates that plasma p-tau181, p-tau217, and p-tau231 begin to change on the abnormality of Aβ-PET, with p-tau231 changing the earliest among these markers [54]. Studies suggest that changes in plasma p-tau231 occur before those in p-tau217 and p-tau181, thus supporting the observations made in CSF [43,61,62]. Furthermore, plasma p-tau231 exhibits a strong correlation with CSF p-tau231, tau pathology as evaluated by [18F]-MK-6240-PET and Aβ pathology assessed by [18F]-AZD469-PET [63]. The concentration of p-tau species in plasma is lower compared to that of amyloid peptides, with p-tau181 and p-tau 231 showing similar levels of abundance, whereas p-tau 217 demonstrates a much lower concentration [64]. Despite its lower concentration and potential detection challenges, p-tau 217 holds valuable diagnostic potential.

Although plasma p-tau shows potential in AD monitoring, challenges remain in its practical application. First, plasma p-tau measurement is impacted by various factors, including sample handling, assay methods, and patient biological differences, affecting accuracy and reproducibility. Moreover, further research and validation are needed to enhance plasma p-tau's ability to differentiate AD from other neurodegenerative diseases. Despite a study indicating plasma p-tau's high specificity [65], determining a method to accurately distinguish AD from other neurodegenerative diseases remains a key research goal. There are currently several high-performance plasma p-tau immunoassays with comparable efficacy, indicating a promising potential for successful clinical implementation [66,67]. Future research should aim at promoting the broad clinical application and practical benefits of plasma p-tau.

Tau-PET

Tau-PET imaging employs specialized radioactive tracers, among which the first-generation tracers such as [11C]-PBB3, [18F]-AV1451, and [18F]-THK5351 have been extensively analyzed *in vitro* and *in vivo*. These tracers possess the ability to bind specifically to tau protein tangles within the brain. Using PET scans, the spatial distribution and density of tau proteins can be visualized, providing valuable insights into neural pathology. Notably, [18F]-AV1451-PET has received FDA approval for clinical use in diagnosing AD by assessing NFTs [68]. The diagnostic accuracy of this tracer has been substantiated through an autopsy study exhibiting a sensitivity range from 92 to 100% and specificity between 52 and 92% [69]. Moreover, Passamonti *et al*. [70] highlighted its high specificity for AD-related tau pathology, as opposed to tau pathology associated with progressive supranuclear palsy (PSP).

Although [18F]-AV1451 reflects late tau pathology in AD, its detection capability for early pathology formation is weak, whereas CSF p-tau is more sensitive for early pathology detection. This difference may relate to the recognition capabilities of the methods used for the detection of aberrant tau protein forms [71]. Therefore, the potential value of [18F]-AV1451 in the early clinical pathology stages of AD requires further investigation. Specifically, clinical data on the binding patterns of [18F]-AV1451 with tau protein in patients with early stages of AD need to be validated for effectiveness and specificity. Additionally, more research is required to evaluate the performance of [18F]-AV1451 across different AD subtypes, aiming to enhance its application in early diagnosis and disease monitoring in real-world settings. In the individuals with Aβ-PET-positive signal, those with MCI, or in patients with AD dementia, plasma p-tau217, and tau PET, particularly [18F]-AV1451-PET, show a strong correlation [72]. These findings emphasize the potential utility of plasma p-tau217 and [18F]-AV1451-PET as biomarkers in the clinical assessment of Aβ-positive patients with cognitive impairment. Compared with CSF p-tau, the uptake of [18F]-AV1451 is a more precise predictor of cognitive decline and neurodegeneration in patients with AD [73]. Nonetheless, an increase in CSF p-tau levels occurs before the positivity of [18F]-AV1451-PET at the onset and progression of cognitive decline associated with AD [74].

New-generation tau radioactive tracers, such as [18F]-MK-6240, [18F]-RO-948, [18F]-PM-PBB3, [18F]-GTP-1, and [18F]-PI-2620, exhibit less off-target binding and high specificity for tau neurofibrils in the human study [75]. These characteristics suggest potential for AD diagnosis, especially in juxtaposition with other tau pathologies. Among them, [18F]-MK-6240 and [18F]-PI-2620 are being

widely focused on by clinicians. A head-to-head comparison of [18F]-AV1451, [18F]-MK-6240, and [18F]-PI-2620 tau PET tracers showed high consistency in binding characteristics. Consequently, all three tracers hold promise as *in vivo* biomarkers for tau protein aggregation in AD, despite differences in their non-specific binding profiles [76]. Another investigation revealed that [18F]-MK-6240 binds strongly to NFTs in AD while demonstrating negligible binding to tau aggregates in most non-AD tauopathies, or to lesions containing Aβ, α-synuclein, or TDP-43, suggesting its high specificity [77]. This characteristic makes [18F]-MK-6240 an effective instrument for assessing NFT buildup throughout AD. Furthermore, PET tracers [18F]-AV1451 and [18F]-MK-6240 show similar *in vivo* retention characteristics and comparable diagnostic efficacy, distinguishing patients with AD from those with no cognitive impairment [78]. Evaluations of [18F]-MK-6240's long-term test-retest reliability in HC and patients with MCI, as well as its longitudinal application in HC and patients with AD, support its utility in measuring longitudinal changes in NFTs [79]. The SUVr dynamic range of [18F]-MK-6240 in target regions is roughly double that of [18F]-AV1451, potentially offering a superior capability to detect early tau pathology or to conduct longitudinal studies that observe minor changes over time, thus overcoming the limitation of [18F]-AV1451 in identifying early AD pathology.

The distinct advantages of [18F]-PI-2620 PET imaging technology have captured significant attention for its ability to detect tau protein deposition in AD with high sensitivity and specificity. [18F]-PI-2620 PET effectively differentiates patients with AD from HC, notably in the challenging early stages of AD and in other neurodegenerative disorders [80]. Furthermore, Blazhenets *et al.* [81] demonstrated that [18F]-PI-2620 PET not only produced strong signals in patients with AD but also showed distinct binding patterns in non-AD tauopathies. For example, in patients with PSP and corticobasal syndrome, [18F]-PI-2620 primarily exhibited increased uptake in the globus pallidus, corresponding with the clinical and pathological characteristics of these conditions. This feature may aid clinicians in distinguishing AD from other tauopathies in a clinical setting.

The study points out that [18F]-PI-2620 PET is highly effective in detecting various tau protein isoforms, such as 3R and 4R tau, which are essential for accurate AD diagnosis and classification of pathological types. A significant challenge in the clinical application of [18F]-PI-2620 PET lies in its complex interpretation. The variability in tau protein distribution and accumulation patterns across individuals can make image analysis difficult. Furthermore, [18F]-PI-2620 PET may exhibit higher background signals compared to other PET tracers, potentially hindering the early detection of tau protein accumulation [82]. To overcome these challenges, combining [18F]-PI-2620 PET with other imaging modalities like MRI or CT could enhance diagnostic accuracy and reliability. Additionally, the development of more advanced image analysis algorithms could improve the accuracy and efficiency of interpreting [18F]-PI-2620 PET images [83].

Tau-PET exhibits high specificity and sensitivity in AD diagnosis. Current research primarily focuses on tackling off-target binding issues with tau PET tracers. An ideal tau PET tracer should possess key attributes such as high specificity and selectivity to minimize off-target binding, favorable pharmacokinetic properties for rapid distribution and clearance in the brain, and a high dynamic range and stability to yield reliable quantitative results. These characteristics would enhance the accuracy and reproducibility of PET imaging, playing a crucial role in diagnosing and studying AD and other neurodegenerative diseases.

MTBR-tau243

The microtubule-binding region (MTBR) of a tau protein-containing residue 243 (MTBR-tau243) is a novel CSF biomarker, emerging later and showing specificity for insoluble tau aggregates. Commonly used p-tau biomarkers such as p-tau181, p-tau217, and p-tau231 closely correlate with Aβ [84], whereas MTBR-tau243 has the strongest correlation with tau-PET and cognition, and the lowest with Aβ-PET. CSF MTBR-tau243 is a specific biomarker for tau aggregation pathology.

Horie *et al.* [85] evaluated the efficacy of MTBR-tau243 in comparison to other p-tau measurements in CSF, including p-tau181, p-tau205, p-tau217, and p-tau231. The findings revealed that MTBR-tau243 exhibited the strongest correlation with tau-PET imaging. Moreover, the study suggested that the combination of MTBR-tau243 and p-tau205 provides the most accurate prediction of tau-PET indices and cognitive decline. Consequently, the authors recommended considering MTBR-tau243 as a superior alternative to traditional p-tau measurements for AD fluid biomarkers.

In another investigation, the SuStaIn algorithm was employed alongside a spectrum of CSF biomarkers, such as MTBR-tau243, to develop an innovative disease-staging model. This model integrates biomarker levels and clinical data, yielding a more precise depiction of AD pathological progression [86]. Although present research concentrates on CSF MTBR-tau243, forthcoming studies might explore the possibility of detecting MTBR-tau243 in blood samples.

Although current studies show a high correlation of MTBR-tau243 with tau PET and cognitive measurements, further research is needed to understand its specific influence on AD pathology progression. Future research should explore the distribution of MTBR-tau243 in the AD brain and its specific links to pathological changes through more precise biomarker analysis and imaging technologies.

Tau Protein-Based Immunotherapy

In recent years, treatment strategies targeting tau protein have made some progress. Researchers have proposed various potential treatments, including inhibiting tau protein phosphorylation, proteolysis, and aggregation; promoting tau clearance intra- and extracellularly; and stabilizing microtubules. Currently, most tau-targeted drugs in clinical trials are immunotherapies.

The unsuccessful progression of anti-Aβ immunotherapy has prompted researchers to investigate immunotherapy approaches that target tau pathology. In this context, two vaccines, AADVac1 and ACI-35, are currently being developed as potential immunogens [87]. A randomized, placebo-controlled, double-blind, parallel-group, multicenter Phase II trial of AADVac1 in patients with mild AD revealed the vaccine's safety; however, it did not show a significant effect on cognitive assessments [88]. However, the trial for ACI-35S is still underway.

Passive immunotherapies targeting tau proteins in AD are advancing. Unlike active immunization, passive immunization offers benefits such as minimizing adverse immune reactions and enhancing specificity for particular epitopes. This specificity decreases the risk of targeting non-pathological tau proteins and allows for treatment adjustments based on different stages or types of tau pathology. Presently, several passive immunotherapies targeting tau are under development. Reports indicate that 14 anti-tau antibodies have entered human clinical trials, with nine specifically being tested for PSP and AD. These include semorinemab, bepranemab, E2814, JNJ-63733657, Lu AF87908, APNmAb005, MK-2214, PNT00, and PRX005. Despite this progress, none have reached Phase III clinical trials. Among them, semorinemab stands out as the most advanced anti-tau monoclonal antibody for AD treatment. The continued validation of passive immunotherapy for primary and secondary tauopathies depends on the outcomes of ongoing Phase I/II clinical trials [89].

The Challenges and Prospects of Tau Protein Immunotherapy

As of now, the completion of clinical trials is pending, which prevents us from drawing definitive conclusions about the therapeutic benefits of targeting tau pathology.

Tau protein exists in multiple isoforms, each with unique functions and pathological traits in different brain regions and at various stages of disease. This diversity complicates the precise targeting of pathogenic tau in immunotherapy while preserving the normal, functional tau. Moreover, pathological modifications of tau not only increase its heterogeneity but may also affect the specificity and effectiveness of immunotherapy [90]. Additionally, the complex mechanisms by which tau spreads between neurons make it difficult for single-target immunotherapies to completely stop tau propagation, thereby limiting their overall therapeutic efficacy [91].

Most mAbs that recognize N-terminal epitopes have not been validated successfully in clinical trials for anti-tau immunotherapy. The industry is now concentrating on developing mAbs that target the mid-region of tau or p-tau, which might be more effective in stopping tau seeding and propagation.

Non-Specific Biomarkers Involved in the Pathophysiology of AD

NfL

In the ATX(N) framework, t-tau is classified as a biomarker of neurodegeneration but lacks specificity for AD. As a result, NfL has gained attention as a biomarker for axonal damage and is frequently utilized in clinical and research settings. One study demonstrated that NfL levels were correlated with cognitive performance and pathological markers in the CSF of patients with AD [92]. Additionally, NfL levels are linked to the clinical progression of AD and can potentially aid in evaluating disease prognosis. Another study investigated the relationship between NfL and other AD biomarkers, finding that NfL levels correlated well with t-tau and p-tau in CSF [93]. This suggests that NfL is associated not only with the extent of neuronal damage but also with tau pathology in AD. Notably, elevated NfL levels are observed not only in AD but also in other neurodegenerative diseases such as amyotrophic lateral sclerosis, FTLD, traumatic brain injury, and peripheral neuropathy [94]. This indicates that NfL shows widespread changes across various neurological conditions. Furthermore, NfL levels are closely correlated with aging, necessitating consideration of age-related effects in clinical applications. Age-related variability might affect the specificity and sensitivity of diagnosing AD. As the research indicates, this characteristic of NfL could limit its effectiveness as a standalone biomarker [95], because age-related factors need to be considered when interpreting NfL levels. This factor limits NfL's specificity in diagnosing AD but underscores its value in staging and prognosis of the disease.

Biomarkers of Astrocyte Activation

GFAP

GFAP is an intermediate filament protein primarily expressed in astrocytes, which is related to cytoskeletal stability and glial cell activity regulation. Studies show that plasma GFAP levels significantly increase in patients with AD and positively correlate with Aβ deposition but are less associated with tau pathology [96]. Supporting this view, a recent study reported that lower CSF Aβ42/40 was directly correlated with higher plasma GFAP concentration [97]. GFAP is also involved in Aβ-induced neuroinflam-

mation, possibly exacerbating the pathological process by regulating immune cell activity and pro-inflammatory cytokine release. The study further found that plasma GFAP outperformed CSF GFAP in predicting Aβ-PET positivity [96]. GFAP expression changes are also correlated with other clinical features of AD. For instance, in autosomal dominant AD, GFAP concentration is higher in mutation carriers, detectable approximately 10 years before symptom onset, further supporting GFAP's potential as an early AD biomarker [98]. However, further validation with larger sample sizes is needed. Although GFAP is not specific to AD, its changes in AD are more significant compared to those in non-AD neurodegenerative diseases such as FTLD [99]. Prospective and longitudinal studies are needed to confirm GFAP's effectiveness in early AD diagnosis and disease course monitoring.

Other Astrocyte Activation Biomarkers

GFAP is currently the sole astrocyte activation biomarker recommended for clinical practice according to the 2023 NIA-AA guidelines. Nonetheless, additional astrocyte biomarkers within the ATI(N) framework possess substantial research significance despite not being endorsed for clinical use. Among these, S100B and YKL-40 stand out [100]. The role of S100B in AD diagnosis and monitoring remains ambiguous. First, serum S100B levels have been found to correlate with disease severity in patients with AD, suggesting its potential as a biomarker. However, its expression is susceptible to various factors, such as inflammation and neuronal injury, which can undermine its specificity and sensitivity for AD diagnosis [101].

Moreover, elevated S100B levels are not exclusive to AD and are observed in other neurodegenerative diseases, complicating its utility as an independent AD biomarker. Therefore, although S100B has shown some relevance in the context of AD, its clinical efficacy for diagnostic and monitoring purposes requires further investigation and validation. In contrast, the diagnostic value of YKL-40 in AD is hampered by its non-specific expression. To improve its diagnostic utility, YKL-40 may need to be combined with other biomarkers such as Aβ42/40, p-tau, and t-tau. Additionally, complement proteins found in astrocyte-derived exosomes (ADE) are being investigated as potential biomarkers for astrocyte activation in AD. Emerging research suggests that levels of ADE-derived C3b and C5b-9 are significantly elevated in patients with AD and are associated with cognitive decline [102].

Astrocytes engage in complex interactions with other glial cells, such as microglia, and neurons. These interactions are pivotal in AD development, although our understanding remains incomplete. The soluble triggering receptor expressed on myeloid cells 2 (sTREM2) is a biomarker for microglial activation that rises during the preclinical stages of AD. This increase might signify early microglial activation preceding the onset of AD [103]. Nevertheless, sTREM2 levels may decline during the later stages of AD, suggesting that microglial responses evolve as the disease progresses [104].

Additionally, sTREM2 is associated with various non-Aβ and non-tau CSF cytokines and complement factors, implying that cytokines and complements could serve as CSF biomarkers for astrocyte and microglial activation [105]. Research into microglial and astrocyte activation, particularly through PET imaging, is advancing. Notably, PET radioligands targeting monoamine oxidase-B and imidazoline 2 binding sites are undergoing clinical trials [106]. Despite the ongoing research, current ligands are not yet viable for clinical application. Future studies should focus on the interactions between astrocytes and other cell types. Improved biomarker specificity, enhanced detection techniques, and a deeper understanding of cell-cell interactions are essential for developing more effective strategies for early AD diagnosis and treatment.

Non-AD Comorbid Biomarkers

Cerebrovascular Injury

In the 2023 NIA-AA framework, cerebrovascular injury has been incorporated under the "V" classification, creating the expanded ATV(N) framework for AD. This framework includes anatomical brain infarcts, WMH, and significant perivascular space dilation. WMH are areas of increased signal intensity in the white matter observed on MRI, typically presenting as high signal on T2-FLAIR and T2-weighted images and appearing iso- or hypointense on T1-weighted scans [107]. These regions are commonly found in older adults and are generally linked to vascular disease. However, recent research indicates that WMH may also be associated with AD pathology. Evidence suggests that, in addition to vascular-origin WMH, there exists a subset of WMH, referred to as AD-related WMH, which is secondary to AD pathology. Therefore, two types of WMH exist in AD: vascular-related and non-vascular AD-related WMH.

In the dominantly inherited Alzheimer's network study, asymptomatic mutation carriers exhibited greater total WMH volume compared to non-carriers, with this increase becoming apparent approximately 6 years before expected symptom onset. The parietal and occipital lobes were most affected, with group differences noticeable approximately 22 years before onset [108]. This suggests that vascular mechanisms alone may not explain WMH volume differences. Another study indicated that mutation carriers could detect early WMH signs in middle age compared to non-carriers, further suggesting other non-vascular factors in WMH development [109]. These findings indicate that WMH appears earlier in AD than in normal aging and is partially independent of vascular risk factors, supporting the hypothesis that WMH contributes to AD pathology. WMH is correlated with other AD-related biomarkers, particularly

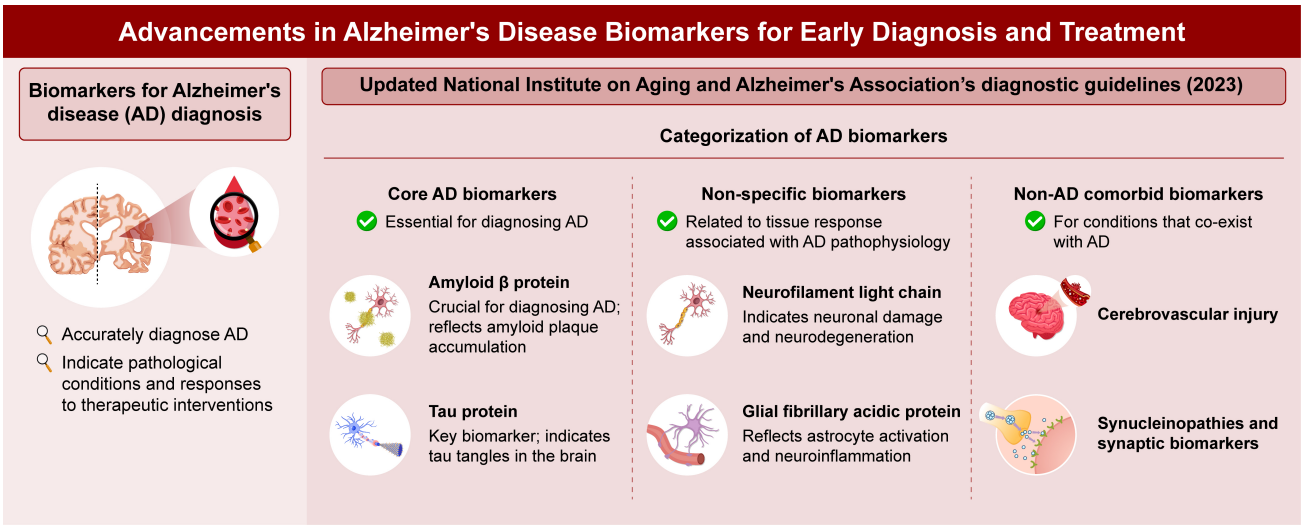

Fig. 1. Advancements in Alzheimer's disease biomarkers for early diagnosis and treatment (Created in Adobe Illustrator, Version 28.7, Adobe, San Jose, CA, USA).

Aβ. A 2019 study indicated that Aβ load was correlated with specific WMH topological patterns in non-demented older adults. Aβ-related WMH region closely related to lobar microbleeds indicated that cerebral amyloid angiopathy might contribute to the relationship between Aβ and WMH regions [110]. Specifically, Aβ42 predicts increased WMH regions, gray matter atrophy, and cognitive decline [109]. However, this study did not find a significant association between increased tau burden and WMH burden.

Although severe WMH load likely indicates a vascular origin, considering vascular involvement alone may overestimate its importance in AD-related WMH cases. At present, clinical practice lacks effective fluid biomarkers specifically for identifying cerebrovascular damage. Nevertheless, platelet-derived growth factor receptor-beta found in CSF is recognized in research settings as a potential biomarker.

Synucleinopathies

α-Synuclein is implicated not only in the pathogenesis of AD but also shows potential as a biomarker. Synaptic dysfunction is recognized as a key pathological mechanism in AD. Hence, by incorporating "S" for synucleinopathies into the ATX(N) framework, we propose an ATS(N) framework to underscore the importance of synaptic dysfunction in AD. Research has demonstrated that CSF levels of α-synuclein are elevated in patients with AD and correlated with tau and Aβ levels [111]. The CSF αSyn-SAA has garnered attention as a diagnostic biomarker for Parkinson's disease and dementia with Lewy bodies [112]. Recent studies suggest that concurrent CSF α-synuclein pathology may occur in a significant number of patients with AD even in the early stages, potentially influencing their clinical presentation. Longitudinal investigations are necessary to assess its importance in the progression of AD [113]. Currently, αSyn-SAA in blood has not been adopted in clinical settings. Efforts are underway to develop α-synuclein PET ligands, but no ligands are yet available for detecting α-synuclein comorbidity in patients with AD.

Other Neuronal Synaptic Biomarkers

Synaptic dysfunction is generally considered the most accurate indicator of cognitive decline associated with AD. Neurogranin (Ng), a marker of synaptic function, shows elevated levels in CSF correlated with cognitive decline in AD. This increase in Ng reflects a loss of Ng within the brain, confirming CSF Ng as a viable biomarker for AD and cognitive decline, even in healthy aging individuals [114]. Furthermore, Ng levels exhibit correlations with other biomarkers such as t-tau and p-tau. Notably, plasma Ng levels do not correspond with CSF Ng levels, likely owing to the presence of Ng synthesis in non-brain tissues. In addition to α-synuclein, attention is also directed towards β-synuclein. Elevated levels of β-synuclein are detected in the blood and CSF of patients with AD [115], with significant associations to brain atrophy observed in AD, distinguishing it from other neurodegenerative conditions such as FTLD [116]. Among patients with Lewy body disease (LBD), those with concomitant AD pathology (LBD/AT(N)+) demonstrate β-synuclein levels similar to those in patients with AD. In contrast, the LBD/AT(N)- subgroup, which lacks AD comorbidity, maintains normal β-synuclein levels [117].

Synaptic vesicle glycoprotein 2A (SV2A), located in presynaptic terminals, is considered the first *in vivo* marker of synaptic density. PET tracers such as [11C]-UCB-J [118] and [18F]-UCB-H [119] have been used to observe SV2A levels in the hippocampus of subjects to identify patients with early stages of AD, suggesting that PET detection of SV2A could be a promising biomarker for AD synaptic

Table 2. Classification of Fluid and Imaging Biomarkers under the ATX(N) Framework.

Biomarker category	Fluid	Imaging
Core biomarkers		
Core1		
A (Aβ proteinopathy)	Aβ42	Aβ PET
T1 (phosphorylated and secreted AD tau)	p-tau181, p-tau231, p-tau217	
Core2		
T2 (AD tau proteinopathy)	MTBR-tau243, other phosphorylated tau forms (e.g., p-tau205), non-phosphorylated mid-region tau fragments	tau PET
Non-specific biomarkers of tissue response related to AD pathophysiology		
N (neurodegeneration)	NfL	Anatomic MRI, FDG PET
I (inflammation)	GFAP	
Biomarkers for non-AD comorbidities		
V (vascular brain injury)		Infarction on MRI or CT, WMH
S (synucleinopathy)	αSyn-SAA	

ATX(N), amyloid/tau/x/neurodegeneration; AD, Alzheimer's disease; CT, computed tomography; MRI, magnetic resonance imaging; NfL, neurofilament light chain; PET, positron emission tomography; WMH, white matter hyperintensity; FDG, fluorodeoxyglucose; p-tau, phosphorylated tau; MTBR, microtubule-binding region; GFAP, glial fibrillary acidic protein; αSyn-SAA, α-synuclein seed amplification assay.

density. Nonetheless, hematological research on SV2A remains limited. Recent findings have demonstrated that CSF and serum SV2A levels are significantly and positively associated with cognitive performance in patients with AD, with these levels diminishing as the disease advances. In particular, serum SV2A has exhibited excellent diagnostic capabilities for amnestic MCI, achieving a sensitivity of 97.8%, which is notably higher than that of NfL, GFAP, and p-tau217 [120]. Consequently, serum SV2A emerges as an ideal early diagnostic biomarker for AD, effectively differentiating individuals at high risk of AD from those with unimpaired cognition.

In summary, research on synaptic dysfunction biomarkers in AD has made some progress but still faces challenges. Current research concentrates on a few specific proteins and epigenetic markers, whereas synaptic dysfunction involves various molecules and signaling pathways. Future research needs to expand to more types of biomarkers and their specific mechanisms in AD.

Discussion

The ATX(N) framework is designed to be flexible and adaptable (Fig. 1). As our comprehension of AD pathophysiology advances, it is expected that this framework will be refined further by integrating new biomarkers into the current pathophysiological categories (A, T, and N) and by introducing new categories, represented by the X component of ATX(N) (Table 2).

The insights gained from clinical trials are expected to offer valuable lessons on the potential future application of the ATX(N) framework in clinical practice. A significant challenge in early prevention is the need for dependable prognostic markers for early-stage disease. The complexity of late-stage disease complicates later preventive efforts, possibly requiring multi-target combination therapies that go beyond just A and T [121,122]. More broadly, the development of late-stage biomarkers that more comprehensively represent the complexity of advanced AD will enhance the translatability of basic research. Therefore, further investigation into additional X components is crucial for effective multi-target treatment.

Once DMT is accessible, the ATX(N) framework can be utilized in precision medicine to match patients' biomarker profiles with the drugs' mechanisms of action, thereby assigning suitable treatments. Individuals who are A+ within the AD continuum are specifically candidates for Aβ-targeted therapy, whereas biomarkers should also guide the initiation of tau-targeted therapy. For A+T- individuals, the therapeutic goal is to prevent tau accumulation, whereas for A+T+ individuals, the objective is to eliminate tau [123]. Enhancing the AT(N) system to the ATX(N) framework will improve the depth and precision of biomarker characterization in individuals within the AD continuum [124], thereby guiding precision medicine for AD.

Although genetic markers are excluded from the ATX(N) framework, they are crucial for personalized medicine. The *APOEε4* gene stands as the most significant known risk factor. By analyzing extended haplotypes and non-coding variants in the APOE-LD region, high-risk individuals can be identified, and the age of onset for early symptoms can be estimated [125].

We must acknowledge that despite substantial progress in biomarkers and precision medicine, diagnosing and treating AD continues to face numerous challenges. First, the variability of biomarkers and the absence of standardized testing criteria hinder the creation of uniform diagnostic standards. Second, the intricacy of data integration and analysis complicates the development of diagnostic and therapeutic strategies [126]. Additionally, the design and execution of clinical trials encounter various obstacles, including patient heterogeneity, high costs, and prolonged durations [127].

Future research should prioritize the integration of multidimensional biomarkers, encompassing genomic, proteomic, metabolomic, and imaging data. The multimodal diagnostic approach, by integrating various biomarkers from blood, CSF, and imaging technologies, with particular emphasis on the application of blood biomarkers, has significantly enhanced the accuracy and early detection capabilities of AD diagnosis [128]. Additionally, it provides a crucial foundation for the development of personalized treatment plans. In ATX(N) research, there is an urgent need for coordinated ATX(N) measurements and longitudinal studies encompassing large populations of diverse ages, ethnicities, and risk factors. These studies are crucial for a deeper understanding of the biological-clinical trajectory starting from A-T-N- cognitively healthy individuals.

Technological innovation serves as a crucial catalyst for advancing AD research. Future investigations should focus on developing and applying new technologies to improve the accuracy and efficiency of biomarker detection. Emerging machine learning algorithms have the potential to surpass the limitations of traditional statistical methods by identifying genetically and biologically homogeneous subgroups within specific ATX(N) categories and capturing multi-layer associations in large-scale clusters [129]. Moreover, creating open data-sharing platforms to promote data exchange and collaboration among diverse research teams will greatly accelerate research progress and the development of new therapies.

Conclusion

As a neurodegenerative disease, the early and accurate diagnosis of AD is crucial. The 2023 NIA-AA guidelines have further refined the ATX(N) framework by incorporating newly developed blood biomarkers and clearly specifying the three representative biomarkers of "X" in clinical practice. Although there remain challenges in translating current biomarkers into clinical treatment, considerable breakthroughs have been made in AD biomarkers suitable for clinical use, showing promising prospects for AD diagnosis, follow-up, monitoring, tracking treatment responses, and population screening. Future research is expected to delve deeper into refining the ATX(N) framework, alongside making efforts to develop more robust detection methods. Detecting AD at an early clinical stage through biomarkers could pave the way for more promising therapeutic drugs and bring significant benefits to patients with AD.

Availability of Data and Materials

Not applicable.

Author Contributions

HC and EY conceived and planned the layout and content of this review. HC, EY, WZ, and JL contributed to the literature review and drafting of individual sections of this work. WZ was involved in revising the manuscript. All authors were involved in the drafting and critical revision of the manuscript, provided final approval of the version to be submitted for review and publication, and agreed to be accountable for all aspects of the work including ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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