

Comments received for the first draft (July 2023) through the Alzheimer's Association website, through communication to one of the committee members or during the presentation at AAIC 2023. Although organization names have not been removed, other identifying information has been removed.

Comments below reflect those received for the first round of open commentary either through the online website or directly to committee members in writing in response to the AAIC presentation and the associated draft documents. Updated drafts are now available at alz.org/diagnosticcriteria for open comments.

Comments received as of October 1, 2023 include:

1. The BBB vision is excellent. I conducted a survey of 500 US and EU physicians that found 74% would order blood tests if they were accurate for diagnosis or staging. Patients and the field would benefit due to low cost and ready point of care access. However as you imply all this depends on the accuracy. There are no clear binary cut-offs established for any blood marker for diagnosis or prognosis. None are FDA approved for diagnosis in practice. With 4 core BBB markers, there would be 16 possible permutations of positive/negative or high/low. It would be useful to know how doctors in the real world would interpret such results. Also there are >50 BBB tests in development. Cancer staging blood markers are tested in large field trials before launching widely. e.g. the NCI is doing a field trial of cancer blood markers in 240000 people as part of the cancer moonshot. In addition to research validation studies, I recommend the committee consider doing a field trial of BBBs for AD in general practice and neurology settings to understand real world performance. Such a trial will help determine how blood test results for AD/MCI should be interpreted, and determine a standard approach to patient screening as companies flood the field with new tests.
2. On behalf of the team at C2N Diagnostics, I applaud the efforts of Alzheimer's Association, the National Institute on Aging, and all the esteemed authors and other members of the expert committee who contributed to this draft document that seeks to provided updated criteria for diagnosis and staging of Alzheimer's disease. In general, the document was very well-written, concise, evidence-driven, and commensurate with the changing times in the field of Alzheimer's disease and cognition health. Our comments to this draft manuscript stem from our experience and steadfast commitment spanning over 15 years of existence. At C2N, we have a singular focus to translate disease epidemiology, biological mechanisms, scientific integrity, and technology innovation into meaningful healthcare solutions that improve the lives of individuals dealing with cognitive impairment and other neurological disorders. We appreciate the opportunity to share our comments to the draft manuscript and will be happy to discuss any of our comments outlined here in further detail, as desired by the authors / committee. 1) Given the importance of ApoE as the strongest genetic

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risk factor for sporadic Alzheimer's disease and the recognition that ApoE4 carrier status now has a proven role in clinical decision making in the era of disease-modifying therapies (DMTs) / anti-amyloid therapies (AAT), we were surprised to see the lack of attention provided to ApoE throughout the document. Clear evidence supports a pathophysiological role for ApoE status as well as a prognostic role. If the committee is seeking to stay away from "genetic information" because of concerns for potential discrimination or untoward effects of disclosure, we anticipate that patients will find similar risk of discrimination with AD biomarkers, including those that do not include genetic information. For instance, in the asymptomatic "high-risk" individual who is identified to have brain amyloid and tau pathology regardless of ApoE status, what are the implications for that person in other aspects of his/her life? 2) It would be beneficial to see PET and CSF and blood all referred to as biomarkers. This would allow these forms of amyloid and tau assessment to be on equal footing. The term "PET assay" is unclear. 3) If "fluid ptau" is "often" discordant with tau PET in a significant proportion of patients, how helpful is the test in a patient in whom AD is on the differential. A+pT+ seems to be more consistent with amyloid pathology rather than tau tangle positivity. In C2N's experience, extensive education is still required for both the clinical care and research community about how best to interpret abnormal p-tau markers. 4) Two CSF assays for A β -amyloid have FDA and IVDR-CE approval for clinical use. Many current plasma assays for both A β and tau are listed as suitable for research use (Table 3). Some of these may advance to general clinical use, but at this point that is difficult to determine and will ultimately depend on utility assessments by users. Of note the PrecivityAD blood test has been commercially available since 2020. Reference: <https://precivityad.com/>. A recent clinical utility paper, published within this past month, has also shown the value of the test in general clinical use as well as utility assessment by users. Reference: Monane M, Johnson KG, Snider BJ, et al. A blood biomarker test for brain amyloid impacts the clinical evaluation of cognitive impairment [published online ahead of print, 2023 Aug 7]. *Ann Clin Transl Neurol.* 2023;10.1002/acn3.51863. doi:10.1002/acn3.5186 5) The degree of renal function should be taken into account when measuring and comparing any biomarker dependent on renal clearance, which includes both total tau as well as subtypes. A ratio that normalizes p-tau quantitation to total tau levels (or non-p-tau) has now been shown in multiple studies that it offers a more robust measure than any specific p-tau value by accounting for renal clearance. Renal function naturally declines with age due to nephron loss and represents an important confounder for the clinical care community to understand in the course of interpreting plasma biomarker values. 6) Regarding the sentence in the draft manuscript: "... Biofluid assays do not require FDA approval; the much-less rigorous CLIA or CAP (in the US) certifications do not require autopsy validation ...": This sentence should

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say "biofluid assays don't require AUTOPSY for FDA approval." 7)

Regarding the sentence in the draft manuscript, "... We recommend conservative interpretation of values near cutpoints and we recommend employing an indeterminate zone around a normal/abnormal biomarker cutpoint..." : "The creation of an indeterminate zone begs other questions - how large is the zone? What is the next step for patients with indeterminate scores? By creating an indeterminate zone, one has created another challenge on the management of patients with values near the normal/indeterminate zone as well as indeterminate/abnormal zone. With an indeterminate zone, there are now two cutoffs the clinician must be mindful of rather than one cutoff." "Further, regardless of one cutoff value or two cutoff values, clinicians will always examine values closer to the defined cutoffs. Cutoffs are a natural phenomenon of most diagnostic tests in clinical medicine, and for this reason, it seems important to emphasize that any single test result and interpretation should always be evaluated in the context of the patients' entire clinical presentation, along with other examinations, tests, other variables impacting a clinician's confidence in making a proper diagnosis. Thank you again for the opportunity to comment on this robust draft document that will aid the global research and clinical communities immensely, and ultimately lead to better care for patients.

3. 1. The NIA-AA Workgroup is to be applauded for being bold and encouraging the transition of the guidelines from a research framework to clinical use. We note that the new draft guidelines go beyond translating the 2018 NIA-AA Research Framework into clinical diagnostic criteria, as the Workgroup has made a substantial change, from requiring A+T+ to proposing that "AD may be diagnosed by any abnormal core AD biomarker." This departs from arguments previously made in favour of the 2018 NIA-AA framework over the IWG criteria based on the risk of clinical deterioration among A+T+ individuals. (For example Ossenkopp et al. (2022) showed that cognitively unimpaired A+T+ individuals had clearly increased risk and showed steep trajectories of cognitive decline, which supported the 2018 NIA-AA criteria-based classification "especially when "T" is defined by PET." The same paper (and others) show that individuals who are only A+T- have considerably lower risk of progression and slower decline in memory scores). If there is a need to relax the requirement for A+T+, perhaps a compromise could be to require A+ together with any of T+, N+ or C+? 2. The evolution of plasma biomarkers is attention-grabbing and hopeful. Nonetheless It seems that there remain unresolved questions around thresholds, race and ethnicity (genetic background?), co-morbidities (e.g. chronic kidney disease), etc. In table 1, only aSyn-SAA is given the footnote that it requires CSF rather than plasma, yet the question remains whether the blood based tests are ready for general clinical use. At this transitional stage of biomarker adoption, one solution could be to temper the recommendation to "exploratory"

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clinical use for plasma testing, and in the meantime relocate plasma biomarkers from the table 1 (clinical) to table 3 (‘‘research and possibly for future clinical use’’)? The status of fluid tests and their equivalence in this rapidly changing field also creates problems for consistency across the recommendations - as the NIA-AA draft itself notes, ‘‘PET and fluid measures are not equivalent’’, and accordingly, fluid ptau (181, 217 and 231) measures appear in the same section of table 4 as A+T- PET (‘‘initial stage’’); but this appears inconsistent with categorising fluid ptau 181, 217 as a (clinical) biomarker of T in table 1 in the same row as Tau PET. 3. In the new categorisation, derived from the ATX(N) structure, it seems appropriate that both inflammation (Kinney et al PMID:30406177) and vascular pathology (Lee et al PMID:27016429) should be considered together with the neuropathology of Alzheimer’s disease. However, while synucleinopathy is a common copathology it is not present in the same way that inflammatory effects are, nor as the directly toxic effects of amyloid on vascular tissue. Synuclein inclusions are present in the neuropathology of about 70% of AD brains (Crews et al Neurotox Res. 2009). 4. In section 3, a disconnect between the biomarker framework and the neuropathology is suggested. This seems to be a concern as prior evidence shows that clinical diagnosis is neuropathologically incorrect 25-30% of the time. In fact the wording here indicates two slightly different scenarios: a) a loss of consistency between neuropathology and in vivo biomarkers (‘‘the link between the pathologic gold standard and the in vivo definition will not always be consistent’’) or (b) a failure of biomarker positive individuals to ‘‘qualify for a pathological diagnosis of intermediate/high AD neuropathologic change’’. Text Box 4 observes that ‘‘Biomarkers are less sensitive than neuropathology for detection of mild/early pathology’’. Whereas, Section 3 states that Biomarker positive individuals may fail to ‘‘qualify for a pathological diagnosis of intermediate/high AD neuropathologic change’’, which is intended to indicate biomarkers are sensitive to early change preceding intermediate neuropathologic change. Although these statements are not contradictory they may be seen as confusing. Is it helpful to clarify that T biomarkers are less sensitive for mild/early pathology while A biomarker positive individuals may not qualify for intermediate/high AD neuropathologic change? It might be considered sufficiently important to have ‘‘the in vivo definition of AD aligned with the established neuropathological definition’’ to warrant keeping the A+T+ requirement. 5. Since its introduction in 2010, the ‘‘hypothetical model’’ or ‘‘Jack Curve’’ diagram and its revisions have been hugely influential; differences in the version in Figure 1A to earlier versions therefore deserve further discussion. (a) Although clearly a schematic, variation in the spacing of the new Jack curves has been included (for example the orange, pink, and green lines for Advanced T, N, and C) which a reader will assume is meaningful. If this is so, then the wide and even spacing of the A and multiple T curves deserves

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further consideration. It seems that Early T should be closer to A (see Jack, 2022 Lancet Neurology) and there should be more space between N and C. Similarly, the 2013 update noted that "subcortical tauopathy is the first AD pathophysiological process to arise in many individuals" and showed this (below the detection threshold) in its Figure 6, but this aspect seems to have been ped in the latest version. (b) If splitting T into 3 parts (early, inter, adv) it would be appropriate to split N into 2 parts (Nmicro, Nmacro) as in Weston et al. (2015). It can be understood that micro will always precede macro, and imaging evidence reinforces this (Torso et al., 2022; Spotorno et al. 2023), to be specific, Nmicro can include biomarkers across the 3 main biomarker modalities: dMRI, NfL and SV2A PET (and potentially other synaptic PET imaging markers). (There could also be an M for Metabolism (FDG-PET), perhaps falling between the other two N curves) (c) It might be considered that C should also be split into earlier (vulnerable), typical, and later (reserve/resilient), or the high-risk/low-risk band that appeared in Fig.6 of the 2013 update. (d) Inflammation might also be a shaded band starting after A but spanning the T lines, reflecting the understanding that there is an inflammatory response to amyloid plaques themselves, and to the subsequent tauopathy. 6. It is acknowledged in the text that the draft guidelines are particularly influenced by the imminent blood/fluid tests and this may have led to an emphasis on early stages. Table 6 currently enumerates clinical stages 1, 2 and 3 but then groups together stages 4-6. However if the guidelines are shifting from a research framework to be considered as clinical guidelines then they should not concern themselves only with the early stages. Condensing the descriptors from Table 5 (1 asymptomatic, 2 transitional (e.g. SCD), 3 cognitive impairment (e.g. MCI), 4 mild dementia, 5 moderate dementia, 6 severe dementia), it is not clear that there are no biological differences between mild and severe, nor that there is no interest in differentiating them in terms of biomarkers (eg. several drugs target MCI together with mild dementia but not moderate or severe). In parallel with expanding the columns of table 6, the Workgroup could consider expanding the rows by adding inflammation and/or neurodegeneration (ideally separated into Nmicro and Nmacro) beyond the current limit of "Advanced" tau pathology; expanding Table 6 from the current 4x4(5) table into a 6 or 7 x 6(7) table could helpfully create a more complete picture without an undue increase in its complexity. 7. Table 2. Why is there no row for V biomarkers? (ie. for "Staging, prognosis, treatment" ?) White matter hyperintensities (WMH) in MRI are commonly used and seem helpful for staging and prognosis of vascular pathology/dementia. Papers on autosomal dominant AD have also argued that WMH is a core feature of AD rather than a fully independent cardiovascular contributor. WMH is also a valuable biomarker for treatment trials, both directly as an efficacy endpoint for treatment targeting vascular pathways, and indirectly for safety as an ARIA-E measure for anti-amyloid treatments. 8. For "Identification of co-pathology"

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it would be appropriate to distinguish between single dimension and multi-dimensional biomarkers; fluid markers typically provide a single dimension of quantity, for example NfL, whereas imaging data provides quantity with anatomical distribution, for example MRI and FDG PET regional patterns can distinguish different pathologies. 9. Inflammation has a rather poor selection of biomarkers in Table 3, but good evidence suggests that dMRI can be a biomarker for inflammation (at least conceptually “ as in footnotes to tables 5 and 6) Yi et al. (2019; PMID:30837826) [Microglial Density] Garcia-Hernandez et al. (2022; PMID:35622913) [microglia and astrocyte activation] Oestreich & Sullivan (2022; PMID:35051668) [Astrocytic processes ~ neurite density index] 10. The text refers to the concept that “therapies may alter relationships between biomarkers” and makes the example of a distinction between the brain’s “steady state” in the natural history of the disease and, by implication, the treated state. (a) is it correct to define the natural history of disease as a “steady state” rather than a progressive state? (b) perhaps it may be worth giving further consideration to the use of different biomarkers for assessment and monitoring of progression in treated patients; for example, macrostructural atrophy appears to be confounded by opposing effects of reduced neurodegeneration and accelerated volume loss or pseudoatrophy, perhaps due to clearance of damaged tissue and/or fluid shifts, but microstructural measures of neurodegeneration could be informative, and the combination of multiple measures (e.g. micro, macro and metabolic) could further help to characterise treatment-related dynamics. 11. The text in line 9 refers to principles regarded as “fundamental tenants” “ presumably this should be “fundamental tenets” .

4. On behalf of The Gerontological Society of America (GSA), thank you for the opportunity to provide comments to the Draft NIA-AA Revised Clinical Guidelines for Alzheimer’s. Our mission at GSA is to cultivate excellence in interdisciplinary aging research to advance innovations in practice and policy. GSA’s 5,400 members include gerontologists, health professionals, behavioral & social scientists, biologists, demographers, economists, and many other disciplines. These experts study all facets of aging with a life-course orientation. The multidisciplinary nature of the GSA membership is a valued strength, enabling the Society to provide a 360-degree perspective on the issues facing our population as we age. GSA is advancing major initiatives related to improving adult immunization rates, earlier detection of cognitive impairment, improving oral, hearing, and vision health, framing our language to improve the public’s understanding of aging, and understanding the impact of the longevity economy. As a professional membership society with a long-standing commitment to translating research to inform evidence-based care for persons with dementia, GSA has developed The GSA KAER Toolkit. This work is

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intended to support primary care teams in implementing a comprehensive approach to initiating conversations about brain health, detecting and diagnosing dementia, and providing individuals with community-based supports. We are currently working with the University of Washington and the Centers for Disease Controls' Alzheimer's Disease and Healthy Aging Program to pilot the Toolkit in a primary care system. Likewise, GSA members and staff actively participate in and serve as members federal councils such as the Advisory Council on Alzheimer's Research, Care, and Services. GSA appreciates that we are all focused on improving care for persons living with dementia and their loved ones while advancing innovation and access to pharmacologic and non-pharmacologic therapies for prevention and treatment of Alzheimer's disease and related dementias (ARD). GSA focuses our comments in the following areas. Overall comments: The draft guidelines are clearly written and very informative with respect to the proposed criteria and the reasons behind them. It is exciting to learn about the increases in knowledge and other factors that have created the motivation for this update. While progress and breakthroughs in biomarker discovery are exciting and promising, we recognize that as we identify people with early cognitive impairment, the clinician and care provider communities must be prepared to provide the services and supports to ensure what matters most to the individual. In particular, clinicians will need to be prepared to observe, assess, and implement standards of care to support individuals with cognitive impairment to maintain optimal function and quality of life. Biomarker categorization: The draft discusses common, non-AD co-pathologies, including vascular pathologies and non-AD neuropathologies, such as Lewy Body pathology and TDP-43, at many places in the text, beginning on p. 4. The draft notes that no biomarkers are currently available for some of these diseases, ((p. 8 (section 2.3), p. 10 (line 227) and p.11)), where the text says: "Biomarkers are not available for all relevant neuropathologies, therefore it cannot be known with certainty in vivo what neuropathologies in addition to AD are present in any individual or what the proportional neuropathologic burden is among various pathologies." GSA appreciates this acknowledgement of non-AD co-pathologies and neurodegenerative diseases in the draft. However, many clinicians and others are probably not aware of their extent. GSA suggests that clear information about the extent of non-AD co-pathologies should be added early in the document, perhaps in connection with Figure 1. Some of the many research articles that provide such information include: Boyle et al., 2021; Boyle et al., 2013; Boyle et al., 2018; Filshtein et al., 2019; Kawas et al., 2015; Rabinovici et al., 2017; Schneider et al., 2007, and White et al., 2016. The information in these articles is based primarily on autopsy data but would, nevertheless, help readers and users of the proposed guidelines understand the extent of these non-AD conditions, the heterogeneity and complexity of cognitive impairment and dementia in older people, and the

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implications for detection, diagnosis, treatment, and care. As noted in the draft guidelines, non-AD co-pathologies are much more frequent in older than younger people with AD. Very large proportions of people living with AD are older, however, and understanding the extent of non-AD co-pathologies is important for those who are providing clinical care and other services for such people. Some of the articles listed below point out that "pure AD" is much less common than might be expected in findings from autopsy studies (Boyle et al., 2018; Schneider et al., 2007). Most of the articles provide graphs and other figures that illustrate the extent of non-AD co-pathologies. Adding one or more of these graphs or figures to the draft would be useful in increasing understanding about the various causes of cognitive impairment and dementia in older people. The draft discusses the importance of addressing diversity in studies of non-AD co-pathologies (p. 27). Two of the references listed below point out differences among diverse populations in the types and average extent of non-AD co-pathologies (Filshtein et al., 2019; White et al., 2016). Through Primary Care we can use biomarkers to detect Dementia disease prior to the illness and is important to include minorities and underrepresented communities which historically have not been included in major research. It is important to incentivize our underserved communities to participate in this type of research. Clinical staging: GSA notes it is important for primary care practitioners to be informed and provided with screening resources to inform their patients and conduct functional assessments, given that clinical staging is proposed. Diversity and need for more representative cohorts: GSA agrees that there is an increased need for more representative cohorts. More importantly, local, and inclusive projects in our own communities are the way to improve brain health promotion and screening for and early detection of ADRD. ADRD are becoming increasingly common as we age. Most individuals with dementia will first present for care and assessment in primary care settings. High quality primary care is the foundation of the health care systems where there is a need for dementia screening that can accurately diagnose cognitive impairment and dementia in underrepresented populations. According to a survey by the American Board of Internal Medicine Foundation, consumers trust their primary care physicians more than the healthcare systems. The Centers for Medicare & Medicaid Services (CMS) and NIH rate early detection of cognitive impairment as a top research priority. Although most individuals with cognitive impairment will first present for care and assessment in primary care settings, primary care practitioners are unaware of cognitive impairment in more than 40% of their cognitively impaired patients. The problem of underdiagnosis and late detection is more prevalent among older African Americans and Hispanics than older whites. Importantly, disparities in access to early cognitive impairment detection and screening include quality and thoroughness of clinical evaluations, affordability of care, lack of insurance coverage, and lack of access to community healthcare services. Furthermore,

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disparities in dementia care have been reported in African American and Hispanic older adults with significantly lower odds of receiving a timely diagnosis compared to those identified as white. Populations identified as racial or ethnic minorities, that live in rural areas and have low social economic status have been underrepresented in clinical dementia trials despite overwhelming evidence that such populations are at increased risk for developing dementia. More importantly, Morris and colleagues noted that racial differences in Alzheimer biomarkers suggest possible race-dependent biological mechanisms that contribute to expression of disease , further highlighting the importance of primary care practitioner to continue to elevate brain health as an important aspect of overall health with patients during any office visit, including the Annual Wellness Visit Per the Alzheimer's Association older adults from African American and Hispanic backgrounds are twice and one- and one-half times, respectively, as likely to have Alzheimer's or other dementias as older white adults. Four in ten Americans will talk to their doctor right away when experiencing early memory or cognitive loss, and seven in ten will want to know early if they have Alzheimer's disease if it could allow for earlier treatment. ? We note that among persons that have mild cognitive impairment about 15% develop dementia after two years. About one third develop dementia due to Alzheimer's within five years. Overall primary care practitioners can provide a connection for the patient with subspecialties with specialty referrals, enroll patients and family members in clinical trials (including biomarker assessment, disease specific biomarkers), and provide the patient and caregiver with community resources. Future Direction: The draft emphasizes that the proposed criteria are for clinical use. While acknowledging that the draft guidelines are clearly written and very informative, GSA's main concern is about how these new and complex ideas will be conveyed to clinicians, non-clinician care providers, and the extensive array of public information sources that cover AD and dementia. There is currently considerable lack of understanding about AD and non-AD diseases and conditions in the U.S. and elsewhere. The proposed changes will increase that problem exponentially. NIA is currently providing valuable information about AD, as are many other government and private sector organizations and individuals. Leadership is needed to begin now to create training, materials, and messages to convey accurate understanding about the implications of the new criteria among all groups. GSA is ready to participate fully in the process of creating and delivering such training, materials, and messages. GSA suggests that important participants in the process should include NINDS, HRSA, ACL, VA, CDC, and other federal government agencies with relevant expertise and experience in creating the needed training, materials, and messages. Likewise, many professional organizations, state, county, and local government organizations and home and community-based agencies should be involved. Additionally, we support the recommendations of the National Task Group on Intellectual

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Disabilities and Dementia Practices related to NIA's investment in DS-AD biomarkers research. We recommend extending this investment to determining the nature of Alzheimer's disease biomarkers in the adult population with lifelong intellectual disabilities. Specifically, we also recommend research to determine the applicability and effectiveness of biomarker findings noted in the proposed clinical guidelines relevant to diagnosing Alzheimer's disease in adults with intellectual disabilities other than Down syndrome. Also, such biomarker research should include other genetic syndromes associated with intellectual disability that may offer particular risk for Alzheimer's disease or other diseases associated with dementia. We further recommend, if biomarker parameters vary with respect to Down syndrome, that the guidelines note biomarker parameters that may be idiosyncratic to Down syndrome. We also recommend that the guidelines provide clinical equivalencies to staging factors and functioning for adults with intellectual disabilities. i. Seitz DP, Chan CC, Newton HT, Gill SS, Herrmann N, Smailagic N, Nikolaou V, Fage BA. Mini-Cog for the diagnosis of Alzheimer's disease dementia and other dementias within a primary care setting. *Cochrane Database Syst Rev.* 2018 Feb 22;2(2):CD011415. doi: 10.1002/14651858.CD011415.pub2. Update in: *Cochrane Database Syst Rev.* 2021 Jul 14;7:CD011415. PMID: 29470861; PMCID: PMC6491332. ii NORC at the University of Chicago. Surveys of trust in the U.S. Health Care System Amerispeak Survey. American Board of Internal Medicine. https://www.norc.org/content/dam/norc-org/pdfs/20210520_NORC_ABIM_Foundation_Trust%20in%20Healthcare_Part%201.pdf. Accessed 8/31/2023. iii National academies of sciences E, and medicine. Preventing cognitive decline and dementia: A way forward. Press TNA, Ed. Washington DC 2017. iv 2023 Alzheimers Disease Facts and Figures <https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf> v Tsoy E, Kiekhofer RE, Guterman EL, et al. Assessment of racial/ethnic disparities in timeliness and comprehensiveness up dementia diagnosis in California. *JAMA neurology.* 2021;78(6)657. Doi:10.1001/jamaneurol.2021.0399 vi Indorewalla KK, O'Connor MK, Budson AE, Guess DiTerlizzi C, Jackson J. Modifiable Barriers for Recruitment and Retention of Older Adults Participants from Underrepresented Minorities in Alzheimer's Disease Research. *J Alzheimers Dis.* 2021;80(3):927-940. doi: 10.3233/JAD-201081. PMID: 33612540; PMCID: PMC8150544. vii Morris JC, Schindler SE, McCue LM, et al. Assessment of Racial Disparities in Biomarkers for Alzheimer Disease. *JAMA Neurol.* 2019;76(3):264-273. doi:10.1001/jamaneurol.2018.4249

5. Thank you very much for your exceptional work. Here are some suggestions for consideration: I recommend refraining from using the term "inflammation" (very unspecific) and instead utilizing "immune processes" when referring to " ". Similarly, it would be advisable to

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avoid the term "activation" and instead opt for astrocyte or microglia "reactivity". While I am a strong supporter of the biological definition of AD and I have used the "Preclinical AD" concept for research purposes, I propose refraining from using the term "disease" in biomarker-positive cognitively unimpaired patients in clinical settings. Terms such as "Preclinical Alzheimer's" (excluding the term "disease"), "at-risk of Alzheimer's disease," or simply "amyloid-positive" might be more appropriate. Drawing a parallel with "HIV-positive" vs. "AIDS" could serve as a useful precedent. I concur with the decision to dichotomize the core AD biomarkers as (+/-), while acknowledging the existence of an intermediate range. However, I question the necessity of binarizing the N, I, or V biomarkers. I believe a continuous measurement or a scale (akin to cognitive assessments) might be more fitting. A notable feature of the new criteria is the inclusion of the notion that "AD may be diagnosed based on any abnormal core biomarker," rendering the positivity of both amyloid and tau biomarkers (A+T+) no longer obligatory. Nonetheless, I posit that a patient with symptomatic AD should exhibit A+T+ status; otherwise, alternative explanations may better account for their symptoms. Consider these case scenarios with a biomarker profile of A+T-: - A 90-year-old patient presenting non-amnesic MCI and small vessel disease in MRI. - A 75-year-old patient displaying PSP-Richardson syndrome. - An 85-year-old patient with highly suggestive Lewy Body Disease. - A 68-year-old patient with frontal syndrome and confirmed PGRN mutation. In these cases, is it reasonable to attribute their symptoms to AD if Tau is still negative? Would a biological AD diagnosis be warranted? Building upon the previous point, even if a patient is A+T+, it is always important to indicate whether the clinician believes the symptoms primarily stem from this pathology. While a patient might have preclinical AD (A+T+), cognitive symptoms could result from an alternative neurological disorder. I propose segregating synaptic "S" biomarkers from "N" biomarkers. It is worth highlighting that, unlike other biomarkers, plasma GFAP more accurately detects amyloid-positivity than CSF GFAP. Lastly, I underscore the importance of acknowledging that most patients and clinicians lack access to biomarker data. This should not, however, hinder them from diagnosing Alzheimer's disease. While I firmly advocate for a biological definition of AD, it is crucial to develop language that accommodates diagnoses without biological confirmation. The previously employed terms "possible" or "probable AD" could be re-introduced. Additionally, contemplate using the following phrasing for a diagnosis: "Patient with amnesic syndrome in dementia stage (Stage 4), likely attributable to AD." I greatly appreciate your valuable contributions once more.

6. I would like to thank all contributors for this timely update of the NIA-AA criteria. I have one comment that refers to the categorization of biomarkers evaluated

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suitable for use in clinical practice as shown in Table 1. Compared to the other categories (A,N,I,V,S), the "T" category in Table 1 reflects the most heterogeneous pool of changes. "T" means that there could be changes in tau biology (without neurofibrillary tangle pathology/tau deposits) and/or neurofibrillary tangle pathology/tau deposits. As I understand that all changes listed in the "T" category base on changes of the tau protein and therefore agree to arrange them in one category "T", a sub-division of the "T" category in sub-categories indicating tau changes with and without neurofibrillary tangle pathology/tau deposits may account for the heterogeneity in this category. This may be for example realized by the terms "tau pathophysiology" (tau changes without neurofibrillary tangle pathology/tau deposition) and "tau pathology" (neurofibrillary tangle pathology/tau deposition). Thank you very much for the opportunity to comment on this draft.

7. Early identification of cognitive impairment and brain pathology associated with Alzheimer's disease is essential to maximize benefits from evidence-based lifestyle interventions and accelerate access to emerging disease-modifying treatments. While establishing blood biomarker status is necessary and important, it is not sufficient alone as a diagnostic approach to Alzheimer's and other dementias for several key reasons: 1. Potential for unnecessary treatment and testing: It is important to understand both if an individual has blood biomarkers present AND if they are experiencing or are likely to experience an impact to their cognitive function. Research shows that at least 20% of older adults have amyloid and/or tau in their brains, but never go on to experience Alzheimer's symptoms. Detecting blood biomarkers alone could thus result in significant unnecessary testing, treatment (which can be associated with serious side effect risks). 2. Potential for unintended consequences: Detecting biomarkers of Alzheimer's disease does not mean the patient will ever develop cognitive impairment. This opens up significant ethical implications given the potential of creating a self-fulfilling prophecy. A growing body of evidence shows that negative thinking, despair, and lack of hope " commonly associated with a diagnosis of Alzheimer's disease " leads to increased risk of dementia and greater disability. 3. Lack of coverage for MCI and dementia unrelated to amyloid: Many people experience mild cognitive impairment and dementia, but do not have amyloid in their brains. In fact, a large study found that about 45% of PET scans of people with MCI and about 30% of PET scans in people with dementia are amyloid negative. Over-indexing on blood biomarkers could thus result in an incomplete diagnostic approach with a focus that results in a substantial share of patients missing opportunities for diagnosis and intervention for their specific conditions. 4. Patient access / health system strain: Widespread use of blood biomarkers alone for screening " and the additional diagnostic evaluations and potentially unnecessary treatment resulting from it "

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would exacerbate the already significant strain on specialist resources and lengthy wait times to access them. Better solutions for informed triage are needed to avoid worsening the supply-demand gap by overwhelming the system and risking eligible patients progressing outside of the early stage treatment window. 5. Feasibility, efficiency, and cost issues: New treatments rely on early detection for patients to take advantage of them and this relies on broader detection in and triage from primary care. Blood tests do not provide results at the point of care like efficient and sensitive cognitive assessments do. Performing widespread digital cognitive assessments would enable PCPs to better identify and triage patients needing specialist care and help streamline treatment access for those eligible. Advanced digital cognitive assessments also provide a practical, efficient, and cost-effective option to support equitable access. 6. Lack of actionability: Blood tests only provide only a result, whereas cognitive assessment solutions pair cognitive function results with clinical guidance. In order to truly empower PCPs to perform effective screening and streamline access to treatment, both are essential. While appealing in its simplicity and a key component in moving the needle on early detection in Alzheimer's™s, an approach that focuses only on blood biomarkers brings the risk of gaps in care, access equity issues, and serious negative psychological impact on patients (and their families) for those who do not and would not ultimately need treatment (or for whom the risks outweigh the benefits). A better approach would optimize the use of both blood biomarkers and AI-enhanced digital cognitive assessments "capable of detecting cognitive impairment before symptoms arise" with a focus on primary care settings, to help providers best identify, triage, and prioritize candidates for treatment.

8. We appreciate the initiative to update the NIA-AA 2018 research framework considering the latest achievements in the field of Alzheimer's disease (AD) biomarkers and therapies. The attempt to reveal the interaction of the biological staging and clinical manifestation is crucial for a more detailed diagnosis and understanding of the disease. Also standardized, detailed, individual classification is mandatory for a targeted treatment of AD patients. AD is a disease with a complex underlying biology, being influenced by different clinical conditions and external factors in each individual case. The combination of biomarkers and clinical assessment allows to display the complex patterns of the AD continuum more effectively and reflects what is already done by experts in everyday clinical practice. Nevertheless, details such as whether the diagnosis of AD requires A+ alone or A+T+ or can even be made in the constellation A-T+ are, in our view, still too imprecisely defined. Some biomarkers are still lacking standardization and thorough validation at the present time and should therefore,

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in our view, not be introduced into a clinical classification system yet. The proposal of explicitly dividing AD biomarkers into fluid-based modalities and imaging modalities is to be fully supported, since there is a qualitative difference between them. As the biological definition of AD involves two proteinopathies, from our point of view, a biomarker profile including the corresponding core biomarkers must be analyzed in order to evaluate the underlying pathology in its entirety and to allow for (differential) diagnosis of the disease, staging, therapy selection and monitoring the course of the disease as well as treatment effects. Regarding therapeutic approaches, neurodegenerative diseases in general are similarly complex to tumorous diseases. Based on the analogy, one can also assume that there will be a very dedicated patient cohort that will benefit the most and equally there will be patients with profiles that will be more prone to adverse events or less efficient therapeutic effects. Evaluating only one of the core biomarkers, which serves as a surrogate marker for amyloid beta pathology, can lead to an incomplete picture with missing information as it will certainly not reflect the whole pathology in its entirety. Also, possibly in the future there will not be one single drug that fits all patients, but there will rather be a patient-adapted, individual and very well-selected combination of drugs and symptomatic therapy approaches. In order to fully capture an individual patient's profile biochemically and to stratify patients before and during therapy, diagnostic means must allow to analyze all relevant biological parameters as precisely and completely as possible. Imaging modalities, like Amyloid-PET scans are not comparable to cerebrospinal fluid CSF-based analysis in terms of quality and comprehensiveness. In addition, imaging modalities are still to a certain degree examiner-dependent, not suited to provide information on several biomarkers at once and might lack sensitivity. Besides the fact that they are burdensome for patients due to radioactive tracers, they are cost ineffective and not widely accessible. In general, in vitro-diagnostic analysis in body fluids allows for more precise quantification of multiple biomarkers, since automated systems are more or less operator-independent. Still bodily fluids for diagnostic purposes also differ to a great extent regarding their diagnostic quality. CSF is a fluid which, by its very nature, has direct contact with the region of interest, the brain. It has been shown to allow for the sensitive and precise detection of AD relevant proteins and exclusion of AD as well as supporting differential diagnosis of non-AD associated neuropathologies. Unfortunately, lumbar puncture for CSF analysis is not suitable for early detection in the presymptomatic phase or measurements over time for obvious reasons. So, from a scientific and diagnostic point of view, CSF is the perfect matrix to detect pathophysiological process in the central nervous system, but is limited by its invasiveness, its limited accessibility and is thus not suitable for screening or monitoring. Thus, less invasive, repeatable, and scalable diagnostic procedures with at least the same quality as CSF analysis are needed to display the relevant spectrum of brain derived biomarkers.

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Research on plasma-based approaches has been going on for decades and the progress that has been made, e.g. with pTau217 and NfL, especially on the basis of increasingly sensitive methods, is immense and very gratifying. In the NIA-AA revised Clinical Guidelines for Alzheimer's Disease, plasma-based tests are proposed as a valid alternative for CSF-based testing. This needs to be addressed and discussed with absolute caution. Plasma, as a bodily fluid for analysis of biomarkers for neurodegenerative diseases such as AD or other brain diseases, has severe limitations. The brain is shielded from the blood circulation by the blood-brain barrier for very good reasons. Thus, analyte concentrations in plasma are usually low, sometimes in the femtomolar range and analysis of these low analyte levels requires extremely sensitive and robust methods and analytical systems. Furthermore, the pattern of analyte aggregates which carry an important diagnostic information might be impacted heavily by the transition through the blood-brain barrier. In addition, blood components are metabolized by various organs that change the composition of the relevant analytes in an uncontrolled manner and thus can affect biomarker concentrations and their detection. This might explain the relatively poor correlation of plasma-based biomarkers with CSF levels and the small effect sizes. When there is only a small difference in biomarker levels between affected and non-affected individuals, even small (pre-) analytical errors or technical variations of the measurement platforms can reduce the clinical performance to a level where it is no longer clinically useful. The suggested plasma-based core biomarkers for amyloid pathology show reasonable, yet not excellent diagnostic performance, with mass spectrometry (MS)-based assays being in the lead. Still the number of false positives and false negatives needs to be addressed with caution in addition to the fact, that MS-based assays are not widely available yet. Moreover, there is also a lack of standardization between different methods and there is no certified reference material or measurement procedure available. It is still too early to encourage plasma-based testing in general and it would even endanger patients. Even more importantly, to our knowledge, evidence from large prospective studies in real-world settings is still largely missing and, thus, the diagnostic accuracy of plasma-based tests, mainly assessed in highly selected cohorts, might currently be overestimated. In particular, the impact of chronic diseases such as metabolic syndrome, cardiovascular diseases or chronic diseases affecting the liver or kidney as well as the intake of commonly used drugs in the target patient population, e.g. anticoagulation or antihypertensive medication is already sufficiently evident from the current data. It is of utmost importance that patients of different races, genders and with different clinical conditions (metabolic syndrome, nephropathies etc.) are diagnosed accurately. With the availability of disease-modifying medications, physicians might rely on plasma-based tests alone to determine eligibility of patients for disease-modifying treatment. In absence of confirmatory testing, a potentially high false-positive rate

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would result in overtreatment with therapeutic drugs that can have severe side effects, not to mention the financial aspects. In case of false-negative results, on the other hand, patients who could hypothetically benefit from a therapy would not be recognized by the test. In conclusion, the diagnostic performance and the level of evidence remains insufficient for plasma-based biomarkers to be used as a sole diagnostic or for use cases such as staging and monitoring. The data that is currently available is not considered sufficient to support such a broad application of plasma-based tests. Instead, all plasma tests should, in our view, at least be interpreted in the context of a patient's history and other diagnostic procedures and would need confirmatory testing with CSF and or amyloid PET. We acknowledge the fact, that there is still an unmet need for a non-invasive diagnostic procedure that detects multiple AD-specific biomarkers to allow for diagnosis, staging and monitoring that is broadly available. To our opinion and corresponding data, there is an alternative body fluid that is extraordinarily well-suited to the purpose and has the potential to fulfil the above-mentioned requirements: nasal secretion. The frontal brain communicates through the Brain Nose interface (BNI) with the nose across a large area that is directly and non-invasively accessible. It allows to assess the emerging pathology in the brain at closest distance. At the same time, the area is involved very early in the course of the disease, so that both anatomically and functionally the ideal conditions are given for a simple, early, precise and comprehensive analysis of brain-derived biomarkers obtained at the site of closest possible proximity, including ones that are highly relevant to disease pathology such as soluble amyloid-beta oligomers that might not be detectable in plasma. Noselab has developed a proprietary diagnostic workflow to enable a precise and comprehensive analysis of multiple markers (amyloid proteins and tau proteins) from nasal secretion comparable to CSF based samples while at the same time leveraging all the benefits of a minimal invasive, scalable and early applicable method. They are now in the process of analytical and clinical validation in multicentric, prospective cohort studies to prove its diagnostic performance in heterogeneous patient cohorts independently of clinical conditions, cognition status and any other above mentioned influencing factors such as medication. In our view, there is currently not enough data available for plasma-based tests to be recommended as a first-line test with equal performance as CSF-based tests into clinical routine. Therefore, we believe that it would be too early and risky to support this proposal at this point of time.

9. The recent proposal to update Alzheimer's disease (AD) diagnostic criteria has prompted discussions in the scientific community. While the goal of improving diagnostic accuracy and integrating new insights is laudable, a closer examination of the suggested changes reveals possible limitations. This critique aims to express our concerns regarding the proposed revisions. The revised

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criteria heavily lean on biomarkers like amyloid and tau proteins for Alzheimer's disease diagnosis and staging. Although these biomarkers have shown promise in research settings, their practical utility in clinical settings remains uncertain. The limitations of using blood-based assessments for Abeta have been extensively discussed in existing literature, and we intend to highlight the most pertinent issues here (Angioni et al., 2022; Hansson et al., 2022; Karikari et al., 2022; Pan et al., 2023). In the case of plasma A β 42/40 ratios, it is noteworthy (as acknowledged by the Alzheimer's Association) that there is significant overlap between different groups, with a mean 8.6%-fold change observed between individuals with positive and negative amyloid PET scans, compared to the substantial 37.5% change in cerebrospinal fluid (CSF) (Hansson et al., 2022; Karikari et al., 2022). This overlap has implications for the reliability of the assay, any biases during the pre-analytical or analytical phases could potentially influence the final outcomes (Karikari et al., 2022). The suggested requirement for a narrow between-assay coefficient of variation of around $\pm 3\%$ for the A β 42/40 test presents challenges in clinical settings due to limited tolerance for variability (Benedet et al., 2022; Rabe et al., 2023; Plasma A β "First Sign of AD, But Tough to Measure Prospectively? | ALZFORUM, n.d.). Even when considered as a preliminary screening tool, there exists a need for additional supporting evidence for plasma Abeta 42/40 (Rabe et al., 2023). Efforts to improve these tests often involve incorporating APOE into the equation, but this approach remains controversial as APOE genotype primarily indicates risk rather than direct Abeta pathology (Lautner et al., 2014; Hansson et al., 2022). The lack of robustness has led to ongoing searches for more dependable blood-based biomarkers (BBMs), either singly or in combination, along with the necessity for real-world studies, particularly across diverse populations (Hansson et al., 2022). In contrast to Abeta, pTau displays a significant increase as the disease advances, ranging from 250% to 650% (Palmqvist et al., 2020; Thijssen et al., 2021; Hansson et al., 2022). This pronounced elevation inherently allows a higher tolerance for errors in laboratory analyses, be they clinical or otherwise, rendering these tests more resilient to pre-analytical and analytical biases. Furthermore, due to the elevated levels of pTau in the presence of the disease, whether found in CSF or blood, it can be effectively measured using high-throughput technologies (ELISA, Cobra, SIMOA, etc.), thereby reducing costs and expediting test turnaround time. Nevertheless, the two recommended pTau markers for clinical use, pTau181 and pTau217, as core AD biomarkers in accordance with the revised NIA-AA guidelines, exhibit robust associations with conditions such as chronic kidney disease, hypertension, stroke, or myocardial infarction (Mielke et al., 2022), all of which are common among older adults. Like Abeta, it is pivotal to meticulously account for the "biological factors that might adversely impact diagnostic accuracy" (Karikari et al., 2022), aligning with the Alzheimer's Association's current stance on blood biomarkers in AD (Hansson et

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al., 2022). Notwithstanding the link between pTau and comorbidities more prevalent among the elderly, the revised guidelines advocate for their integration into clinical settings. During the panel discussions at AAIC in July of this year, questions were raised by the audience regarding the interpretation of positive test results. In response, the panel emphasized the responsibility of physicians to analyze such outcomes within the broader medical context. Dr. Reisa Sperling stated, "That's why us clinicians will still have jobs" (Revised Again: Alzheimer's Diagnostic Criteria Get Another Makeover | ALZFORUM, n.d.). However, we find the stance of the NIA-AA and Dr. Sperling's comment inappropriate within the realm of clinical care, potentially downplaying the challenges and confusion that these tests could introduce for patients and their physicians (e.g., interpreting results in the background of metabolic syndrome). This approach seems to shift the responsibility and burden to physicians in primary and secondary care, as well as to community-based neurologists. Our final concern emerges from a narrative shared by Dr. Clifford Jack during the AAIC 2023 panel discussions about the under-consideration NIA-AA guidelines. Dr. Jack recounted a case involving a patient with dementia who exhibited A+T-N+ biomarkers (Revised Again: Alzheimer's Diagnostic Criteria Get Another Makeover | ALZFORUM, n.d.). As per the revised guidelines, this individual would be classified as having AD. However, Dr. Jack's comprehensive analysis revealed a more intricate scenario where the dementia could be attributed to advanced limbic predominant age-related TDP-43 encephalopathy (LATE). Beyond the care offered by experts like Dr. Jack in specialized memory clinics, the question arises whether patients might receive inadequate diagnoses based on blood tests in the context of insurance-driven managed care, characterized by time and financial constraints. This is especially concerning given the concerns about validation, robustness, and comorbidities. Furthermore, the appropriateness of therapy for an individual whose dementia is caused by LATE is open to debate, underscoring the risk of marginalizing clinical expertise and patient history in favor of an exclusive biomarker focus. In light of these considerations, the recent proposed revisions by the NIA-AA regarding Alzheimer's disease diagnostics give rise to several concerns that warrant careful attention. The report from the Alzheimer's Association emphasizes the limited readiness of BBMs for widespread clinical application, suggesting cautious utilization in specialized memory clinics for diagnostic purposes (Hansson et al., 2022). This standpoint, echoed by the CTAD report (Angioni et al., 2022), calls into question the appropriateness of the NIA-AA's decision to introduce BBMs into primary and secondary care settings. Interestingly, the NIA-AA document seems to lack any caution against such usage. Despite the NIA-AA panel's assertion that these biomarkers are "sufficiently validated for clinical use" (Revised Again: Alzheimer's Diagnostic Criteria Get Another Makeover | ALZFORUM, n.d.), we respectfully hold a different view, particularly

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concerning BBMs. The existing issues related to robustness, comorbidities, and real-world validation raise doubts about their reliability, even when considered as preliminary screening tools or ancillary to the diagnostic pathway. Of significant note is that these BBMs have primarily been developed based on studies within Western populations (mainly Caucasian), leaving the applicability of the criteria uncertain for non-Western populations where genetic, cultural, and environmental factors can significantly influence disease presentation. Furthermore, the NIA-AA's admission that cutoff values for fluid biomarkers are absent gives rise to concerns about the practical implementation of the proposed staging. The claim of their sufficient validation clashes with this ambiguity, particularly when these markers do not closely align with clinical impairment. The potential for misdiagnosis becomes evident when biomarkers fail to correlate with cognitive decline. While the importance of synaptic biomarkers is acknowledged as a future direction in the field, the draft document seems to give them minimal attention, despite the existence of viable markers (Chirila et al., 2013; Colom-Cadena et al., 2020). Cognitive testing, often overlooked, constitutes a critical component, especially given that only 25% of Medicare beneficiaries recall undergoing such assessments (Jacobson et al., 2020). The emphasis on biomarkers, without substantial validation, operational clarity, and alignment with cognitive decline, raises concerns about their reliability and the potential for misinterpretation. Dr. Sperling's assertion that "Not everyone with amyloid will develop symptoms, but we take [its presence] seriously, and we want to treat it" resonates with our perspective (Revised Again: Alzheimer's Diagnostic Criteria Get Another Makeover | ALZFORUM, n.d.). We advocate for comprehensive lifestyle interventions and the management of comorbidities, both of which contribute significantly to dementia cases (Livingston et al., 2020; Rolandi et al., 2020). Recognizing the elevated mortality rate in the elderly population, where the majority do not progress to AD (Brookmeyer and Abdalla, 2018; Brookmeyer et al., 2018; Jack et al., 2018), we draw parallels with conditions like heart disease and diabetes. In these cases, high cholesterol and blood sugar levels function as biomarkers, indicating risk and justifying lifestyle interventions before resorting to pharmacological treatments. While the proposed revisions to Alzheimer's disease diagnostic criteria aim to incorporate recent scientific advancements, they concurrently give rise to significant concerns that merit thorough consideration. Given the limited and inconclusive evidence regarding the clinical and diagnostic benefits of BBMs, the potential burdens on both physicians and patients, the challenges associated with timely care management following a positive BBM result, and the potential financial implications, it becomes imperative to exercise caution in adopting the revised NIA-AA guidelines. We strongly recommend reiterating the Alzheimer's Association's recommendation to limit BBMs to specialized memory clinics as an essential aspect missing from the current draft. Furthermore, we urge the NIA-AA

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to endorse annual cognitive testing (Jacobson et al., 2020), and considering the Alzheimer's Association's highlighting of the absence of routine cognitive screening (Hansson et al., 2022). These measures can be further augmented by proactive management of comorbidities and lifestyle adjustments earlier in the diagnostic process (Livingston et al., 2020; Rolandi et al., 2020). We appreciate your consideration of these arguments and remain committed to collaborative efforts with the NIA-AA and stakeholders to establish evidence-driven policies that enhance healthcare outcomes for all beneficiaries.

10. Dear NIA-AA Workgroup: We have one principal modification and a number of relatively minor modifications/clarifications to suggest for the draft guidelines. The principal modification has to do with the distinctions drawn between the use cases of the core biomarkers from non-core biomarkers. In particular, there is ample evidence that combining one or more core biomarkers with one or more non-core biomarkers can significantly enhance the diagnostic accuracy of a blood test, yet the draft guidelines do not recognize the use of non-core biomarkers as adjuncts to core biomarkers for diagnosis. This could put limits on the development, utilization, and reimbursement of multi-variable tests that combine both core and non-core biomarkers to improve the diagnostic performance of the test. Specific verbiage to address this critical point is suggested below. The relatively minor modifications have to do with improving the clarity of meaning or the removal of unnecessary terminology that could impede the adoption of blood based biomarkers. These edits are listed in detail below. Principal Modification: Diagnostic Usefulness of Non-Core Biomarkers: Table 2 limits the use case of non-core fluid biomarkers (NfL, GFAP) to "Staging, prognosis, as an indicator of biological treatment effect." While these biomarkers are not specific to AD pathology, numerous studies have shown that when used in conjunction with one or more core biomarkers diagnostic performance can be significantly improved. As examples, Chatterjee et al [1] found that the detection accuracy for amyloid pathology in cognitively unimpaired individuals (AIBL cohort) was enhanced from a maximum AUC of 0.84 with single plasma core biomarkers to 0.91 when adding plasma GFAP and NfL to the model. Bucci et al [2] recently reported that amyloid pathology detection accuracy in a cohort of MCI patients from a tertiary care clinic increased from a maximum AUC of 0.65 with single or combined plasma core biomarkers to 0.93 when adding plasma GFAP and NfL to the model. We would like to suggest the following edits to address this. Line 284 and Text Box 3 "We'd like to suggest that the sentence on means to diagnose AD be expanded to include "either alone or in combination with each other or other validated biomarkers (e.g., GFAP, NfL)." Note that the requirement of an abnormal core biomarker for amyloid pathology/AD diagnosis remains intact; the proposed verbiage simply expands the requirement to permit potential inclusion

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of adjunctive non-core biomarkers if they are shown to improve diagnostic performance. Minor modifications: Line 383 “ We™d like to suggest that this be altered slightly to read: “The zone of uncertainty thus divides the continuous range of values into 3 result categories that would correlate with confidently normal, confidently abnormal, and indeterminant. These zones can be achieved through qualitative, quantitative, or algorithmic risk thresholds” . As originally worded, it implies results should only carry “normal/abnormal/indeterminant” titles and may not be able to be reported as a risk score. Given some of the current validated fluid biomarker assays combine amyloid ratio and tau in algorithmic fashion, we want to avoid wording that may be interpreted too tightly to allow for algorithmic outputs. Line 431-432 “ We would like to suggest this line be expanded to say “An important principle is that biological staging of AD applies only to individuals in whom the disease has been diagnosed by an abnormal core biomarker, either on a previous result or as part of a combined core and non-core fluid biomarker panel” . Panels of core with non-core biomarkers could allow for simultaneous tiered reporting of AD diagnosis and staging on the same result, eliminating the need for additional blood draws/visits, and time to results. Lines 707 “ We would like to suggest that “appear as ATNISV with +/-” be adjusted to say “appear as some combination of ATNISV with results indicated as appropriate for each category” . Given some of the non-core biomarkers are only available by one means or another (e.g., GFAP by fluid, aSyn-SAA by CSF, or vascular injury by imaging) it may not be possible for any one test to look at all 6 at one time, nor may it be necessary to yield clinically useful and accurate results, depending on the test development. Also, by removing the +/- designation, it again opens these tests to be more continuously reported with algorithmic risk scores that can be interpreted along with clinical input to help inform decisions, rather than be suggested that strict +/- results are the best way to report. Line 746 “ It is mentioned that profiling may be used for exclusionary criteria in trials, but there is no mention of using biomarkers as a means of monitoring for drug efficacy/stoppage, which is an important potential use case. Lines 781-783 We suggest including “research” in the sentence “The staging schemes we outlined earlier therefore should be regarded as tools for diagnosis, staging/prognosis, and treatment assignment pretreatment...” The staging schemes can also be used as post-treatment research tools to better understand the interaction between therapeutic approaches and their effects on AD pathology. Future Directions section, Line 817. We recommend explicitly addressing the current paradigm of autopsy as the gold standard, which propagates a view that a test is not fully validated until it is validated against autopsy. If we believe that fluid and imaging biomarkers are sufficient for diagnosis, use of validated methods (amyloid PET, CSF) can adequately serve the purpose of validating subsequent methods (blood-based biomarkers) without

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a requirement of autopsy. Thus, "autopsy confirmation" may need to be overturned as an impractical standard of truth. "Figure and Tables" document, line 103-104 - We recommend adding the following footnote beneath lines 103/104: "The diagnostic use case is not limited to core biomarkers and may include one or more non-core biomarkers when used in conjunction with one or more core biomarkers." "Figures and Tables" document, line 240 - The footnote referencing the need for plasma amyloid ratio to include CSF, amyloid PET, or fluid tau for staging, may get lost and we believe it is important enough to draw attention to - at least by reference- in the text. This also supports some of our suggestions about adjusting the wording to allow combinations and algorithmic output. "Figures and Tables" document, line 195 "What does "assay standardization" mean? Harmonization across methods is unrealistic, even with consensus reference standards. As an example, extensive efforts to harmonize troponin methods were not successful, yet the test is broadly used clinically. One doesn't need a consensus reference standard for a validated test that is suitable for clinical use. We recommend deleting reference to "assay standardization", as this is unnecessary and may impede acceptance of blood tests. References 1. Chatterjee P, Pedrini S, Doecke JD, et al. Plasma A β 42/40 ratio, p-tau181, GFAP, and NfL across the Alzheimer's disease continuum: A cross-sectional and longitudinal study in the AIBL cohort. *Alzheimers Dement.* 2023;19(4):1117-1134. 2. Bucci, M., Bluma, M., Savitcheva, I. et al. Profiling of plasma biomarkers in the context of memory assessment in a tertiary memory clinic. *Transl Psychiatry* 13, 268 (2023).

11. August 30, 2023 RE: NDSS Comment on the Draft NIA-AA Revised Clinical Guidelines for Alzheimer's Dear National Institute on Aging and Alzheimer's Association: The National Down Syndrome Society (NDSS) empowers individuals with Down syndrome and their families by driving policy change, providing resources, engaging with local communities, and shifting public perceptions. We write today in response to the National Institute on Aging (NIA) and Alzheimer's Association draft Revised Clinical Guidelines for Alzheimer's. Given that there has been significant progress in the scientific and clinical understanding and diagnosis of Alzheimer's disease since 2018, NDSS applauds the NIA and the Alzheimer's Association for convening a workgroup to update and enhance the clinical guidelines for Alzheimer's disease. Furthermore, NDSS strongly supports the explicit inclusion of the Down syndrome community in the guidelines (Section 5.2, Stage 0 and genetics) and urges the NIA and the Alzheimer's Association to ensure that these new diagnostic criteria are included in the final guidelines. Individuals with Down syndrome are uniquely situated in the Alzheimer's landscape because they

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have an extra copy of chromosome 21. The 21st chromosome carries the amyloid precursor protein (APP) gene, which is strongly associated with the formation of amyloid peptides and plaques, a hallmark of Alzheimer's disease. As a result, individuals with Down syndrome have an elevated lifetime risk "higher than 90%" for developing Alzheimer's disease (the combination is referred to as DSAD), with the onset of symptoms coming earlier and progressing faster than in the general population. In fact, Alzheimer's disease is the number one cause of death for individuals with Down syndrome. Unfortunately, Alzheimer's disease is commonly misdiagnosed in patients with Down syndrome. Often, this is because Alzheimer's Disease and Down syndrome share some observable traits, leading many physicians to attribute behaviors to Down syndrome without testing to see if these traits are because of the onset of Alzheimer's Disease - an issue called diagnostic overshadowing. Given this prognosis, it is critical that researchers, clinicians, and community members have an increased understanding of the diagnosis of Alzheimer's disease in the general population and DSAD that is rooted in clinical evidence and pathology, not just observable traits. Section 5.2, Stage 0 and genetics, proposes the addition of stage 0, which explicitly acknowledges the unique genetic predisposition that individuals with autosomal dominance and Down syndrome (Trisomy 21) have for developing symptoms and the clinical onset of Alzheimer's disease. Furthermore, the creation of stage 0 stipulates that an individual would only move to stage 1 "when core biomarker(s) become positive." For individuals with Down syndrome who present across a broad spectrum of cognitive and executive function, this focus on objective diagnostic biomarkers helps ensure diagnostic overshadowing does not negatively impact an individual's diagnosis or subsequent treatment for Alzheimer's disease but instead, these diagnoses and treatment plans are based in clinical pathology. As noted in the guidelines, this proposal is consistent with recent proposals for diseases such as Huntington's and Parkinson's disease and is warranted given the latest advancements in the understanding and diagnosis of Alzheimer's disease. As researchers' understanding of the genetic and pathologic indicators of the disease improves, so too should the criteria clinicians use to diagnose the disease so that more accurate diagnoses can be made and ultimately treatment plans and outcomes can be more informed and effective. The inclusion of Section 5.2 could positively impact the Down syndrome community in a number of ways including, but not limited to "increasing education, diagnostic efficacy, and access for those with DSAD, elevating the status of the Down syndrome community within the field, and changing the narrative around quality of life and expected outcomes of DSAD. Increasing Education, Diagnostic Efficacy, and Access Despite being adversely affected by Alzheimer's at a rate that is markedly higher than that of the general population, or any other underserved population, the Down syndrome

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community continues to face barriers to accessing high quality diagnostic, treatment, and care options. This can be attributed, at least in part, to the lack of education and awareness of the connection between Down syndrome and Alzheimer's disease and how DSAD compares to Alzheimer's disease in the general population. Research supports that there is little to no genetic difference between Alzheimer's disease in the general population and DSAD, and therefore, drugs and treatments that are found to be effective for the general population will very likely have the same efficacy for those with DSAD. These treatments may, however, pose additional or increased rates of symptoms or complications from what is typically observed in the general population. Inclusion in clinical trials and the development of safety data that is inclusive of, and specific to, the Down syndrome community is necessary to ensure individuals with DSAD can safely benefit from the drugs and treatments the general population is prescribed. The cornerstone to establishing these trials and the prescription of any promising drug or treatment is a clinician's ability to diagnose Alzheimer's disease confidently and objectively in a patient with Down syndrome. Thus, the inclusion of diagnostic criteria specific to DSAD in the revised clinical guidelines is paramount to ensuring that clinicians are educated about DSAD, have the tools necessary to diagnose DSAD based on the clinical pathology, and ultimately, that individuals with DSAD will have equitable access to the interventions they so desperately need. Lastly, these clinical guidelines will also be invaluable to the INCLUDE (INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndromE) Project, a trans-NIH research initiative on critical health and quality-of-life needs for individuals with Down syndrome that promotes the inclusion of people with Down syndrome into clinical trials, and the NIA's Alzheimer's Clinical Trials Consortium's Down syndrome (ACTC-DS), which exemplifies the innovative efforts needed to ensure that people with Down syndrome benefit from recent advances in Alzheimer's disease diagnostics and therapeutics. The incorporation of diagnostic criteria specific to the Down syndrome community will support these two entities in their work to advance clinical trials designed for people with Down syndrome. Elevating the Status Beyond the immediate impact on clinicians, the inclusion of this proposed diagnostic criteria also has the potential to have a wide-reaching impact on regulatory agencies, drug developers, researchers, and families. Criteria specific to the diagnosis of DSAD will help bring familiarity of the co-occurrence to drug developers that could lead to further developments in the treatment and cure of Alzheimer's disease for patients with Down syndrome. Additionally, bringing a focus to the unique presentation and diagnosis of DSAD could urge researchers and clinicians to consider new and innovative treatments that could potentially impact thousands of people within the Down syndrome community. Lastly, as the understanding of DSAD continues to develop, it is our hope that regulatory agencies will ensure that individuals with Down syndrome

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are able to fully access all drugs and treatments available and prescribed by their physician following a diagnosis based in clinical pathology and individualized assessment. Including the Down syndrome community in the revised clinical guidelines is the first step toward elevating the status of the Down syndrome community throughout the field and ensuring that all decision makers have the information they need to make informed decisions about the development, prescription, and coverage of drugs and treatments. Changing the Narrative For far too long, the same somber sentiment has been echoed within the Down syndrome community "it is not if, but when." With lifetime risk rates for developing Alzheimer's disease climbing over 90%, parents, caregivers, and individuals with Down syndrome too often live in fear of an intangible disease that they have been told they or their loved one will almost certainly develop. As a community-facing advocacy organization, NDSS feels strongly that it is the role of organizations like ours to partner with agencies, researchers, drug developers, and clinicians to change this narrative. A disease that once seemed untreatable is now becoming treatable. A community that has for so long lived in fear can now begin to live in hope. Once again, we urge the NIA and the Alzheimer's Association to ensure that Section 5.2 of the proposed guidelines is included in the final revised clinical guidelines and to work with organizations like ours to continue changing the narrative for the Down syndrome community. NDSS strives to ensure all individuals with Down syndrome are assured their human rights and valued by a more inclusive society. We applaud the Alzheimer's Association, the NIA, and the working group for their work on this critical issue and look forward to continuing to work together toward a more equitable and inclusive diagnostic landscape for the Down syndrome community-National Down Syndrome Society

12. Dear writing team, I am very supportive of this update and find the grouping of biomarkers into 3 broad categories particularly useful. I will primarily comment on the vascular markers in the group "biomarkers of common non-AD co-pathologies", because that is my area of expertise. My main suggestion would be to carefully look at the terminology, some of the terms used are not really well accepted in the stroke field. I would suggest to stay consistent with STRIVE-2 terminology where possible. Specifically: -line 239: "Macroscopic cerebral infarctions, including both large cortical and subcortical (lacunar) infarctions, on anatomic MRI"; suggest to change to: "Macroscopic cerebral infarctions, including both large cortical and subcortical infarctions and lacunes"; large infarcts can be subcortical and not all subcortical infarcts are lacunes. -line 240: The term "Anatomic MRI" is also not commonly used in this context and these infarcts can also be seen on CT. The sentence could be "Macroscopic cerebral infarctions, including both large cortical and subcortical infarctions and lacunes, on MRI or CT". (also applies to

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terminology table 1) -line 239/tables: Why accept infarcts as contributor to cognitive decline and vascular injury but not primary intracerebral haemorrhage? I would recommend to add ICH. -Table 1; "abundant dilated perivascular spaces" ; in my view these are indeed an indicator of small vessel disease. Yet, abundant PVS can occasionally be seen in people without any indication of brain injury, also at young ages. They likely not always reflect vascular injury. Their relation with cognition is weaker and the knowledge base on their etiology is smaller than for infarcts/ICH/WMH. I would recommend to move the PVS to table 3, "Additional biomarkers" .

13. Given the NIA's progress toward more accessible diagnostic criteria, the NTG appreciates the work of the NIA-AA working group and lauds its contribution to improving the efficiency and accuracy of diagnosis and staging of Alzheimer's disease. To this end, we offer our support to formalizing the clinical standards for determining Alzheimer's disease diagnosis using plasma biomarkers and support further investments in research in a range of biomarkers for other forms of dementia in the adult population. We concur with the specific recognition on page 22, line 645 ff. (5.2 "Stage 0 and genetics") of the contribution of genetics inherent in Trisomy 21, Down syndrome, to the eventual presentation of brain amyloid accumulation and expression of Alzheimer's disease, and we laud the working group for recognizing this special situation and for providing a clinical basis for pre-symptomatic Down syndrome associated Alzheimer's disease. We also laud the NIA's investment in DS-AD biomarkers research. We recommend extending this investment to determining the nature of Alzheimer's disease biomarkers in the adult population with lifelong intellectual disabilities. Specifically, we also recommend research to determine the applicability and effectiveness of biomarker findings noted in the proposed clinical guidelines relevant to diagnosing Alzheimer's disease in adults with intellectual disabilities other than Down syndrome. Also, such biomarker research should include other genetic syndromes associated with intellectual disability that may offer particular risk for Alzheimer's disease or other diseases associated with dementia. We further recommend, if biomarker parameters vary with respect to Down syndrome, that the guidelines note biomarker parameters that may be idiosyncratic to Down syndrome. We also recommend that the guidelines provide clinical equivalencies to staging factors and functioning for adults with intellectual disability. Finally, we recommend adding language to Item (9), page 27, line 800 ff., "Diversity and need for more representative cohorts," to include adults with various lifelong neuroatypical conditions, including intellectual disabilities, in observational studies and clinical trials. Treatment studies, considering the contribution of social determinants of health, also should include diversity "reflecting cognitive impairments associated with neuroatypical conditions."

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14. We are pleased to see inclusion of fluid biomarkers within the NIA Clinical Guideline framework; however, we want to highlight our concern around vague and inaccurate language used in the discussion of CSF and blood-based biomarkers. Furthermore, there is insufficient peer-review data to support the use of CSF and blood-based biomarkers interchangeably as implied throughout the document. Specific feedback by page/line number is provided below, however, we also strongly recommend seeking feedback from the appropriate laboratory medicine specialty in North America prior to finalization of this document. These experts will be able to provide guidance on appropriateness of the recommendations based on the current standards and regulatory requirements for implementation of these tests in clinical laboratories. In laboratory medicine, AD biofluid testing is most commonly overseen by Clinical Chemists (DABCC, FCACB, or equivalent designations outside of North America). The appropriate professional associations for consultation in North America include the Association for Diagnostic and Laboratory Medicine and the Canadian Society of Clinical Chemists.
1. Terminology used throughout document:
- a. biomarkers diagnostic of AD v. biomarkers diagnostic of AD pathology Earlier international consensus efforts (e.g., DOI: 10.1002/alz.12545) have emphasized the need to refer to AD biomarkers as being reflective of the pathology and not the disease. The language in the draft document presents a deviation from this consensus and rationale for this change in terminology should be provided.
 - b. The draft guideline includes many generalizations between CSF and blood, as if they were interchangeable. This is inaccurate and misleading. We recommend striking the use of "œfluid" as terminology and instead specify CSF and/or blood for each instance where it is relevant throughout the text, text boxes and tables.
 - c. Throughout the document only CSF AB42/40 is referred to as core biomarker but we know that CSF pTau181/AB42 ratio is as good as a measure of amyloid pathology (e.g., DOI: 10.1186/s13195-020-00595-5, DOI: 10.1002/dad2.12190, DOI: 10.1002/dad2.12182). CSF pTau181/AB42 should be specifically listed as a core biomarker. While pTau181/Ab42 does not fit nicely within the ATN framework, it should not be ignored for this reason.
2. Page 1, line 24-25:
- "œplasma-based biomarkers with excellent diagnostic performance have been developed and clinically validated"
- a. Recommend removing reference to "œexcellent diagnostic performance" and replacing with data ranges (for example). From the studies performed in controlled research settings, the diagnostic performance can vary between assays and labs.
 - b. Recommend removing "œclinically validated." This is misleading. The majority of plasma biomarkers are in development. While these assays may have been analytically validated, they have not been fully clinically validated. Therefore cut-points relevant for interpretation of the results based on the context of use have not

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been establish for interpretation in clinical practice. 3. Page 3, line 72: "The most significant advance in AD diagnostics in recent years has been the development of plasma biomarkers with excellent diagnostic performance." This phrasing ("excellent diagnostic performance") is vague/inaccurate. The document should acknowledge that not all plasma assays perform the same clinically or analytically. 4. Page 3, line 74: "This now makes biological diagnosis of AD (which previously required PET or CSF assays) generally accessible and is projected to revolutionize research and clinical care. It is correctly stated that moving to diagnosing AD with a blood test has the potential to drastically change clinical care. With the impact of this change in mind, before blood-based biomarkers are included in clinical guidelines, clinical utility needs to be established, and assay specific performance of blood tests, as compared to PET and CSF testing, needs to be transparent to patients and providers. Inappropriate utility of blood tests or misunderstanding of the limitations of these tests will lead to many false positive and false negative results. Care needs to be taken to ensure that this "revolution to clinical care" helps patients, rather than harms them. 5. Page 4, line 116-119 "Biomarkers were placed into Tables 1,2 vs Table 3 based on the committee's assessment of the strength of available evidence of high diagnostic accuracy (e.g., sensitivity, specificity) compared to a valid gold standard, high reproducibility, and diagnostic utility based on clinical studies in real world settings.14,15" Notably, both papers cited (ref 14 and 15) discuss work that still needs to be done before these assays are ready to be used clinically. If a claim is to be made that these biomarkers have been tested and have "high reproducibility and high diagnostic utility in real world settings" , then references need to be included pointing to those specific studies (including transparency on how the studies were performed" , the specific assays used in those studies, their cutoffs, and their diagnostic performance compared to imaging and CSF. The majority of the data on sensitivity/specificity of these assays was determined in research settings, using batch testing, without pre-established cutoffs. This is far from equivalent to a "real world setting in a clinical laboratory." 6. Page 5, line 142: "Plasma and CSF A β 42/40 both correlate with amyloid PET and predict clinical progression: however, the fold difference between individuals is around 50% for CSF A β 42/40 but 10%-15% for plasma A β 42/40." a. This passage inadequately addresses that plasma and CSF AB42/40 do not equally correlate with amyloid PET. CSF performance is superior (AUC in mid 0.90) compared to blood (AUC in the 0.70 to mid 0.80 depending on the assay) (PMID: 34542571). Further, various plasma AB42/40 assays show significantly different correlations with amyloid PET. The guideline should not be written from the perspective of the best performing plasma assays, while ignoring the variability in performance of what is in the literature (e. g. DOI: 10.1001/jamaneurol.2021.3180, DOI: 10.1093/brain/awac333). Additionally, performance was determined in research

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settings, under the best possible conditions (i.e., batch testing, careful sample handling, etc.) Performance needs to be determined in a clinical setting, with exposure to common preanalytical variables that are likely to decrease assay precision, thereby decreasing diagnostic performance (PMID: 35130933). b. The acknowledgement of a 50% difference in CSF vs 10-15% difference in plasma is an opportunity to address the limitations of plasma assays. For plasma assay™s performance to be equivalent to CSF, the requirements for precision and accuracy are much higher in plasma. The small difference between "disease" and "healthy" in plasma, means that small increases in imprecision as the assays transitions from a research setting to a clinical setting has the potential to result in big decreases in diagnostic performance (PMID: 35130933). 7. Page 6, line 151: "Two CSF assays for A β -amyloid have FDA and IVDR-CE approval for clinical use." It would be helpful to add that these assays assess the presence of amyloid pathology by different means, that is pTau/Abeta 42 v. Abeta42/40, yet have similar diagnostic accuracy for AD pathology. 8. Page 11 line 327: "Biofluid assays do not require FDA approval; the much-less rigorous CLIA or CAP (in the US) certifications do not require autopsy validation." We recommend striking this sentence or otherwise rewording and providing appropriate citations. 9. Page 12 line 349: "Diagnosing AD by an abnormal core biomarker demands a high level of fidelity when applied clinically. However, any diagnostic test value, fluid or imaging, has a degree of uncertainty associated with it. We therefore recommend 3 protections against misdiagnoses!" a. "Fidelity" is not an acceptable laboratory medicine term. We recommend the use of the term "accuracy". b. This paragraph is an opportunity to request from companies and laboratories for clarity on the performance of the biomarkers they are offering and the need for transparency on how these metrics were determined so that physicians can interpret the test findings. Asking for "rigorous validation standards" is too vague. There are established validation standards; however, there are no standards relating to the public transparency in the reporting of these validation metrics. 10. Page 13 line 364: "prescribing specific performance metrics; however, fluid or PET biomarkers used for diagnosis should meet high standards for sensitivity, specificity, and precision" a. Here it is accurately stated that clinical use of plasma biomarkers is in active development (NB: if they are in development, then they are not likely appropriate to mention in detail in a clinical guideline). However, the use of the word "fluid" is misleading. Grouping plasma and CSF assay as "fluids" may lead to the incorrect inferences that they can be (1) used interchangeably, and (2) are at the same stage of development. b. Many plasma assays in development are being positioned as a screening test instead of diagnostic tests for amyloid pathology. (This is even suggested on page 10, lines 289-292, where it is noted that biomarkers can detect AD pathophysiology "...even though onset

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of symptoms may be years in the future.) When blood tests are added to clinical guidelines, the appropriate utility of the tests should be clearly outlined in the guidelines. These guidelines should state that blood tests are not yet ready to be used clinically in asymptomatic patients. As prevalence decreases so does performance. There is not yet evidence supporting the use of blood tests as clinical screening tools. c. Clinical validation needs to include: (1) identifying set points, (2) defining context of use, including the appropriate patient population (3) determining (and being transparent about) the performance of the assay in the defined context of use. 11. Page 13 line 383: "The zone of uncertainty thus divides the continuous range of values into confidently normal, confidently abnormal, and indeterminant. In addition, incorporating a zone of uncertainty may lessen fluid/ PET discordances, particularly for A biomarkers. We recommend rewording guidance on the reporting of indeterminant zones. Depending on the assay used, the lab's informatics system, and whether the relevant data is provided by the manufacturer AND is relevant to the population the laboratory serves, reporting an "indeterminant zone" may not be feasible. Instead of advocating for reporting "indeterminant zones" we recommend (1) advocating for physician education in the interpretation of AD biomarker findings, and (2) advocating for manufacturers and labs running LDTs to provide detailed performance data around their assays' medical decision limits. 12. Page 17 line 39: "The onset of abnormal ptau 181, 217 and 231 seems to occur around the time of amyloid PET and much earlier than neocortical tau PET abnormalities" Conflating established fluid biomarkers (pTau 181 and 217) and research stage biomarkers (pTau205, pTau231, MTBR) would imply that they perform similarly and that their use is supported by an equivalent level of scientific evidence. This is inaccurate. Research-grade biomarkers should not be found in clinical guideline, other than perhaps a "Future Directions" section where it should be noted that the biomarkers therein have not been rigorously evaluated for clinical use. 13. Page 19 line 574: "We have identified specific fluid biomarkers to denote the early, intermediate, and advanced fluid stages. However, these fluid biomarkers have not yet been widely tested" We recommend striking the use of "fluid" as terminology and instead specify CSF and/or blood for each instance where it is relevant. 14. Page 25, Section 8 "Treatment effects" We recommend simplifying this section to indicate that at this point there is not enough data to provide specific recommendations of the use of biomarkers for monitoring treatments effects. The numerous data points in the draft document on this topic without specific statement on readiness may lead to misinterpretation or potential misuse. 15. Text box 1: Change "diagnostic of AD" to "diagnostic of AD pathology" 16. Text box 2: "Development of plasma biomarkers with excellent diagnostic performance" Vague and overly simplistic language. There is promising data for plasma biomarkers but as stated in the appropriate use recommendations,

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there are issues specific to blood-based biomarkers that need to be addressed before these can be established as diagnostic biomarkers. 17. Text box 3: a. Change "diagnosis of AD"™ to "diagnosis of AD pathology"™ b. "Stringently validated biomarkers (fluid or PET)" is too broad i. Recommend differentiating between CSF and blood, and pointing out what is needed before blood tests are ready for clinical use (DOI: 10.1002/alz.12756, DOI: 10.1002/alz.13026). 18. Table 1 a. CSF and blood should have their own columns as the performance is not interchangeable. b. A caveat should be included that (1) performance varies greatly by assay when using blood, (2) most blood assays are not yet clinically validated, and (3) publicly available clinical validation studies for blood assays have focused on symptomatic patients in a specialist setting, yet the guidelines suggest these tests can be ordered by general practitioners, where prevalence of AD pathology will be lower. c. For CSF fluid, ptau181/ab42 and tTau/Ab42 need to be mentioned as they have similar performance to (in CSF) to Abeta42/40. The fact that they do not fit in nicely with the ATN acronym should not be part of the rationale for their exclusion from a clinical guideline. d. For pTau181 and tTau mentioning their strong correlation would be valuable, so as to convey that one or the other could be used (but both may be unnecessary) in combination with Abeta42. 19. Table 2: a. CSF and blood should have their own columns. i. A caveat should be included that (1) performance varies greatly by assay when using blood and (2) blood assays are not yet clinically validated. b. There is no rationale provided for the inclusion of NfL and GFAP in this table. Under what context should they be used clinically for the diagnosis/prognosis/staging of AD? 20. Table 3: a. CSF and blood should have their own columns. b. Research grade biomarkers should be d " it is unclear why there is a mix of research use and clinical use assays as this is a clinical guideline and not a review paper of the status of AD biomarkers. 21. Table 4: a. Tau/abeta42 ratios should be added and Abeta 42 should also be listed as a biomarker like ptau181. b. CSF and blood should have their own rows.

15. We thank the NIA-AA for the opportunity to review and comment on the latest modular installment of recommended diagnostic criteria for Alzheimer's Disease (AD). The proposed module is an exciting and thorough adjustment to the scientific and disease landscape surrounding AD diagnostics and treatment. We appreciate the accelerated revision of criteria in keeping with previous commitments in the 2018 revision. The proposed staging, which integrates clinical and biomarker profiles, is a welcomed addition and helps reframe and accelerate the understanding of the AD continuum. Below, we provide comments, challenges, and recommendations. We hope that these constructive comments aid in the revision of criteria. Apart from a notable missing asterisk on

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pTau205 in Table 4 and citations needed at lines 132-135, our comments focus on clarifying ambiguous language in areas that address fluid biomarkers, both in sample type and method. We also focus on differentiating fluid biomarker platforms, because not all methods are equal. The criteria begin by claiming that the update was prompted by the development of plasma biomarkers with “excellent diagnostic performance.” However, the criteria later claim wide variability in plasma biomarker performance, citing head-to-head comparisons for both amyloid and pTau assays. The cited literature (Janelidze 40, 41) indicates that mass spectrometry (MS)-based plasma assays demonstrate superior performance and less variability than immunoassays for pTau and amyloid. Though we agree that rigorous performance validation must be undertaken regardless of platform, a generalized statement regarding the variability of all platforms may be misleading. We feel that using “fluid” as a term is needed, especially for staging. However, we found that its interchangeable use to denote plasma, CSF, and combined plasma/CSF assays or sample types may be confusing. For example, at lines 176 and 177 on page 6, the use of “fluid” after specifying CSF and plasma, prompts the reader to assume there is a distinction between CSF and plasma assays and fluid assays: “CSF and plasma total tau begin to increase early in the disease course in autosomal dominant AD 18 and closely correlate with fluid ptau in autosomal dominant and sporadic AD 53.” We suggest that “fluid” be pre-defined as encompassing CSF and plasma biomarkers, but that “CSF” or “plasma” be used when details and distinctions are necessary. Research-use only (RUO) biomarkers pTau205, tau fragments, and MTBR243 are used in integrated staging denotations (Supplementary Table 2) but are omitted from the use cases in Table 2, which displays those biomarkers that are “currently suitable for clinical practice.” We seek to understand the reasons for the apparent discrepancy. Plasma-based biomarkers analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) demonstrate comparable performance to CSF and PET testing across laboratories and cohorts. The criteria indirectly recognize their high performance, but ultimately defer to CSF, PET, or fluid pTau for a final ruling of amyloid status as noted in Supplementary Table 2: “to established stage Fa with a 42/40 assay: CSF Ab 42/40 is sufficient alone, but plasma Ab42/40 should be accompanied by a positive CSF Ab 42/40, amyloid PET or fluid ptau assay as well.” For many individuals, this confirmation may not be necessary, especially those with unambiguously in-range or abnormal LC-MS/MS results who could avoid the need for invasive and costly CSF or PET procedures. We would appreciate the NIA-AA team specifically addressing the clinical utility of plasma biomarkers. Thank you for your consideration.

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16. As FDG PET hypometabolism is included as a non-specific biomarker in N section I suggest including rCBF SPECT hypometabolism as well, as rCBF SPECT is much more accessible than PET in many centres. Medial temporal hypometabolism is typical for AD.
17. The new criteria reflect the exciting progress in biomarkers that has been made over the last few years. We really are at a great time for Alzheimer's research. But, defining this disease by biomarkers only when (a) so little is understood about timing and trajectories and (b) in most cases, the disease is one of a series of comorbidities which greatly impacts the progression and onset times of clinically meaningful cognitive change, is dangerous. The timing of these new criteria, with the anti-amyloid medications getting approvals, is concerning, especially given the many conflicts of interest of those proposing the criteria and the advisors. The number of potential customers that these new criteria will generate, and the self-fulfilling prophecies ("they didn't decline, it worked!") generated are very concerning. These criteria are jumping ahead of the science and will generate a large income and other successes for many of those involved, while offering limited benefits to sufferers and their families. We see biomarker positive cases frequently in clinic who are doing well or improve when modifiable risk factors are tackled. Pathologizing their current status would lead to distress. I have friends, collaborators and colleagues among those proposing these criteria, and support both the NIA and AA, but I know my concerns are shared by many in the community.
18. The links provided on your web site to the 2018 research framework do not work (all the references on the target site are from 2011-12).
19. I read the draft NIA-AA Framework for Alzheimer's diagnosis. I agree with American Geriatrics Society comments that making Alzheimer's diagnosis purely biological/marker based is premature. Blood/CSF tests are not easily available/covered by insurance yet to my knowledge as a PCP/geriatrician. See also the book "How Not to Study A Disease" by Dr Karl Herrup for criticism of conflating Alzheimer's clinically with Amyloid pathology given the disappointing clinical trial results which show only modest decline. I think this book made some very good points.
20. I have three concerns regarding the proposed updated criteria. The first is that the new criteria do not seem to build upon the 2011 criteria, which are the criteria that we primarily rely on clinically (in addition to the DSM-5 neurocognitive disorder criteria). Clinically, it is useful to have more detailed guidance on assessment and interpretation of cognitive and functional decline. There is significant neuropsychological literature on the cognitive assessment and typical

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cognitive profile of Alzheimer's disease that is missing from this document. The second concern is a more practical one in that these new criteria feel disconnected from the clinical setting. As both a memory clinic provider and researcher, I can absolutely appreciate the benefit of improved precision of biomarker testing and staging, but as a clinic provider this is not relevant to me because we rarely get biomarker testing in the clinic. Criteria that only allow for a diagnosis of Alzheimer's disease with a biomarker test are not very relevant to the current clinical setting. Given this, it might be helpful to incorporate the 2011 criteria as "core clinical criteria" and include the biomarker testing/staging as "indicative" or "supportive" biomarkers, rather than requiring the biomarker testing for diagnosis. Lastly, the draft states that biomarker testing should not be ordered or interpreted in the absence of the clinical context, but also includes interpretation of biomarkers in asymptomatic people as having AD; some clarification on this and the language used to describe elevated biomarkers in asymptomatic populations is needed (especially given heterogeneity of cognitive trajectories in this group). Overall, I appreciate the continued precision and I think these criteria are a nice update to the NIA-AA Research Framework, but they feel premature as clinical criteria for AD.

21. First of all, congrats on a thought leading and excellent draft -- I thoroughly enjoyed reading it! I would like to point out one inaccuracy at Line 773: "Individuals followed after cessation of A β immunotherapy have shown reversal of CSF A β 42/40 normalization, some clinical progression, and eventual recurrent accumulation of amyloid on PET". The reference is McDade et. al. *Alzheimers Res Ther.* 2022;14(1):191. It should be "plasma Abeta 42/40" as opposed to CSF.
22. Re these two sentences: 674: The symptomatic consequence of biological AD is modified by interindividual differences in co-pathologies, resistance, and reserve (i.e., education other social determinants of health). 691: Individuals who lie below the diagonal (i.e., better clinical stage than expected for biological stage) may have exceptional resistance or cognitive reserve. Suggestions: 1. The term "resistance" has typically been used in reference to not developing AD pathology, as opposed to resilience in the presence of pathology) (e.g, Arenaza-Urquijo EM, Vemuri P. Resistance vs resilience to Alzheimer disease: Clarifying terminology for preclinical studies. *Neurology.* 2018 Apr 10;90(15):695-703. doi: 10.1212/WNL.0000000000005303. Epub 2018 Mar 28. PMID: 29592885; PMCID: PMC5894932.) I suggest replacing the word "resistance" with "resilience." 2. I would also suggest using the term "cognitive reserve" in both of these sentences, since "reserve" is a more general concept that might encompass both brain and cognitive reserve.

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23. I applaud the efforts by the workgroup to move the world of Alzheimer's Disease diagnosis and care into a biomarker-based approach and to a biological definition of disease. It is truly remarkable that the science has progressed so much. That being said, I am wary of the decision to define AD by the presence of any single abnormal core biomarker, particularly amyloid in the absence of tau. The pathology of the disease has always been defined by the presence of both amyloid and tau pathologies, and in the clinical realm we know that not everyone with amyloid positivity will develop clinical symptoms. With the newly proposed definition of disease, the logical next step is to use amyloid as a screening test for AD and to then potentially use it to guide treatment decisions for asymptomatic individuals. This could easily lead to unnecessary harm to patients both with regards to the treatment for isolated amyloid deposits and the psychological harm of being given an AD diagnosis in the absence of symptoms. An analogy has been drawn between amyloid and HbA1c for diabetes. I would suggest that a more appropriate biomarker analogy is to prostate-specific antigen (PSA), which can be a marker of prostate cancer but is not specific to prostate cancer and has led to overdiagnosis and overtreatment. I urge the committee to reconsider the use of amyloid positivity as an isolated diagnostic biomarker.

24. Overall, I think the committee has done great work in integrating a rapidly evolving field. The use of staging will substantially refine the understanding of AD. However, there are some points I am concerned about: I am apprehensive about the lack of distinction between CSF and plasma biomarkers. Some plasma biomarkers (in my opinion, only p-tau217) have performance that can reasonably approximate CSF (and only in some settings). Most other plasma biomarkers are substantially inferior to CSF (Ashton et al, Alzheimer's & Dementia 2022; Therriault et al Alzheimer's & Dementia 2023, Palmqvist et al JAMA 2020), and a lack of distinction here stands to create substantial confusion and error. I understand that the committee does not want to make broad statements on specific biomarkers, but perhaps some more guidance on minimal accuracy (let alone PPV or NPV) of specific biomarkers would be useful. Especially if these biomarkers will be used for diagnosis: as we have seen, many companies are rushing to the market with very low performance plasma tests. Perhaps more clarity surrounding the "rigorous validation standards" (page 12 line 353) would be helpful here to guide their use. Also, I am concerned about the need for only one core biomarker for diagnosis. I agree that two PET scans is not realistic (let alone one in most cases). However, if CSF biomarker tests are ordered (which typically include AB42 and p-tau at the very least), would a profile of A+/p-tau- be

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considered "AD"? This does not fit with my intuitions, and there is potential for confusion here. Some guidance would be helpful. Thank you for all your work.

25. The American Geriatrics Society (AGS) appreciates the opportunity to provide feedback on the draft National Institute on Aging-Alzheimer's Association (NIA-AA) Revised Clinical Criteria for Alzheimer's Disease (AD) which is an update of the NIA-AA Revised Clinical Guidelines for Alzheimer's Disease. AGS believes that the rapid evolutions in our knowledge of Alzheimer's Disease and Related Dementias (ADRD) will necessarily (and hopefully) lead to future shifts in clinical practice and revisions to how we diagnose, and label conditions and pathologies associated with ADRD. We recognize that defining AD as a biological construct has advantages for research. We therefore agreed with the following definition that was articulated in the 2018 guidelines given its stated purpose: "This unifying update is labeled a "research framework" because its intended use is for observational and interventional research, not routine clinical care." The draft 2023 update of the guidelines proposes to expand their use into clinical care: "A major new direction therefore is to expand the 2018 framework from a research-only focus to one that provides recommendations that are applicable for both research and clinical care. The title of this modular update, NIA-AA Revised Clinical Criteria for Alzheimer's Disease, reflects this progression in focus." The AGS believes that the proposed expansion of the 2023 guidelines to include use in clinical practice is premature. Practitioners, patients, and society have not been sufficiently prepared for this shift, and the current evidence base is underdeveloped to support it. The reality is that there is no current evidence that discovery of biomarker positivity in a cognitively normal individual should lead to initiation of a specific clinical intervention. While discovery of an asymptomatic cancer during a routine screening colonoscopy justifies a diagnosis of colon cancer and initiation of specific treatment, as of now, there is no evidence that removing amyloid helps a cognitively normal person who is biomarker positive. We are concerned that the proposed expansion of the NIA-AA guidelines to include usage in clinical care will place many older and multimorbid people at risk of overdiagnosis, which in turn could lead to initiation of treatments with limited benefit and high potential for harm in this population. Unintended harms that this expansion could cause also include potential requirements from insurance companies, employers, and others that individuals be tested as a condition of insurance or employment. We believe that the risk of these potential harms is greater due to the proposal that the guidelines continue to carry the imprimatur of two well-respected organizations " NIA and AA. We outline our specific concerns in more detail below. General Concerns We have three general concerns related to this, the third modular update of the NIA-AA Guidelines: " The first concern is the composition of the workgroup that is proposing the guidelines be expanded to include use in clinical

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practice. According to the AA website (<https://aaic.alz.org/nia-aa.asp>), seven of the workgroup members are from the industry, and a number of other members have disclosed significant conflicts of interest. The makeup of the workgroup may be appropriate for a framework aimed solely at research criteria but is wholly inappropriate for a clinical guideline that includes recommendations for clinical practice. The Council of Medical Specialty Societies (CMSS) Principles for Clinical Practice Guidelines (<https://cmss.org/wp-content/uploads/2017/11/Revised-CMSS-Principles-for-Clinical-Practice-Guideline-Development.pdf>) recommend that clinical guideline panels be comprised of members who are free of conflicts of interest and that there be a process for identifying and resolving any potential conflicts. The CMSS principles build upon the 2011 recommendations from the Institute of Medicine of the National Academies of Science, Clinical Practice Guidelines We Can Trust (<https://www.ncbi.nlm.nih.gov/books/NBK209539/>). In this proposed update, the guidelines document itself is lacking a disclosure of the workgroup members' conflicts, nor is there any description of how the conflicts inherent in industry representation on the workgroup were resolved and how the conflicts of other workgroup members were mitigated. At minimum, the guidelines document should be revised to include the following directly in the document: (1) a list of workgroup members inclusive of their disclosures; (2) a description of how conflicts were addressed with respect to industry representatives; and (3) how any conflicts of other workgroup members were mitigated. Unfortunately, this will not address the major flaw which is the presence of industry representatives on the workgroup in the first place. ¶ The second concern is the guideline's disregard of important distinctions across fields of 'clinical practice.' Clinical practice in cognitive neurology is not like clinical practice in geriatrics, family medicine, or internal medicine. Statements about 'adoption of biomarker diagnosis in clinical practice' should specify which disciplines would be adopting this, the circumstances under which seeking a biomarker diagnosis would be appropriate, and how the practicing clinician is to guide person-centered decision-making about appropriate use of biomarker information in life planning. Further, the guidelines should account for the very substantial differences between medical disciplines in purpose, context, societal function, and population impact. It should also address the potential impact of a change in diagnostic standards on the coding of dementia diagnoses in medical records, and on the willingness of non-specialist clinicians to enter any cognitive diagnosis in a patient's chart. Simply put, it is not enough, as the revised guidelines do, to state that this 2018 research framework is now ready for use in clinical care. ¶ The third concern is that the draft text of this proposed expansion does not reflect the same level of collaboration between AA and the NIA that was evident in the 2011 guidelines and the 2018 modular updates which had the intended purpose of providing a research framework, a usage that is consistent with the

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mission of the NIA. For both earlier editions, the expert workgroups were co-convened by AA and the NIA, whereas for this update, AA has indicated that it is the sole convener of the guidelines workgroup and has stated that comments received during this comment period will only be reviewed by the workgroup. Given the organizational structure and the statement about who is responsible for review of the comments, our perception is that AA is in full control of the content of the proposed updated guidelines. We recognize that there is ex officio representation from the National Institute on Aging (NIA) at the National Institutes of Health on the Steering Committee and on the workgroup. What is missing from the document is a description of how the NIA was and is engaged in the work of updating these guidelines and whether the NIA has any decision-making authority over the recommendations that are being made. In the absence of an explicit definition of NIA's role, it appears that AA is proposing continued branding to both AA and NIA. This branding signals to clinicians, policymakers, and the public that the NIA is a full partner in this modular update inclusive of authority over the final content of the guidelines. For transparency, we recommend that the workgroup add an explicit statement about how the NIA has been engaged in this proposed update that is specific as to NIA's role in the development, review, and approval of any recommendations that are made in these guidelines. Further, as noted earlier in these comments, the proposed expanded usage of the guidelines is inconsistent with the NIA's mission and AGS recommends that the NIA consider whether the NIA-AA Revised Clinical Criteria for Alzheimer's Disease (AD) should continue to carry the NIA name. Concerns around adoption of biomarker-based diagnosis in clinical practice AGS appreciates the benefits of diagnosing neurodegenerative pathologies separate from and in parallel with clinical syndromes of cognitive impairment or dementia. We agree that there is an emerging understanding of the biological basis that is associated with characteristic brain pathology. However, we believe it is premature to make currently available single biomarkers of amyloid or tau a basis for clinical diagnosis, or to label all people with amyloid biomarkers or AD-associated tau markers as having Alzheimer's disease. o The proposed guidelines state that the impetus for the proposed change was that several therapies targeting the biology of AD have received regulatory approval since the 2018 guidelines was published, and these approved treatments target only AD. This requires a method of diagnosing AD with high specificity in cognitively impaired individuals; however, there are no targeted therapies to date that have been shown to improve patient level outcomes in individuals who are biomarker positive but cognitively normal. o The guidelines outline use cases for biomarkers (<https://aaic.alz.org/downloads2023/NIA-AA-Revised-Clinical-Criteria-Figures-and-Tables-AAIC-2023.pdf>) and in Table 1 (Biomarker Categorization) and Table 2 (Use Cases), the guidelines note that the biomarkers listed are currently suitable for clinical use, while biomarkers available for research use can be seen in Table

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3 (Additional biomarkers currently suitable for AD research and possible for future clinical use). As stated in the guidelines, “Biomarkers were placed into Tables 1, 2 vs. Table 3 based on the committee’s assessment of the strength of available evidence of high diagnostic accuracy (sensitivity, specificity) compared to a valid gold standard, high reproducibility, and diagnostic utility based on clinical studies in real world settings.” We believe it is important to understand and have in writing the criteria for assessing the strength of the evidence and process used by the workgroup to do this assessment and make these recommendations. The proposed guidelines rely heavily on evidence derived from population-based data that may not be representative of the racial and ethnic diversity and age distribution of people living with ADRD (DOI:10.1016/j.jalz.2018.06.3063). More biomarker studies representing diverse study populations need to be conducted in order to test the validity of the cut-off values of amyloid and tau (A/T) biomarkers across different populations and age strata. Much remains to be learned about how plasma-based biomarkers perform as true indicators of specific brain pathologies in broad clinical populations, including those with various comorbid conditions (DOI:10.1038/s41591-022-01822-2), before implementation into routine clinical care. Much more thought needs to be given to the potential exacerbation of inequities in diagnosis and care that might result from recommending biomarker-based diagnosis as a single criterion for diagnosing AD. It is well known that several minoritized populations are both disproportionately affected by ADRD and disproportionately underdiagnosed. Dementia specialists, pharmaceutical companies, and AD advocates have been highly successful in catastrophizing AD for the general public. We are deeply concerned the guidelines fail to address what a biomarker-based AD diagnosis can convey for personal identity. Due to heterogeneity in cognitive prognosis associated with biomarker positivity, the workgroup may want to consider how best to avoid assigning a clinical diagnosis of AD to biomarker-positive, asymptomatic individuals with normal cognition. Not only do many biomarker-positive individuals never develop cognitive impairment, (DOI:10.1016/j.jalz.2018.03.005; DOI:10.1001/jamaneurol.2018.0629; DOI:10.1001/jamaneurol.2021.5216; DOI:10.1001/jamaneurol.2023.2338) but most people who die with dementia die with, not of, dementia. It may be useful, however, to create a medically codable designator for “elevated risk state” to facilitate clinical tracking over time and we would encourage the AA to consider how to move this concept forward into practice. The AGS understands the heavy toll of Alzheimer’s disease on patients, caregivers, and their families and we are gratified to see promising new therapeutic options on the horizon with the potential to reduce the significant impact associated with ADRD. Additionally, we applaud ongoing work to develop therapies that may be deployed early in neurodegenerative processes, which we hope will one day prevent or delay cognitive changes associated with dementia.

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We are excited to see advances in technologies for earlier diagnosis, efforts to pinpoint the molecular mechanisms that underlie dementing illnesses, and more attention to how the exposome influences brain health in ways that often lead to health disparities in dementia. In the future, if significant evidence supports implementing biomarker-based diagnosis into clinical practice, our community will need to engage in intensive public and professional education efforts that prepare society that some people may be diagnosed with Alzheimer's disease yet never live to develop objective evidence of cognitive impairment or progress to meet clinical criteria for dementia. Significant evidence now supports recommendations that cancer screening and treatment should not be applied uniformly in all populations; in contrast, we do not have the evidence to guide how biomarker-based diagnosis of Alzheimer's disease should be handled in all clinical populations. Until then, purely biomarker-based diagnoses could result in significant psychological and practical harm.

26. In the foregoing criteria, dementia should be present for the diagnosis of Alzheimer's disease (AD). For the past nearly 50 years we have taught the entire population about the malignancy of AD: a major killer. And now we are going to diagnose biological AD, with the same name, even in an asymptomatic individual, who may never experience cognitive decline. What should we do with an individual with subjective cognitive decline who come to us with the diagnosis of biological AD? Should we say he/she should resign from the role as CEO or professor? Or stop running for senate? What if the company, the university or the press have access to this diagnosis? Is it convenient to use the same name for two very different conditions? Biological AD is a risk factor for cognitive decline and dementia due to AD. We need to note that HIV positive is not the same as AIDS, high level of PSA is not prostate cancer. We need to find different names for the two different conditions or we need to explain our new criteria to society before starting to use it.

27. I want to first say that I find the update to these criteria to be very sensible and in keeping with our current understanding of AD pathophysiology, its links to currently available biomarkers, and downstream relationships to cognition. The framing is certainly how I and many of my colleagues think about and interpret biomarkers in our patients. My only concern is the degree to which much of the nuance described will be translated to broader clinical practice, as is the intention of this update, particularly in the setting of therapeutics. To this effect, I would be pleased if the group considered the below comments. I think the importance of "clinical context" could be emphasized a bit more with regard to the neurobehavioral syndrome that a clinician is evaluating. In particular, given the commonality of AD pathology in older adults, it is likely many with other neurodegenerative conditions may have concomitant markers of amyloid, but

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that it is reflective of "preclinical" disease. Certain clinical syndromes that map reasonably well on to non-AD pathologies, such as semantic-variant PPA, may be instances where assessment for AD biomarkers may be less appropriate and could produce results that would be confusing to patients and families. In these instances, presence of amyloid pathology would be unlikely to be meaningful. Similarly, individuals with behavioral phenotypes suggestive of FTLTD would also need some consideration of which AD marker would be most valuable. While there can be some overlap in phenotype with AD, these would be cases where simply knowing amyloid status may misinform about the likelihood of FTLTD as the underlying pathology. In these cases, a tau marker may be of more diagnostic value to at least support the notion that AD pathology is or is not playing a role. A dementia expert may be savvy to these considerations, but many clinicians may not. Along these lines, on page 12 in the diagnosis section, it is noted that "the proportion of the observed cognitive deficit in any individual that is attributable to AD vs other neuropathologies cannot be known with certainty given the present state of technology." While I think this statement is broadly true, I think there are instances when we can feel fairly confident AD is NOT the driver of cognitive impairment; namely when individuals have evidence of amyloid pathology, but are tau negative. There is considerable data that in preclinical stages, these individuals tend not to progress for at least a number of years, supporting the conceptual point that tau pathology is a more proximal driver of neurodegeneration and cognitive symptoms than amyloid. I think all of above is really part of a larger point. Since these criteria are meant to be applied in a broader clinical context with regard to the clinicians that will be users of it relative to the prior criteria which was directed at the research setting, the use of a single biomarker for "diagnosis" should emphasize important caveats. Specifically, in isolation, a positive amyloid study may indicate AD (AD pathophysiology in the prior framing), but does not independently provide information about the role of AD in the cognitive status of a patient and may be completely incidental to cognitive impairment. Clinical context helps "an amnesic, multi-domain presentation would serve as a prior, increasing the probability that a positive amyloid test is likely to suggest AD as the disease driving diagnosis. Other contextual information may also provide support (e.g. family history, course, and, particularly, information about biological staging as is proposed). I think while the decoupling of AD from clinical status is made throughout the document " I think this is made more obvious in the setting of asymptomatic individuals. I think it is important for us to specifically point out the limitations of just measuring amyloid in symptomatic individuals in which "diagnosing" AD would potentially confuse. While experts in the field would be well-aware of this, many in practice will equate a diagnosis of AD based on amyloid status as equivalent to AD being the etiology of a patient with cognitive impairment. Again " the document does discuss such issues, particularly

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around staging and multi-modal biomarker use, but could be made more explicit in this regard.

28. Dear Workgroup members, The draft report of the "NIA-AA Revised Clinical Criteria for Alzheimer's Disease" published in July 2023 provides a superb review of the evidence for evidence for CSF -based, and the coming generation for plasma-based, diagnostics for Alzheimer's Disease (AD) and the opportunity that they represent for its management. However, we feel that reference could, and indeed should, be made for salivary diagnostics, as a complement to the diagnostic toolkit for AD given its unique properties and benefits. Further, we believe that there is sufficient evidence for the inclusion of saliva-based diagnostics for future clinical use. Saliva is one of the important body fluids and its composition reflects both normal and abnormal states of health for a range of conditions, including cancers, cardiovascular disorders, and neurological disorders and including a broad range of molecules. Saliva sampling is supremely practical being low cost, non-invasive, convenient, fast and very easily accepted by patients compared to CSF draws or even blood sampling. This makes implementation in non-traditional healthcare settings significantly more feasible and so is more suited to the epidemiological and logistical challenges that Alzheimer's™s will bring in coming years. Neurological disorders such as Parkinson's Disease use saliva testing already for a-synuclein, as is referenced in table 3 of the document. DNA methylation is another example where saliva to brain correlation has been shown to be higher than for plasma for various mental disorders¹. Finally, Lactoferrin has shown to perform in a similar manner to PET to discriminate Alzheimer's Disease (AD) from non-AD samples²⁻⁴. We respectfully suggest that saliva-based diagnostics be considered for specific mention for future clinical use in the final document. Thank you very much for your attention. ¹ Cui et al 2022. Developments in diagnostic applications of saliva in human organ diseases. *Medicine in Novel Technology and Devices* 13 (2022) 100115. <https://doi.org/10.1016/j.medntd.2022.100115>. ² González-Sánchez et al 2020. Decreased salivary lactoferrin levels are specific to Alzheimer's™s Disease. *EBioMedicine* 57(2020)102834. <https://doi.org/10.1016/j.ebiom.2020.102834> ³ Bermejo-pareja et al 2020. Salivary lactoferrin as biomarker for Alzheimer's™s disease: Brain-immunity interactions. *Alzheimer's™s Dement.* 2020;16:9. DOI: 10.1002/alz.12107. ⁴ Bartolome et al 2021. Standardizing salivary lactoferrin measurements to obtain a robust diagnostic biomarker for Alzheimer's™s disease. *Alzheimer's™s Dement.* 2021;13:e12173. DOI: 10.1002/dad2.12173.

29. Alzheimer's™s disease should not be solely defined by biological measures as enough is not understand as to the pathology. Furthermore, tau is more correlated with clinical outcomes and a sole focus on amyloid will continue to

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produce medications that solely target amyloid without much clinical benefit.

Lastly, diagnosing AD based solely on amyloidosis will lead to many being needlessly diagnosed with negative consequences on their wellbeing and rights.

30. seems like a big jump between stages 4 and 5. Four says still completely independent in basic ADLs and only mild instrumental ADL impairment. Then you would expect stage 5 to be "moderate impairment in instrumental ADL and mild effect on basic ADLs, but instead it is "moderate functional impairment in BASIC ADLs"? Maybe this makes sense, but if there is some way to clarify it just a little, explaining that stage 4, while still independent in basic ADL, may still have some mild effect on them

31. The initiative to update the 2018 NIA-AA criteria is important and timely due to recent advances in biomarkers and treatments of AD. The distinction between fluid biomarkers and imaging biomarkers is valuable. The acknowledgement of markers for co-pathology and the notion that reserve and resilience impact on the extend of symptoms in the presence of different levels of pathology is important. There are, however, critical points, which in my view, limit or even prevent the usage of the framework in the current version as clinical criteria. The first point is the extension of a biomarker definition of AD from A+T+ to also A+T- or A-T+ with the latter two extending beyond the neuropathological definition of AD. The authors acknowledge that in this case, the biomarker AD diagnosis will not always be in concordance with the neuropathological diagnosis. Does that mean that the biomarker diagnosis is the new standard? If the authors consider this, it should be stated very clearly. If the authors mean that pTau and positive tau PET imply (indirectly) amyloid positivity, then this should be explained. The criteria say that A-T+ can define AD, if neocortical Tau in PET is positive. This should be stated clearly throughout the manuscript. In many places, the authors talk about Tau-PET in general. What about an MTL-only positive PET and A-. Is this considered non-AD PART as in neuropathology? How does this relate to the early stage of AD (table 4), where MTL-only positive PET and A+ is AD. This is an example of the complexity of the proposal, which is not explained sufficiently in the manuscript, and which will be confusing for those, who are not fully familiar with all details of the rapidly evolving field. There is also a conceptual error: The AD staging pathology always requires A+ (table 4), whereas the diagnosis itself can be based on T+ only. A critical problem is the definition of AD by A+ only, when based on the plasma A β ₄₂/A β ₄₀ ratio. The authors say that the change in the concentration of A β ₄₂/A β ₄₀ in plasma is 10%-15%. According to the paper by Janelidze et al. (JAMA Neurol., 2021) the inter-assay CV of all tested assays in that paper with the exception of Elecsys are at least 5% per test (A β ₄₀ and A β ₄₂). This means that the expected change in plasma to define A+ by the A β ₄₂/A β ₄₀ ratio is more or less in the range of the CV of the assay,

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yielding a very high likelihood of false positive or false negative results. This is also documented by the correlation coefficients between CSF and plasma A β ₄₂/A β ₄₀ reported by Janelidze et al. (JAMA Neurol., 2021), which are below 0.5 or even below 0.4 for all non-MS-assays and only reach 0.6 for the best MS-assay. This shows that plasma A β ₄₂/A β ₄₀ is not useful to detect amyloid positivity on an individual case base. When thinking about testing individuals, the positive predictive value (PPV) of a test is critical. The highest AUC obtained by a non-MS-assay to detect CSF A+ is around 0.75 (Janelidze et al., JAMA Neurol., 2021). Given a test with a sensitivity of 75% and specificity of 75% and a prevalence of A+ of 25% in the population of people over 75 years of age, the PPV of the test would be 50%. This means that every second test is a false positive case, which prevents the usage of this test to qualify a person as having AD. There are some critical issue with other biomarkers. Regarding the V category, the authors list MRI in table 2 (clinical use). At the same time they say, that single summary measures for vascular damage are not been widely accepted and that most vascular lesion lie beneath the spatial resolution of clinical MRI. How in this case can MRI than be proposed as a clinical tool to dichotomize a person in V+ or V-? In several parts of the paper, the authors state that different biomarkers lack validation, specifically in non-research cohorts with higher diversity. Also, fluid biomarkers also lack neuropathological validation and many biomarkers have low sensitivity. The authors should very clearly define, what their basic validity and reliability requirements are to propose a biomarker for clinical use. At present, many are recommended in the tables, while on page 12, the authors say that only those should be used, which have met rigorous validation standards. These standards are not clear in the paper. Furthermore, on page 18 it says that cut-offs for plasma biomarkers have not been established. How can they then be put into a clinical framework for making a cut-off-based decision? The authors state that biomarker should only be interpreted in the context of the individual patient history. As the proposal clearly includes the detection of AD pathology by biomarkers in the asymptomatic stage, what is the clinical context, in which they should be interpreted? If this refers to conditions, which may impact on a given biomarker, the field is still learning, particularly regarding plasma biomarkers. The knowledge about the effect of kidney dysfunction is out there, but how many other impacting conditions, which are not yet discovered, are there (comorbidities, medication, nutrition, ethnicity, genetics)? How should a physician outside of a high-end research facility integrate all these variables to make a judgment on an individual case base? The authors propose a grey zone approach in this context, which may partly help, but will still leave substantial room for false interpretations. It is irritating to see that authors the NIA-AA framework propose the use of single plasma biomarkers for diagnosing AD, while some of them recently wrote the blood-based markers (BBMs) appropriate use criteria, where blood-based biomarkers are

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recommended as screeners to identify individuals for inclusion in trials of disease-modifying therapies provided the AD status is confirmed with PET or CSF. The AUR further state that blood-based biomarkers should be cautiously used in specialized memory clinics as part of the diagnostic work-up of patients with cognitive symptoms and the results should be confirmed with CSF or PET, whenever possible. Additional data would be needed before use of blood-based biomarkers as stand-alone diagnostic AD markers, or before considering use in primary care. Given the restricted access to CSF and PET, the proposal of blood-based biomarkers for clinical use, will lead to the use particularly in non-specialized centers without the possibility of validation, thereby achieving the exact opposite, of what was intended by the AUR. Overall, while the criteria are a conceptual progress in many regards, they seem too complex and impractical for clinical use. Also, different from oncology, they are not paralleled by high complexity treatment options, yet. So far only anti-amyloid treatment for early AD is available as a molecular specific AD treatment. For now, the detection of the early clinical syndrome and amyloid positivity is more or less sufficient. It is an open question, when a drug class with a different molecular target, which is reflected in the new NIA-AA system will be available. The update makes also goes beyond what is scientifically justifiable. This accounts specifically for the clinical use of plasma biomarkers, and most importantly for the diagnosis A+ by A β 42/A β 40 only. I don't think that this can be left in the final recommendations without the risk of losing acceptance in large parts of the community. This is particularly the case, because the authors acknowledge an extended lack of validation of some markers and missing cut-off. The extension of the diagnosis of AD to A+ only and T+ is a step, which for the first time goes beyond, what has been considered AD for the past century. A+ only can be justified, if there is the assumption, that eventually this will turn into in A+T+. It needs to be made clear that T+ on biomarkers implicitly always also means A+ (e.g. pTau) . If T+ in itself conceptually qualifies for AD in the view of the authors, this should be clearly stated. But how would that be justified (AD without amyloid?). I think that many issue could be solved, if the recommendation for clinical application would be restricted to biomarkers that are largely validated and for which clinical experience exists (PET, CSF, MRI, potentially NFL and pTau in plasma). Instead of proposing immature plasma biomarkers with the risk of large numbers of false positive cases (maybe then receiving expensive treatment without effect), all the rest should go into a second section labeled as a research framework. This should also include to molecular staging. The biomarkers and the molecular staging scheme should be further validated and should be only release to the clinic once there is a need, for example once new treatments are available.

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32. Separating "syndrome" from "biology" would be reasonable if a) the biomarkers were nearly 100% sensitive and specific; b) they were state (and not trait) markers. The current framework is a terrible hubristic misuse of the diagnostic prerogative. You really should compare the situation to that in Huntington's Disease deliberations and read more Foucault and Canguilhem. Please contemplate on the meaning of diagnosis. Recent data on amyloid-lowering trials also show how rudimentary our understanding of the current non-clinical biomarkers (as well as pathophysiology) is. This should give pause to anyone endorsing their use as sole diagnostic markers.
33. The proposed criteria note that objective cognitive decline must be either demonstrated by longitudinal cognitive testing or via impaired performance. There are other ways to determine change from baseline via obtaining estimated premorbid abilities, such that longitudinal testing is not required. Also, I would specify that objective changes should be based on established and appropriate norms - especially in the earlier stages.
34. I am very happy to comment on the draft NIA-AA clinical criteria and thank the authors and the Alzheimer's Association for this opportunity. Unfortunately, my overall review of the diagnostic criteria is rather negative. The following are my main criticisms. 1. THEORETICAL ASSUMPTION (lines 296-298) "This biological definition of AD is consistent with the distinction between a disease vs illness. A disease is a pathobiological condition that will ultimately manifest with symptoms if an affected individual survives long enough. In contrast the term illness denotes signs and symptoms that result from the disease". As far as I know, this is a very unusual and unsupported theoretical position. The objectively studied signs and symptoms are part of the disease, not the illness. Consequently, I believe that the core principle stated in Box 1 "Symptoms are the result of the disease process, not its definition" is arbitrary and epistemologically incorrect. Any brain disease can be studied objectively according to multiple levels of analysis and related disciplines, e.g., genetics, biology, physiology, anatomy, and behavioral sciences such as neuropsychology. All these levels and disciplines share the scientific method and have equivalent legitimacy in defining disease. Cognitive dysfunction defines AD like amyloid plaques and tau tangles, but at a different level of analysis and at a different time in the disease course. Proteinopathies precede the signs and symptoms and, especially in the case of tau, are found to be quite correlated with the signs and symptoms, but this fact does not make the signs and symptoms conceptually less legitimate to define AD. Defining AD by its biology is an option decided by the authors, not a principle. 2. PRECLINICAL DIAGNOSIS The proposed criteria endorse preclinical diagnosis ("Symptoms are not necessary to diagnose AD"; "In living people the disease is diagnosed by disease specific biomarkers"). This position

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is a kind of revolution in the diagnostic pathway of dementia that needs to be discussed extensively by the authors. i) First, I think this message needs to be made explicit by the authors. It is not acceptable to implicitly propose the preclinical diagnosis without emphasizing this step, and without expressing the authors' opinion about it. ii) Second, making a diagnosis of a devastating disease such as AD, even 10-15 years before the disease manifests clinically (with signs and symptoms), could have extremely negative consequences on the individual's life and society (Inglese S, Lavazza A, Abbate C. Crystal Ball Health Policies: A Case Against Preventive Testing For Alzheimer's Disease. *Frontiers in Aging Neuroscience*. 2022 Feb 15;14:842629). Such consequences cannot be disregarded and discussed when preclinical diagnosis is endorsed. The comparison with diabetes in such a case is clearly untenable. (iii) Third, how is it possible to propose a diagnosis of AD to a patient years before when he or she will manifest signs and symptoms when to date we have no validated drug therapy available for this preclinical stage (e.g., recently Solanezumab, which targets monomeric amyloid in persons with elevated brain amyloid levels, did not slow cognitive decline as compared with placebo over a period of 240 weeks in persons with preclinical Alzheimer's disease. Sperling RA et al. *Trial of Solanezumab in Preclinical Alzheimer's Disease*. *New England Journal of Medicine*. 2023 Jul 17)? (iv) Again, how can we accept a preclinical diagnosis of AD when we cannot predict whether the patient will definitely develop the signs and symptoms, or instead will be one of the resilient patients, with a significant burden of amyloid plaques and tau tangles, but who will not develop cognitive impairment? 3. SYNDROMES (lines 635-637) – Five characteristic AD phenotypes are recognized: amnesic or "atypical", language variant, visuospatial variant, behavioral variant and dysexecutive variant which are reviewed in 183, 184. i) Corticobasal syndrome is missing from this list of AD phenotypes. ii) I do not understand why the authors do not use the terminology well known and accepted in the field to name AD phenotypes. Why language variant instead of logopenic? Why visuospatial variant instead of PCA syndrome or Benson syndrome? (iii) In general, I believe that the role of signs and symptoms, syndromes, and clinical evaluation is too marginal in the current diagnostic criteria. These criteria seem to be based on the assumption that preclinical diagnosis is acceptable. However, if regulatory organizations were to ban preclinical diagnosis of AD, the current criteria would lose much of their meaning. I strongly agree with the authors both that the clinical-anatomical convergence and phenotypic heterogeneity in AD make diagnosis based on syndromes alone inaccurate and that AD diagnosis should be reserved for biomarker-positive patients. However, I firmly believe that preclinical diagnosis of AD is currently unacceptable. Therefore, the first step in the diagnostic pathway should remain the evaluation of signs and symptoms. In this regard, the study of a dementia syndrome could prove to be a powerful diagnostic tool at the time of

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the clinical stage (Abbate C. Research on Alzheimer's syndromes is critical to improve diagnosis, patient management and non-pharmacological treatments, but is under-pursued. *Frontiers in Aging Neuroscience*. 2022 Oct 17;14:1039899.). Indeed, to detect a dementia syndrome, experienced clinicians have used pattern recognition, which allows them to readily recognize complex patterns of behavioral and cognitive characteristics from the detection of a few signs and symptoms, even without or long before the full pattern has manifested. In addition, the assessment of signs and symptoms and eventual recognition of a dementia syndrome are unique to address multiple additional aspects of nonpharmacological management and treatment of patients (Abbate C. Research on Alzheimer's syndromes is critical to improve diagnosis, patient management and non-pharmacological treatments, but is under-pursued. *Frontiers in Aging Neuroscience*. 2022 Oct 17;14:1039899.).

4. FEW DIAGNOSES One of the most important problems to be solved in the field of dementia is that few diagnoses of dementia are made and that they are made too late. We fail to make diagnoses. The proposed criteria imply a diagnostic pathway with advanced instrumental examinations, specialized centers where these examinations can be performed, and hyperspecialists who can interpret the results. It is known that not all of these centers and specialists are available. The availability of rapid and reliable kits to catch variations of biomarkers in the blood does not solve the problem. If preclinical diagnosis is not acceptable today, I do not see how these Kits could solve the problem of few diagnoses. In fact, you still have to first identify the patients to whom you then propose such tests. And patients are identified by studying signs and symptoms just as we do now.

5. HYPERSPECIALIZATION Current diagnostic criteria clearly refer to the work of dementia specialists in memory clinics. I do not understand how this hyperspecialization can be reconciled with the role that has been advocated for years for primary care physicians in the diagnosis of dementia. The reference to simple blood tests is actually hardly credible, because in any case the interpretation of their results implies intervention by specialists.

6. HIGH COSTS At first glance, the diagnostic pathway under the current criteria appears to involve very high costs. It is not hard to imagine that the application of these criteria will divert funds for specialist centers that would otherwise be allocated to post-diagnosis services and families to better manage the signs and symptoms phase. Remember that the disease modifying drugs approved for AD in the United States do not cure the disease, so we will still have the same number of AD patients, each with several years of cognitive decline and care burden for relatives and society ahead of them.

35. -Consider removing Stage 0. While intellectually, the rationale for this category based on genetic status is understood, there are concerns regarding the psychological impact on the individual whose identity may become defined by

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their impending Alzheimer's disease. The Stage 0 diagnosis could leave to a myriad of mental health issues including suicide, without yet clearly changing management in our current therapeutic landscape. In contrast, in cancer, Stage 0 indicates in situ disease has been identified. Overall, these criteria represent a major advance, and the authors should be applauded. Nonetheless, there is concern for broad uptake of these highly scientific criteria for many reasons including the following (and drawing comparisons to cancer diagnosis): -The majority of persons with Alzheimer's disease in the US are diagnosed and treated by non-subspecialists (usually PCPs), many of whom are not up to speed on AD biomarker advances. In cancer, staging is done primarily by subspecialists (oncologists) not PCPs. -Payors currently only reimburse for the CSF AD panel but only for strict circumstances (AD vs FTD). Whereas, these criteria reference numerous other biomarkers which are not reimbursed at this time. Broad uptake of new AD diagnostic criteria by all clinicians who diagnose and treat AD will likely require a simplification of the various categorizations and classifications in these criteria and with greater emphasis on real world accessibility, feasibility, cost, and reimbursement potential.

36. To the authors, and the Alz Association: A group of our team members (behavioral neurologist, behavioral neurology fellow, dementia care NP, memory clinic NP) reviewed the new criteria. We had several concerns/questions: 1) cost of biomarker testing and how it would affect the budget of CMS, 2) is all this testing really necessary to make a diagnosis of moderate dementia, when we don't currently have any disease modifying medications and the field has turned away from disease modifying therapies for moderate/severe AD for the most part, 3) does a panel of Abeta 42/40+, ptau 217+ but ptau 205- look the same clinically in every patient (probably not, just as MoCA 26/30 does not look clinically the same across patients), and therefore, this still doesn't remove the subtlety of diagnosis and how to manage patients. Overall, my comment as a site PI for AD trials/ADNI who has followed the evolution of biomarkers and who understands the logic of needing to screen people earlier and who pushes for more in depth and precise diagnoses in my clinic, I am not sure that this makes diagnosis any easier for general neurologist and definitely not for primary care docs. The framework makes total sense to me and fits like a pieces of a puzzle. But I am not sure this would be easily adopted and I am not convinced that this will help efforts to reduce reliance on specialists for diagnosis. When I look at these tables, I just see primary care docs throwing their hands up and pushing more referrals to us, instead of keeping these patients to do the work up. Maybe this would change when (fingers crossed) we have more disease modifying

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drugs available beyond what we have now, and the drugs aren't too risky and complicated to manage.

37. The clinical criteria revision would benefit from better documentation of the quality of the evidence used to support the different statements. The GRADE system could be applied to the data presented with the use of systematic review. On page 1 line 24 there is a point made about blood-based biomarkers. On looking through the rest of the document I struggle to see how this statement can be made as the evidence to support it is not clearly described in any of the text or tables. I am presuming that "fluid" refers to CSF and not to any other body fluid. As pointed out in the text there is still a gap in the ability to match the imaging and fluid biomarkers against pathology. The pathology and matching to clinical features have challenges. This is a difficult area to evaluate but the GRADE system allows for this, and the level of evidence can be classified.

38. Dear Colleagues The novel NIA-AA revised clinical criteria for Alzheimer's disease (AD) aim to update the previous established research framework in response to three recent developments: the entrance of new disease targeted therapies in clinical practice, the accessibility of plasma biomarkers with excellent diagnostic performance, and the recognition that imaging and fluid biomarkers are not interchangeable. Despite the significant impact that these innovations are having in specialized settings, it is essential to acknowledge that, from a global perspective, using them as the foundation for these new criteria intended for worldwide clinical use presents in our opinion several clinical and theoretical limitations. Notably, new disease-targeted therapies are currently unavailable outside the US, and no plasma biomarker assay has received approval for clinical use, at least in Italy. Moreover, Tau PET (which seems to play a crucial role in the proposed biological staging of AD) is only accessible in a limited number of specialized centers. Nevertheless, the new categorization of biomarkers in three distinct groups (core AD biomarkers, non-specific biomarkers relevant in AD pathogenesis, biomarkers of common non-AD co-pathologies) is a challenging development. This classification rightfully acknowledges the importance of novel biomarker categories beyond the conventional A, T, and N markers and, by incorporating a broader range of biomarkers, holds the potential to offer more comprehensive insights into AD (co)-pathology. Here are some concerns: - Core AD biomarkers and their clinical use for AD diagnosis: It is specified in the manuscript that "AD can be diagnosed by the presence of any abnormal core AD biomarker" (page 10) and that fluid biomarkers of A and T are considered informative and suitable for use in clinical practice when measured both in plasma and CSF (Table 1, Table 2). This raises two main concerns: a) Are currently research-only plasma biomarker assays ready for being used for AD diagnosis on a global scale? The manuscript acknowledges

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the need for rigorous validation standards for clinical use (page 12), but it should be emphasized that the current absence of established cutoffs and validations may hinder widespread application of plasma biomarkers of A and T in the next few years. The manuscript should clarify that, even if their validation will probably happen soon, some time is needed before these assays may be available for clinical use worldwide. b) the criteria state that AD can be diagnosed based on the presence of any abnormal core AD biomarker. However, the scenario of individuals who are A- and T+ (e.g., with elevated levels of CSF p-tau due to high increase in CSF t-tau) is not adequately addressed. This possible occurrence deserves consideration in the criteria to avoid potential misdiagnoses. The manuscript should provide guidance on how to interpret such cases and whether an A+ biomarker is always needed for an accurate diagnosis as well as for anti-amyloid treatment. - Biomarkers that are non-specific but important in AD pathogenesis: The recognition of the roles of N and I biomarkers is proper and commendable. However, it is worth noting that, especially for the I biomarkers, validated assays and cutoffs are lacking. The manuscript seems to focus only on CSF and plasma biomarkers, despite a growing amount of evidence on serum NfL and GFAP (some scientific papers relying on serum biomarkers are even cited in the text, such as ref n. 52). The potential use of serum biomarkers should not be disregarded, especially when considering some biomarkers that are currently under investigations (ie. serum sTREM2 and serum YKL-40). - Biomarkers of common non-AD co-pathologies: It should be considered that, while alpha-synuclein seed amplification assays are currently gaining attention in research settings, they are still not widely available for clinical use. Nevertheless, the manuscript appropriately highlights the role of common non-AD pathologies such as S and V, and it should be noted that other potential future biomarkers will be added when reliable biomarkers for TDP-43, 4R tauopathy and other pathological processes will be discovered. - Biological staging: The authors present two distinct schemes for biological staging, based on PET imaging and fluid biomarkers. Notably, both schemes focus solely on A and T biomarkers, excluding the N biomarkers, which deviates from some concepts of the 2018 research framework due to concerns about the A->T->N sequence's inconsistency. In the context of PET imaging, the A->T sequence is considered valid for biological staging (further reinforcing the notion that an A-T+ profile should not be included in the AD continuum, which should be emphasized in the "Diagnosis" section to avoid potential misinterpretations) and the authors appropriately acknowledge the strict association between tauopathy spreading and disease progression. The interpretation of A-T+ cases (positive pTau 217) is still not explained and it is not clear how these patients should be considered. However, the same concept does not seem to be extended to fluid biomarkers, on the premise that ptau181, 217, and 231 concentrations increase before the appearance of Tau PET abnormalities. While this rationale is provided, it is

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surprising that the A+T- profile, commonly observed in both cognitively unimpaired and cognitively impaired subjects in clinical practice, is not discussed in this context. Addressing the significance of this profile in the context of biological staging for fluid biomarkers would provide a more comprehensive understanding of the criteria's applicability. - Clinical-biological diagnosis and staging: The exclusion of the term "prodromal AD" is fair, but it is still relevant to consider "subjective cognitive impairment" and "mild cognitive impairment" as "at risk for Alzheimer's dementia" to avoid confusion in clinical practice. While reliance on a biomarker-only diagnosis would require dependable evidence of a connection between biomarkers-positivity and an extremely high probability of subsequent clinical progression, the evidence on follow-up of cognitively unimpaired biomarker-positive individuals suggests that most of these individuals do not progress significantly over time. Defining the disease by its pathological lesions only, and not by a clinical phenotype, might cause diagnostic confusion, especially considering how the concept of "Alzheimer's disease" is significantly feared by the general population, which associates it with dementia, dependency, and death. The assumption that Amyloids is pathogenic for all people independently from other aspect that increase resilience is a rather strong statement. Thus, how to consider preclinic subjects from a diagnostic and therapeutical point of view. Dubois and colleagues have long challenged the designation of "preclinical AD" in asymptomatic subjects with positive markers (A+, T+), preferring the designation of "at risk for AD", since the future fate of a subject with positive markers but cognitively intact is not inevitably dementia. I think like them, since the "lifetime risk" of evolving to dementia of an asymptomatic subject with positive markers ranges from only 5 to 42%. We agree that these individuals should be better defined as "at risk". In conclusion, we strongly judge these revised criteria a step towards a biologically defined AD diagnosis and staging. However, the global applicability of these criteria faces challenges related to standardization, harmonization, accessibility, and cost-effectiveness of most of the biomarkers considered. While the use of these novel criteria and the consideration of a wide range of A, T, N, I, S and V biomarkers may be appropriate and commendable in research contexts, some refinements and simplifications may be necessary to enable seamless implementation in clinical contexts globally. Other major points a) The extended ATN(C) framework is grounded on the view that AD-related neuropathology (i.e., brain amyloidosis and tauopathy) still represents the core neurobiological cause of cerebral neural neurodegeneration and cognitive deficits from MCI to dementia. In this cause-effect model, there is surprisingly no mention of the emerging neurophysiological oscillatory property of brain activity and measures of the cortical balance between excitatory and inhibitory neural processes. The extended ATN(C) framework does not deal with and exploit the information on the disruptive effects of AD-related neuropathology on the temporal

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(de)synchronization of the neural activity within large populations of subcortical and cortical neurons. In this line, experts of ISTAART Electrophysiology Professional Interest Area (E-PIA) have recently reviewed converging evidence showing that AD neuropathology early affects the dynamic time course of that (de)synchronization regulates the balance of local cortical inhibition/excitation responsible for the generation of ongoing EEG activity (Babiloni et al., 2020, 2021) b) Keeping in mind the above data and considerations, the ISTAART-EPIA Steering Committee proposes that the extended ATN(C) framework may be enriched with pathophysiological "P" biomarkers and the evaluation of vigilance, sleep-wake cycle, cognitive status, and abilities in the activities of daily living as a global clinical output "O" (Table 1). Specifically, a theoretical proposal for an AD model may include brain amyloidosis (A), tauopathy (T), pathophysiology (P), neuroinflammation (I), brain vascular injury (V), alpha-synucleinopathies (S), neurodegeneration (N), etc. The disease processes within those dimensions may produce a clinical output (O) involving vigilance, wake-sleep cycle, cognitive functions, and abilities in the activities of daily living. Such integration may better explain the neurophysiological link between AD-related neuropathology, neurodegeneration, and clinical manifestations in AD patients at all stages of the disease Other minor points On line 333 authors do not consider several evidence of cognitive paradigms able to detect cognitive dysfunction in preclinical state. To mention the important consideration about copathologies but only defined as biological and not clinical or neuropsychological point of view Transferability of the proposed criteria in clinical practice seems rather difficult even more in less developed countries. The disease model fitting proposed mainly applies to anti Abeta amyloid drugs and do not take in account other targets that could lead to a multitherapeutic approach to the disease in the future. The biomarkers proposed should be validated against a gold standard. Authors repeat this several times but no specification on which are these gold standards and how to plan the study on this is clarified. Since PET "is not interchangeable for many use cases" which could be the gold standard of fluid biomarkers? In several part of the text A+ is defined "biological diagnosis" while more appropriate "pathological diagnosis". Is not clear if biomarkers investigation would be planned also in normal population (stage 1 or 2) and in case what is the final purpose. Cognitive markers are not mentioned, indeed all the literature on preclinical cognitive markers is ignored, which if associated with biomarkers could identify individuals with positive biomarkers who will have cognitive decline in the future. References Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA. *Alzheimers Dement*. 2018;14(4):535-562. Babiloni C, Arakaki X, Azami H, Bennys K, Blinowska K, Bonanni L, Bujan A, Carrillo MC, Cichocki A, de Frutos-Lucas J, Del Percio C, Dubois B, Edelmayer R, Egan G, Epelbaum S, Escudero J, Evans A, Farina F, Fargo K, Fernández A, Ferri R, Frisoni G, Hampel H, Harrington MG, Jelic V,

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39. Some feedback on the draft: I believe future genetic efforts for late-onset Alzheimer's Disease will suffer. If these guidelines go through in their current state, we will set back the field for years to come and there's no mention of it in almost 40 pages of document. There's a big challenge in late-onset AD genetics: it's increasingly harder to identify new variants that can help to explain the disease. We haven't been able to scale genome-wide association studies (GWAS) to the extent other phenotypes such as height, BMI or T2D have because we lack patients. We don't have enough clinically diagnosed AD patients getting genotyped. For understandable reasons. For most patients, you need to convince not only the patient, but their families. And if your loved one suffers with AD, it's likely that your first impulse is not to enrol them in any sort of research study. So what have we done? We started using proxies for AD GWAS derived from the UKBB. What are proxies? If your father or mother has/had dementia or AD, you're a proxy for AD. Your genetics will be as valuable as someone with a clinical diagnosis of AD. The point here is not to discuss how and why we should avoid the use of proxies. But it's not good. It's terrible, in fact. As of now, more than 50% of the "patients" included in the latest GWAS don't have AD. And most of them will never develop AD. Meaning that our desperation for new findings has let us down a rabbit hole in AD genetics: we have identified more genetic risk factors. But at what cost? Increasing sample size at the cost of a poorly defined phenotype? As if AD wasn't complicated enough by itself. Genetic variants that are not directly related to the actual disease will mislead researchers and hinder progress in understanding the real genetic underpinnings of AD. AD is the only disease whose SNP-based heritability has decreased considerably by increasing the sample size. The more we increase sample size, the less we can explain of the disease. How does this issue circle back to the new guidelines? These new guidelines are complicated and highly subjective at their core. But most importantly, they will make it a lot easier to diagnose someone with Alzheimer's Disease. Now individuals will get an AD diagnosis based on "amyloidosis" alone. And given the FDA said to be agnostic to the biomarkers being used for that purpose, you can get a diagnosis based on plasma-based biomarkers. These changes will skyrocket the incidence rate of AD. Over the next 5-10 years, the number of patients diagnosed with AD might triple or quadruple aside from the organic growth of the disease. But this is an issue. How easily people will get an AD diagnosis is a problem for genetics. "Being Alzheimer's matters" "From the moment you get an AD diagnosis, you will be eligible for inclusion in future genetic studies. There is no clarity or mention in the document on how to deal with this. "Stage 0" and "Stage 1" AD are still being labelled as AD. And unless crystal clear guidelines for inclusion are put front and centre in the document, we will stall genetic AD studies for decades to come. This will be no different to using proxies. But now

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these new proxies will get an official AD label. There is sufficient data showing that amyloid poorly predicts disease progression. In the "use cases" for diagnosis, it is said that "in many instances, a single biomarker will be sufficient for clinical diagnosis and trial inclusion. No mention of genetic studies. The document lacks a section highlighting proper guidelines for future genetic studies on the back of these broad and ever more unspecific diagnosis criteria. There is a section called "Stage 0 and genetics". This "Stage 0" is a problem for further genetic efforts. Now are we supposed to include autosomal dominant AD and Down Syndrome as Stage 0 Alzheimer's? So, familial AD and Trisomy 21 will get AD status. We need clear guidelines to avoid including these subjects in future late-onset AD GWAS. Aside, the fact that genetics are completely omitted from these guidelines is a disappointment. Mainly because the argument of "non-determination" status given to genetics is not applied to amyloid burden. The document recognizes that "carriers of risk alleles including some APOE e4/e4 individuals may survive to late life without developing fully manifest AD pathologic change or symptoms". This line of thought is not consistent with the proposed amyloid positive/tau negative (A+/T-) base diagnosis for AD (being changes from the previous A+/T+ minimum requirement for diagnosis). Individuals with high levels of amyloid also survive to late life without developing dementia or fully manifest AD pathologic changes or symptoms. If your life runs as normal and we would only know that the individual presents elevated levels of a certain protein, do they really have the disease if it never manifests? It is not fair for patients, not their families. The only ones benefiting from all of this are clinical trials getting more people enrolled based on dubious diagnoses. Numbers, not quality. Possible solution: Simply do not call "stage a", "stage 0" or "stage 1 A-/T+" Alzheimer's. But since that ship has sailed, set a threshold for the inclusion of patients in genetic studies to avoid "entry-level" clinically diagnosed cases adding noise and contributing to false positives, masking of true positives or inaccurate risk variant identification that might relate more broadly to dementia/other types of dementia, non-AD co-pathologies or non-specific co-existing neurodegenerative pathways common to AD. We need more specificity, not less. The document states that biomarker "sensitivity and specificity are obviously inversely related". It matters for biomarkers, but it doesn't for diagnosis? So we will get more. More cases. More patients. We will finally increase variant discovery significantly. At what cost? Not to mention that this new type of diagnosis is to "identify patients earlier for preventive treatment". So you can now diagnose someone based on an abnormally increased A β 42:40 ratio. So that means if your levels of A β 42 start increasing substantially more than A β 40, you now have Alzheimer's. And the point is to treat them with Lecanemab, the A β -removing monoclonal antibody. So per Lecanemab's own data it increases A β 42 while decreasing A β 40. Therefore Lecanemab causes AD if

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A42:40 ratio is a diagnosis criteria. That's the argument. Aside, fluid biomarkers relate to metabolism and processing. As it is stated in the document: "Fluid biomarkers represent net of rates of production/clearance of analytes in near constructs". A snapshot is not a full picture - If these biomarkers reflect a metabolomic-like state, it is bound to be extremely variable. There are no guidelines for how many "AD positive" you need before you get a diagnosis. No mention of how to control for sleep, circadian rhythm or eating patterns. Different times of day and different physiological conditions will impact the results of these tests. We need clarity on how many tests will be required and in which circumstances to get a diagnosis. Even worse, biofluid assays do not require FDA approval. But you'll get a diagnosis anyway. Possible solution: First, don't use it. We don't need more diagnosis. We need a more accurate diagnosis. But since that ship has also sailed, a 4th protective measure against misdiagnosis at least for fluid assays: should be mandatory to have enough points (3-5) for clinical diagnosis based on the volatile nature of the assay. Assaying at different time points should be based on the half-life of plasma Aβ: around 3 hours. Here are some other idiosyncrasies in the document: 1. It is good that other markers are brought to light, mainly synaptic markers. Why not go for more direct markers of pre- or postsynaptic function that are more broadly used in fundamental research like Synapsin, PSD95, vGlut1, Synaptophysin or Gephyrin? 2. Markers of endolysosomal function should be considered for inclusion given they are well recognized as one of the earliest neuropathological changes in AD (<https://doi.org/10.1111/tra.12889>; <https://doi.org/10.1038/s41593-022-01084-8>; <https://doi.org/10.1186/s40035-023-00362-0>). Markers of lysosomal acidification hold particular promise and should be mentioned in the future directions for satisfactory development for clinical use. 3. Most clinicians outside resourceful countries will have trouble getting access to even "easily accessible" fluid biomarker testing. Distinguishing pre- from postsynaptic is important to understand the molecular mechanisms underlying AD, but it will be everything but informative for disease staging purposes at a stage where we can't even get the basics going. Just makes the whole thing more complicated than can realistically be achieved at this very moment. Possible solution: I'd refrain from including it in anything but disease characterization at this point. 4. The use of GFAP is questionable on the basis of it being "associated with higher risk of incident dementia". Educational attainment is associated with a lower risk of AD but does not predict it (<https://doi.org/10.1038/s41588-022-01016-z>). Neither does educational attainment predict amyloid pathology via PET. Nor does BMI, whose strong association with AD doesn't translate into predicting amyloid pathology. If only amyloid and AD were the same thing. Possible solution: Too broad. Remove it and re-include an inflammation marker whenever one becomes available and proven. Something is not always better than nothing. 5. There is

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considerable mention of T2D as a point of comparison both in the document and in its presentation in #AAIC23. The diagnosis for T2D has changed considerably over the last decade. That means that GWAS for T2D are also a mix bag of diagnoses and criteria. I fear the same will happen with AD. This document will broaden diagnosis criteria. We will welcome many more people into the AD realm, even if sceptically. In some years, we will realize that adding numbers alone does not help. We will realize the harm we've done in incorporating patients whose genetics do not correlate with the AD phenotype. We will get more of less. We'll go back. And we'll be following in the footsteps of everyone that came before us. Why? 6. For the thresholds section, refraining from defining cutpoints and thresholds is dusting off the pressure of setting standards. Without a clear definition, there's no certainty in the phenotype. Possible solution: gather a panel of leading experts in the field of biomarkers. Set standards before wasting 5 or 10 more years travelling on uncertain grounds. If those cutpoints or thresholds later turn out to be too restrictive or too mild, change them. But set the rules. 7. The same issue is raised in the "conservative interpretation of values near a cutpoint" section. The clinical interpretation and consequent diagnosis feel highly subjective at every single level. It is there all the way from assay choice, assay interpretation for fluid assays, ligand interpretation for PET, clinical framing to final diagnosis. 8. The "clinical context" section is of extreme importance and feels underdeveloped. 9. For people complaining the document is too complex, things like "In keeping with recognition of nonequivalence between imaging and fluid biomarkers we propose to separate staging schemes from imaging and fluid but with a common overarching concept" do not help. These guidelines feel too smart for their own good in some sections. Let's not forget that these guidelines are mainly meant for diagnosis and the recipients and beneficiaries of this text might not understand the points being raised the same way the people who wrote them intended. 10. It would be important to start defining acronyms. All throughout #AAIC23, people use early-onset Alzheimer's Disease, early-Alzheimer's, autosomal dominant Alzheimer's Disease, sporadic Alzheimer's Disease or late-onset Alzheimer's Disease. Until they explain what they mean by some of them, one is left wondering if early Alzheimer's is early-onset Alzheimer's, if it means the same as sporadic AD or if it's early-stage late-onset Alzheimer's Disease. Possible solution: take this document to set the nomenclature straight so everyone is on the same page.

40. We believe there is a need to clearly understand the rate of clinical progression to MCI and dementia. This could help and enable clinicians in making a more informed (and patient-centric) decisions, with regards to potential treatment and management, earlier in the patient journey. Certain AD biomarkers can provide

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insight as to the likelihood of a patient to experience cognitive decline, during the AD continuum. These could be CSF(1), plasma(2), and imaging(3) biomarkers. For this purpose, it would be helpful to include, under the category of Staging, prognosis, as an indicator of biological treatment effect a class of P, for predictive biomarkers, i.e., biomarkers, which could predict the rate of clinical deterioration (i.e, the level of risk and the timeframe, during which it can occur). References: 1. Chen et al. Front Aging Neurosci. 2022 2. Smirnov et al. Acta Neuropathol. 2022 3. Zhu et al. J Alzheimers Dis. 2022

41. The working group updated the 2018 NIA-AA research framework to create clinical criteria for AD. This document is sorely needed given the rapidly changing clinical environment, wherein amyloid targeting therapies have been recently approved for clinical use, and I applaud the working group for their efforts. Strengths of the document include making a clear distinction between disease and syndrome, summarizing available biomarkers of A and T, delineating PET vs. fluid markers, and incorporating S and V into the ATN biomarker profile system. At the same time, the science and resources surrounding biomarkers, disease staging, and clinical staging has often not yet advanced to a point that would allow meaningful implementation of these clinical criteria in practice. As a result, I think this document could be reframed to emphasize its limitations at the outset and clarify its goals. First, I believe it needs to be acknowledged early on that many healthcare institutions will not have the capacity or expertise available to competently complete AD diagnosis in the manner described in this article. As an example, I work in a well-respected academic hospital system in the U.S. that serves >220,000 patients, yet we have ~3 physicians with expertise in AD and no established system for obtaining AD biomarkers on a routine basis. I imagine the situation is even less ideal in nations and institutions with fewer resources. It would likely be helpful to state early in the document that there will need to be a serious realignment of healthcare resources and training to accommodate these new criteria. Furthermore, it might be useful to provide suggestions for how to continue to diagnose AD in situations in which inadequate resources or training is currently available. Second, we lack consensus in key areas, such as 1) appropriate selection of biomarkers; 2) interpretation of biomarker cutoffs; 3) biomarker selection and interpretation in diverse groups; and 4) operationalization of clinical stages (especially stages 1 and 2). Given these factors, the document seems to be better characterized as guidelines for AD diagnosis, rather than actionable clinical criteria that can be applied in practice. Perhaps the manuscript could be reframed in this way.

42. Synuclein biomarker(S) (table 3): PET biomarker is not established yet, while MIBG cardiac sympathetic nerve scintigraphy is specific to Lewy body disease. Matsubara T, Kameyama M, Tanaka N, Sengoku R, Orita M, Furuta K, Iwata A,

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Arai T, Maruyama H, Saito Y, Murayama S. Autopsy Validation of the Diagnostic Accuracy of 123I-Metaiodobenzylguanidine Myocardial Scintigraphy for Lewy Body Disease. *Neurology*. 2022 Apr 19;98(16):e1648-e1659. doi: 10.1212/WNL.0000000000200110. Epub 2022 Mar 7. PMID: 35256483; PMCID: PMC9052572.

43. Although the biomarker approach to earlier identification of AD has been very useful, it is a mistake to focus on amyloid and tau alone. Measures of neurodegeneration (via imaging techniques such as MRI) have been incredibly important. Specific regions have been identified as first impacted in AD and more research is needed. Research has also revealed fMRI and DTI to be sensitive to early changes in AD. The argument that these are non-specific does not lead to the conclusion that amyloid should be focused upon. Many people have amyloid build up and no cognitive impairment. It is not a specific enough biomarker either. It is also important to emphasize that neuropsychological assessment is essential to AD research and diagnosis.
44. How do these guidelines address staging for individuals with "mixed" dementia types, e.g., suspected significant contribution of cerebrovascular disease to dementia? Where I work, in Bronx, NY, where the majority of my patients are from historically minoritized racial/ethnic groups (Black and Hispanic) and thus have disparately high rates of diabetes, hypertension, and smoking, the occurrence of mixed dementia is high. Given that patients like mine are the kinds of individuals that we want and need to include in research trials going forward, and want and need to work to achieve health equity for, as well, how do we address the needs of these individuals as regards precise staging, when there is comorbid cerebrovascular disease? The guidelines should at least acknowledge, if not address this (I reviewed the document, but didn't see this discussed).
45. First, I want to congratulate the committee to this excellently elaborated and nicely data-driven step forward. One point of discussion (also brought up during AAIC) is the one on the biomarkers for "T". Biomarkers such as the mentioned pT181 or pTau217 show changes in biological processes that are different from those detected by Tau-PET imaging. Therefore, I have the suggestion to consider splitting the "T" into two parts (following the example given for "I" by seperating "inflammation" into astrocytic and microglial components). This could look like this: T(b): Indicating changes in tau biology. Biomarkers for this could for example be pTau181 or pTau217 that would not necessarily reflect tau deposits but rather an earlier process, potentially increased excretion of certain tau species by neurons. T(d): Indicating deposits of aggregated tau protein in the brain (Tau-PET, potentially also MTBR). I think there is sufficient data available to

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include this concept into Table 2 under the "Staging, prognosis, as an indicator of biological treatment effect" category.

46. This is a step in the wrong direction with horrible implications for patients, families and clinicians. The presence of amyloid, a non-specific biomarker, does not mean an individual currently has or will develop cognitive impairment. As such, labeling of perfectly asymptomatic individuals with AD is incorrect and inhumane. Aside from emotional toll, it will have serious consequences for insurance coverage and premiums. It will lead to more (useless) testing. Finally, "treatment" with anti-amyloid drugs (which it will undoubtedly lead to) is associated with terrible side effects and questionable negligible clinical benefit. This should be reversed immediately. Hope is no reason to abandon scientific rigor and the oath of "primum non nocere" .
47. I strongly recommend using a different staging system for preclinic or subclinical AD (stage 0 or 1). A substantial portion of these patients will never progress to Stage 2. For non-research purposes, we should adopt what we have learned from other diseases (ie trappings of DCIS in breast cancer) and avoid labeling patients as stage 0 or stage 1 Alzheimer's without correlates of clinical disease. This leads to overdiagnosis with potential for anxiety and cascade of testing and treatments that are not indicated at this time.
48. This new set of criteria would be harmful to patients and families with little clinical benefit. Diagnosing a large set of Americans who may NEVER become symptomatic will have rious effects on employment and health/life/long term insurance ins, not to mention emotional/psych health. Imagine being healthy, in the prime of your life and asymptomatic, being frightened into taking biweekly infusions that have NOT shown clinically meaningful benefit over the risks, and certainly not long term clinical benefit (extrapolation of clinical trial data into years would be inappropriate), and yet there is probability that you would never develop symptoms at all! Or perhaps only mild/MCI where you still enjoy life, family and travel. In addition, this new framework harms taxpayers in that the burden on Medicare would be extreme - and likely unnecessary.
49. CanAge would like to acknowledge and express our support for the recently revised clinical guidelines for Alzheimer's™ disease by the National Institute on Aging and the Alzheimer's™ Association (NIA-AA). These revisions provide a modern recontextualization of Alzheimer's™ disease given changes in both medical and regulatory environments. Scientific advancements in biomarker technologies as well as the regulatory approval of disease targeted therapies for Alzheimer's disease have rendered the previous standards and guidelines antiquated, if not irrelevant. CanAge believes that the new scalar methodologies

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for diagnosis and treatment of Alzheimer's disease proposed in the NIA-AA's Clinical Guidelines draft should form the foundations of new standards for academy, industry, and clinical practice. CanAge encourages the adoption and implementation of policies and best-practices aligned with the NIA-AA's recommendations. With increasingly aged populations, the fight against Alzheimer's disease will only intensify. Concerted, coordinated efforts between policy makers, researchers, and medical experts are needed now more than ever.

50. Thank you very much for this wonderful update. I trained at Mass General in Boston and now work in the Costa Rican public system. I wonder if options could be provided to stage patients (within the framework) in locations where there is still limited access to biomarkers. Thank you.
51. As a neurologist involved in dementia diagnosis and PET/fluid biomarker research, I found the revised criteria well thought and flexible enough to diagnose patients even when some biomarker results are unavailable or discordant. If an experienced physician with sufficient knowledge about AD biomarker and other causes of dementia who has access to tau PET when needed use the criteria, I think it's the best we can do at this point. My only question is whether this criteria is intended to be used by only specialists or also by non AD specialists. As discussed in the session, even more clinical judgement seems to be needed to apply the revised criteria appropriately. If it is also intended for non AD specialists starting to use biomarkers, I wonder if there could be more information about when to consider reference to specialists.
52. As pathologist I would like to know: 1. How to deal with biopsy samples taken for other reasons (e.g., meningioma resection, evacuation of an intracerebral bleeding) when amyloid plaques and/or NFTs can be seen? May I consider these lesions as A+ and T+? In my opinion, this should be possible. Probably with a p- before the biological stage as you do for tumors (pT4a,pN1...). 2. What do I do if I only see CAA with Abeta deposition but no plaques in the biopsy? Is it A+ or A-V+? 3. Given that the new disease-modifying drugs against AD represent a real therapeutic option, wouldn't it make sense to include stereotactic cortical biopsies from temporal neocortex as a diagnostic option in addition to the biomarkers? For tumors you do this. The advantage of a temporal neocortex biopsy would be that LATE-NC (stage 2 and higher) co-pathology could be easily diagnosed and a differential diagnosis against FTLD species would be possible. The risk of the biopsy, however, must be justified by the therapeutic profit. It would be great if you could give some guidance on points 1 and 2 in the guidelines. Please consider point 3 more as food for thought. Thank you very much for

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reading my comments in advance and congratulations to a big step into the direction of a biological definition of AD. Kind regards

53. This draft falls short in considering the full range of potential combinations of biomarkers. For example, it would greatly benefit from including recommendations tailored to the A-T+ profile of fluid biomarkers.
54. Is there any plan on including criteria with respect to data harmonization specifically for different tracers since different centers use different tracers? The CentaurZ is barely scratching the surface. From the slides I personally did not recognize any statistician/biostatistician with background in AD in the review panel. This is a Clinical diagnosis development, however the presence of biostatisticians/ statisticians is critical for the purpose of reproducibility, sensitivity, diagnostic criteria cutt-offs, etc.. I personally suggest inclusion of Biostatistics experts that are involved in clinical studies and have experience in AD data analysis.
55. It is important that it is specified that CSF P-tau positive AB42/40 negative is not congruent with an AD diagnosis. Right now the document says that any positive biomarkers is enough for AD.
56. Thanks for the excellent update to the ATN research framework. I think the table (1a/1b/1c/2a/2b/â€¦) makes a lot of sense and is very important because the off-diagonal elements of the table may account for the potential role of the N/I/V markers (including heterogeneity in brain atrophy). It also acknowledges the fact that adding certain N/V/I markers to A & T significantly improve the prediction of AD progression, in addition to other types of dementia that may play a bigger role than the amyloid impact.
57. Did you consider including CSF Ab42/Ptau181 as a marker for A+ since it has a higher agreement with amyloid PET than Ab42/40?

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58. I am very worried that if the EEG biomarkers were not integrated into the A-T-X-N(C) Framework, international sponsors would no longer invest in that topic, and the vigilance/consciousness and sleep disorders of patients with AD will continue to be "neglected" in AD research and clinical applications. Not to mention the drop of interest in the proposals of symposia on the EEG biomarkers in AD in the international conferences on dementia. Indeed, the actual A-T-X-N(C) Framework is spotting the attention of sponsors and scientific committees more and more on the new entries: blood plasma, inflammation/immune reactivity, and brain vascular injury biomarkers. It will be an extraordinary reference document on the state-of-the-art in the use of biomarkers for the assessment of Alzheimer's Disease patients from preclinical to clinical stages of (pre) mild cognitive impairment and dementia. Also, I am offering you my full availability to discuss the possible integration of the category of EEG biomarkers of vigilance-sleep dysfunctions in the current A-T-X-N(C) Framework. A common observation in the assessment of Alzheimer's disease patients is that they are prone to frequent diurnal naps, even in the morning, and show fragmented night sleep with a dramatic impact on their quality of life and that of their caregivers. Indeed, AD patients have difficulties in maintaining a stable, quiet vigilance to follow TV programs and news and have prolonged social conversations. Consistently, they show very abnormal resting-state EEG rhythms in quiet vigilance conditions and changes in the structure and (EEG) microstructure of sleep. Neurologists currently seem not to be aware of brain vigilance dysfunctions and do not treat morning naps or vigilance dysregulations and cognitive fluctuations during the day as these symptoms do not hurt family persons. They only treat sleep disorders, but only when it has significant behavioral manifestations. The change in the Framework I propose may enlighten this important but undercovered aspect of the disease and may promote more research on vigilance/consciousness disorders in Alzheimer's disease patients. ABSTRACT: The extended A-T-X-N(C) Framework successfully expands the biological model of Alzheimer's disease (AD) to include neuropathological, (neuro)inflammatory/immunoreactivity, neurovascular, synucleinopathy, and neurodegenerative biomarkers as an insightful and useful basis for future research and clinical applications. This white paper posits that this Framework may integrate two clinically relevant aspects of AD: (1) The dysregulation of vigilance and sleep as target clinical manifestations and (2) EEG biomarkers reflecting abnormal neurophysiological excitatory/inhibitory balance in the brain. In this line, the core outcome of some recent position papers of The Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART) professional interest areas was summarized. More than 10% of AD patients show non-motor epileptiform activity from ongoing EEG activity (usually without overt seizures) that may predict faster disease progression and may be targeted by pharmacological treatment. AD patients also

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show higher EEG delta (< 4Hz) and theta (4–7Hz) rhythms and lower EEG alpha (8-12 Hz) rhythms during conditions of quiet vigilance and poor task-related EEG delta and theta responses to rare stimulus targets. In AD patients, abnormalities in the sleep-wake cycle are common and related to early neuropathology and neurodegeneration in the neuromodulatory subcortical ascending systems interacting to promote wakefulness and sleep. In conclusion, the extended A-T-X-N(C) Framework may be enriched with the Pathophysiological “P” biomarker category probing abnormalities in EEG oscillations reflecting an altered neurophysiological excitatory/inhibitory balance that significantly affects the sleep-wake cycle and, then, patients’ and caregivers’ quality of life.

59. Congratulations on this update to the diagnostic framework. It is clearly a forward-looking document that will evolve as the science advances. I strongly support the molecular staging of AD and targeting therapies based on molecular and clinical profiles. It is important to distinguish between fluid biomarkers that closely correlate with amyloid plaque build-up and those that correlate better with aggregated forms of tau. The framework also needs to be clear and easy to implement in clinical practice. Amyloid PET and specific fluid assays are good at detecting cerebral amyloidosis and generally your proposal for Core 1 and Core 2 are useful, except that tau staging will become increasingly important to determine who is most likely to benefit from amyloid-lowering antibodies and there could be a scenario where tau PET is obtained first to both diagnose and stage AD. Plasma amyloid and tau measures to diagnose AD are just beginning to be used in clinical practice so the data on real world applications is limited. These measures can be used to prescreen who is most likely to be a candidate for amyloid-lowering therapy but during this transition period I suggest that a confirmatory test be performed, preferably with PET, that can also be used to monitor treatment response. Given the robustness of the assays, I am sure that they will be used to diagnose AD as a standalone in the near future, especially if PET is not needed to guide treatment. Provisions need to be made for describing values near cut-offs and for confirmation with other assays. I like the plan for tau staging but I surprised that characterization of amyloid status is binary, given the wide range of values and their potential impact on diagnosis and treatment. How will missing biomarkers be handled in the staging grid? Thank you for including I, V, S and X in the model. It is not clear how ApoE and other genetic markers might fit, except for dominant mutations.

60. We welcome the proposed revisions as they provide further guidance beyond the research guidelines published in 2018, and offer a pragmatic framework that will

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be useful for both research and clinical routine. In the current fast-changing landscape, we applaud the working group for the timeliness of these updates, as the use of disease modifying therapies and plasma biomarker tests will soon become a reality for many practitioners. In addition, the proposed staging scheme is a recognition that the Alzheimer's disease (AD) field is moving towards a personalized medicine approach, which will have great benefits to patients living with AD, their caregivers, and society. Our feedback focuses on: I. The framework defined for biomarker assays to be deemed appropriate for clinical use, as we consider that the current guidelines lack the regulatory perspective that applies to clinical diagnostic assays; II. The proposed biomarker categorization and staging, which we consider to be complex and based on non-validated biomarkers; and III. The indeterminate zone proposed for result interpretation. We believe the recommendation needs to be clarified and possibly removed, as it creates uncertainty in result interpretation, it may lead to delays in care and unnecessary workups, and it compromises the value of in vitro diagnostics (IVD) products strictly validated and approved with only one cutpoint.

I. Feedback regarding guideline as framework for clinical use A. The proposed guideline mentions that the biomarkers listed in Table 1 and Table 2 (A β 42/40, pTau181, pTau217, GFAP, NfL, Alpha syn-SAA) are "suitable for use in clinical practice" (see Table captions) without mentioning the criteria (eg. clinical or regulatory evidence) the committee used for deeming these biomarkers safe and effective therefore suitable for clinical care use. We believe this needs to be clarified and additional explanations on how to interpret results of these biomarkers included in order to facilitate implementation in clinical practice. There are a range of biomarker assays at different stages of development for use in AD, including IVD devices (or tests/assays), laboratory developed tests (LDTs; also known as in-house devices), and research use only devices (or tests/assays) (RUOs). 1 The majority of AD plasma biomarker tests currently available (and in development) are designated as RUO, thus should only be used for research purposes and not for clinical decision-making or diagnosis. 2 Additionally, inherent limitations of LDTs are that, compared to IVDs, their safety and effectiveness might not have been comprehensively proven; their design, validation and implementation are specific to each lab, therefore the performance of the assays might differ between laboratories. 3 FDA and IVD regulations (IVDR) for clearance/approval in the US and Europe, respectively, provide a framework for stringent validation criteria, including Clinical and Laboratory Standards Institute (CLSI) guidelines for analytical validation and clinical studies. Our recommendation is that the document should mention that, with the exception of CSF assays for the core biomarkers beta-amyloid and pTau181, the rest of the biomarkers included here have not received regulatory approval/clearance for use in clinical practice. B. In addition, on line 152, there is a mention of two CSF FDA approved assays for beta-amyloid. We understand

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that the committee is not recommending specific assays, but the language should be clarified to specify that there are currently FDA approved assay ratios showing concordance with amyloid PET, and therefore able to detect amyloid pathology, which is a requirement to qualify patients for amyloid targeting therapies. The biomarkers and biomarker combinations for which IVD assays with regulatory approval exist should be indicated in the Tables and the committee should consider updating these tables periodically based on information from manufacturers. C. Furthermore, in Table 1 and 2, we propose to distinguish more clearly between CSF and plasma biomarkers, for clarity and especially because of differences in performance. For plasma A β 42/40, there is published evidence that it is not a clinically robust biomarker for worldwide scalability. 4,5 In addition, published evidence shows that, in contrast to other biomarkers (e.g. pTau), plasma A β 42/40 are only stable for a very limited amount of time (max. 2-3 hours) at room temperature in whole blood and plasma. 6-8 Taken together, utilization of plasma A β 42/40 as a biomarker in clinical routine can lead to misclassifications of amyloid status. D. An additional observation in Table 1 and 2, it is unclear why A β 42/40 is listed as a ratio, whereas other biomarkers are listed as single markers only. If only the protein pathological pathway matters, A β 42 should be listed as a single marker (as A β 40 alone does not have diagnostic utility). Alternatively, provided that the A β 42/40 ratio should be kept and given that "biomarkers that are currently suitable for use in clinical practice" appear in Table 1 according to the Committee, other fluid biomarker ratios (pTau181/A β 42 and tTau/A β 42) with rigorously demonstrated equivalence to amyloid PET imaging should be listed. 9-12 Exclusion of the above mentioned biomarkers and ratios in the tables could impact future coverage and reimbursement of these tests and impact patient access to therapy. In addition, we propose that CSF tTau should be included as a non-specific marker of neurodegeneration, since there are reliable IVD assays that measure tTau in CSF. The argument provided in the manuscript (lines 175-182) does not explain why tTau is not included, since the N category is mentioned to be non-AD specific (lines 167-170). Also, we would like to note that recent data suggests pTau181 and pTau217 are actually better biomarkers of A than T, which is why the suggested categorization may be misleading in this regard. 13 E. From a regulatory perspective, stringent validation should always refer to particular assays (or "devices") and not to biomarkers in general. Therefore, we suggest modifying the text throughout the manuscript accordingly (e.g. Textbox 3, line 40 to: "only stringently validated imaging and fluid biomarker devices/assays" and Textbox 4, line 50 to: "validated imaging and fluid biomarker devices/assays"). F. Finally, we would like to draw the committee's attention to the statement in lines 327-328 "Biofluid assays do not require FDA approval" which is not an accurate statement when it refers to clinical use of these assays. The FDA requires that fluid biomarker assays be validated against an approved reference method

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according to the intended use (i.e. currently amyloid PET). In addition to these general observations, we would like to express our particular concerns regarding the following parts of the guidance document: A. Line 174: It is mentioned that "NfL [...] is used clinically in various disorders" despite the fact that there is no IVD-approved NfL assay yet and NfL has not yet been stringently validated for clinical routine use. We propose that this should be more clearly articulated in the tables and the text. B. Line 219-222: aSyn-SAA is not IVD-approved yet, which is why it is unclear as to why it is referred to as a "diagnostic biomarker". We recognize the potential for aSyn-SAA to become a clinically suitable diagnostic biomarker and we propose that the committee clarifies the level of evidence currently available. C. Line 569: "The centiloid scale is the accepted method for quantifying amyloid PET". We appreciate that the centiloid scale is beneficial for quantitative standardization of amyloid deposits. However, currently this method does not have regulatory approval. For the fluid biomarker assays with the intended use of rule in/rule out/confirmatory of amyloid pathology, the regulatory requirement was to compare against visual read (amyloid) PET, which is an accepted method for determining amyloid positivity. We propose that visual read is included in the document, as outlined in the tracer method sheet. II. Feedback regarding biomarker categorization and staging A. In the proposed guidelines revision, the ATN categorization primarily reflects the protein pathology, but its role in disease staging is unclear. If the intent is that an overarching ATN categorization is not meant to be indicative of staging anymore due to the non-equivalence of fluid and imaging markers, this should be made clear and highlighted explicitly, since this is a shift from the previous understanding. In particular, this should also be mentioned in the captions of Tables 1-4. Without this explanation, the tables are difficult to interpret (e.g. a split by column (Tables 1-3) or row (Table 4) is not sufficient to indicate non-equivalence of imaging and fluid markers). Unclear statements or tables can influence the interpretation of the integrated biological and clinical staging proposed in Table 6. If a biomarker stage is defined, the definition criteria needs to be consistent. B. Table 2 lists biomarkers for several intended uses. However, the subgrouping according to ATNIVS stages, given the non-equivalence between fluid and imaging markers, is confusing, in particular since "staging" is listed as one of the use cases. We propose additional clarification around the Use Cases and consistency across the document to avoid confusion and further to consider harmonizing the language to use "Intended Uses" instead of "Use Cases". C. Table 3 lists biomarkers deemed as suitable for AD research. However, there should be a statement that the list is not exhaustive and will be updated with other novel biomarkers, as per the fast advancements in the field. Providing more information on what criteria made these biomarkers suitable for research and also stating that they are suitable for research use only, would help differentiate between clinical care and research use and prevent misuse of research biomarkers for

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routine clinical care. D. It is not clear why biomarkers suitable only for research and not yet validated with regulatory bodies are included in Table 4 (e.g. pTau205, MTBR-243 and non-phosphorylated tau fragments), as that implies they are ready to be used in clinical practice for disease staging. We propose listing them as “exploratory biomarkers” or “biomarkers under investigation” and removing them from tables that are intended for clinical disease management. E. In Table 4 it is not evident if the initial core biomarkers suggested for AD diagnosis (presented in Table 1 and 2) will be positive throughout the different stages. In addition, in the Fluid staging row, if just individual biomarkers are listed, we propose listing A β 42, pTau181, pTau217, pTau231. If assay ratios are considered, pTau181/A β 42 and tTau/A β 42 ratios along with the A β 42/40 ratio assay should be included. F. The recommendations for PET staging in Table 4 carry more validity than those for fluid staging, since fluid biomarkers for staging from stage (b) onwards are not currently validated for clinical practice, implying that staging should be focused on the use of Tau PET, which is neither widely available nor approved outside of the US. In fact, it is not clear from Table 4 whether there are actually cutpoints that can distinguish biomarkers stage (a) from (b) for e.g. ptau-T205 and pTau181. G. Lastly, given the imminent utilization of disease modifying therapies (DMTs), we encourage the Committee to mention the importance of evaluating APOE4 status during or after AD diagnosis and before treatment initiation, as per the reported results related to amyloid-related imaging abnormalities (ARIA) incidence in DMT-treated patients in clinical trials.

14-15 III. Feedback regarding indeterminate zone The proposed guidelines promote the definition of an indeterminate zone in Section 3.3.2

1. Consider aligning wording: indeterminant, indeterminate, intermediate throughout the document.
2. Consider clarifying or potentially removing the recommendation for an intermediate zone (around a cutpoint or in between two cutpoints):

A. Most available clinical assays are able to provide a single validated cutpoint that optimizes sensitivity and specificity for the clinical intended use, without the need for two cutoffs and an indeterminate zone in between. Such an indeterminate zone is difficult to interpret and act on clinically and needs additional guidance for patient management. The percentage of subjects who are expected to be in the indeterminate zone is dependent on the performance of the biomarkers and of the assay/platform. B. In the case of regulatory approved/cleared assays, the interpretation of assay results at the established and validated cutpoints is included in the package insert and is based on the assay's performance in clinical studies and the intended use identified. Therefore, recommending an indeterminate zone around any biomarker cutpoint, outside of manufacturer recommendations can create uncertainties in clinical practice and lead to delays in clinical care and unnecessary workups. C. Consideration should be given to the statistical uncertainty that exists within a measurement and around each validated/established cutpoint. Analytical and biological variability will influence

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the uncertainty of each measurement at the validated/established cutpoint and therefore it is of utmost importance to have clinically validated biomarker assays that have met the rigorous analytical validation procedures required by regulatory agencies to minimize analytical variability around cutpoints. The committee's recommendation on always using the biomarkers' results in the clinical context is very clear, matches the assays manufacturers' recommendations and should suffice. Final Considerations In summary, we welcome the timely and appropriate proposed revisions of the NIA-AA research framework and transition to a research and clinical framework. These updates will be an important step forward for the AD field, and will be an often cited document for future clinical practice. We encourage the Working Group to consider our comments and feedback carefully to ensure that clear recommendations are made, which will be universally interpreted, and have positive impacts on the management of AD patients in the future.

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61. We are pleased to see inclusion of fluid biomarkers within the NIA Clinical Guideline framework; however, we want to highlight our concern around vague and inaccurate language used in the discussion of CSF and blood-based biomarkers. Furthermore, there is insufficient peer-review data to support the use of CSF and blood-based biomarkers interchangeably as implied throughout the document. Specific feedback by page/line number is provided below, however, we also strongly recommend seeking feedback from the appropriate laboratory medicine specialty in North America prior to finalization of this document. These experts will be able to provide guidance on appropriateness of the recommendations based on the current standards and regulatory requirements for implementation of these tests in clinical laboratories. In laboratory medicine, AD biofluid testing is most commonly overseen by Clinical Chemists (or equivalent designations outside of North America). The appropriate professional associations for consultation in North America include the Association for Diagnostic and Laboratory Medicine and the Canadian Society of Clinical Chemists. 1. Terminology used throughout document: a. biomarkers diagnostic of

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AD v. biomarkers diagnostic of AD pathology Earlier international consensus efforts (e.g., DOI: 10.1002/alz.12545) have emphasized the need to refer to AD biomarkers as being reflective of the pathology and not the disease. The language in the draft document presents a deviation from this consensus and rationale for this change in terminology should be provided. b. The draft guideline includes many generalizations between CSF and blood, as if they were interchangeable. This is inaccurate and misleading. We recommend striking the use of "fluid" as terminology and instead specify CSF and/or blood for each instance where it is relevant throughout the text, text boxes and tables. c. Throughout the document only CSF AB42/40 is referred to as core biomarker but we know that CSF pTau181/AB42 ratio is as good as a measure of amyloid pathology (e.g., DOI: 10.1186/s13195-020-00595-5, DOI: 10.1002/dad2.12190, DOI: 10.1002/dad2.12182). CSF pTau181/AB42 should be specifically listed as a core biomarker. While pTau181/Ab42 does not fit nicely within the ATN framework, it should not be ignored for this reason. 2. Page 1, line 24-25: "... plasma-based biomarkers with excellent diagnostic performance have been developed and clinically validated" a. Recommend removing reference to "excellent diagnostic performance" and replacing with data ranges (for example). From the studies performed in controlled research settings, the diagnostic performance can vary between assays and labs. b. Recommend removing "clinically validated." This is misleading. The majority of plasma biomarkers are in development. While these assays may have been analytically validated, they have not been fully clinically validated. Therefore cut- points relevant for interpretation of the results based on the context of use have not been establish for interpretation in clinical practice. 3. Page 3, line 72: "The most significant advance in AD diagnostics in recent years has been the development of plasma biomarkers with excellent diagnostic performance." This phrasing ("excellent diagnostic performance") is vague/inaccurate. The document should acknowledge that not all plasma assays perform the same clinically or analytically. 4. Page 3, line 74: "This now makes biological diagnosis of AD (which previously required PET or CSF assays) generally accessible and is projected to revolutionize research and clinical care. It is correctly stated that moving to diagnosing AD with a blood test has the potential to drastically change clinical care. With the impact of this change in mind, before blood-based biomarkers are included in clinical guidelines, clinical utility needs to be established, and assay specific performance of blood tests, as compared to PET and CSF testing, needs to be transparent to patients and providers. Inappropriate utility of blood tests or misunderstanding of the limitations of these tests will lead to many false positive and false negative results. Care needs to be taken to ensure that this "revolution to clinical care" helps patients, rather than harms them. 5. Page 4, line 116-119 "Biomarkers were placed into Tables 1,2 vs Table 3 based on the committee's assessment of the strength of available evidence of

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high diagnostic accuracy (e.g., sensitivity, specificity) compared to a valid gold standard, high reproducibility, and diagnostic utility based on clinical studies in real world settings.^{14,15} Notably, both papers cited (ref 14 and 15) discuss work that still needs to be done before these assays are ready to be used clinically. If a claim is to be made that these biomarkers have been tested and have “high reproducibility and high diagnostic utility in real world settings”, then references need to be included pointing to those specific studies (including transparency on how the studies were performed”, the specific assays used in those studies, their cutoffs, and their diagnostic performance compared to imaging and CSF. The majority of the data on sensitivity/specificity of these assays was determined in research settings, using batch testing, without pre-established cutoffs. This is far from equivalent to a “real world setting in a clinical laboratory.” 6. Page 5, line 142: “Plasma and CSF A β 42/40 both correlate with amyloid PET and predict clinical progression: however, the fold difference between individuals is around 50% for CSF A β 42/40 but 10%-15% for plasma A β 42/40.” a. This passage inadequately addresses that plasma and CSF A β 42/40 do not equally correlate with amyloid PET. CSF performance is superior (AUC in mid 0.90) compared to blood (AUC in the 0.70 to mid 0.80 depending on the assay) (PMID: 34542571). Further, various plasma A β 42/40 assays show significantly different correlations with amyloid PET. The guideline should not be written from the perspective of the best performing plasma assays, while ignoring the variability in performance of what is in the literature (e. g. DOI: 10.1001/jamaneurol.2021.3180, DOI: 10.1093/brain/awac333). Additionally, performance was determined in research settings, under the best possible conditions (i.e., batch testing, careful sample handling, etc.) Performance needs to be determined in a clinical setting, with exposure to common preanalytical variables that are likely to decrease assay precision, thereby decreasing diagnostic performance (PMID: 35130933). b. The acknowledgement of a 50% difference in CSF vs 10-15% difference in plasma is an opportunity to address the limitations of plasma assays. For plasma assay's performance to be equivalent to CSF, the requirements for precision and accuracy are much higher in plasma. The small difference between “disease” and “healthy” in plasma, means that small increases in imprecision as the assays transitions from a research setting to a clinical setting has the potential to result in big decreases in diagnostic performance (PMID: 35130933). 7. Page 6, line 151: “Two CSF assays for β -amyloid have FDA and IVDR-CE approval for clinical use.” It would be helpful to add that these assays assess the presence of amyloid pathology by different means, that is pTau/Abeta 42 v. Abeta42/40, yet have similar diagnostic accuracy for AD pathology. 8. Page 11 line 327: “Biofluid assays do not require FDA approval; the much-less rigorous CLIA or CAP (in the US) certifications do not require autopsy validation.” We recommend striking this sentence or otherwise rewording and providing appropriate citations. 9. Page 12 line 349:

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“Diagnosing AD by an abnormal core biomarker demands a high level of fidelity when applied clinically. However, any diagnostic test value, fluid or imaging, has a degree of uncertainty associated with it. We therefore recommend 3 protections against misdiagnoses...” a. “Fidelity” is not an acceptable laboratory medicine term. We recommend the use of the term “accuracy”. b. This paragraph is an opportunity to request from companies and laboratories for clarity on the performance of the biomarkers they are offering and the need for transparency on how these metrics were determined so that physicians can interpret the test findings. Asking for “rigorous validation standards” is too vague. There are established validation standards; however, there are no standards relating to the public transparency in the reporting of these validation metrics. 10. Page 13 line 364: “...prescribing specific performance metrics; however, fluid or PET biomarkers used for diagnosis should meet high standards for sensitivity, specificity, and precision” a. Here it is accurately stated that clinical use of plasma biomarkers is in active development (NB: if they are in development, then they are not likely appropriate to mention in detail in a clinical guideline). However, the use of the word “fluid” is misleading. Grouping plasma and CSF assay as “fluids” may lead to the incorrect inferences that they can be (1) used interchangeably, and (2) are at the same stage of development. b. Many plasma assays in development are being positioned as a screening test instead of diagnostic tests for amyloid pathology. (This is even suggested on page 10, lines 289-292, where it is noted that biomarkers can detect AD pathophysiology “...even though onset of symptoms may be years in the future.”) When blood tests are added to clinical guidelines, the appropriate utility of the tests should be clearly outlined in the guidelines. These guidelines should state that blood tests are not yet ready to be used clinically in asymptomatic patients. • As prevalence decreases so does performance. There is not yet evidence supporting the use of blood tests as clinical screening tools. c. Clinical validation needs to include: (1) identifying set points, (2) defining context of use, including the appropriate patient population (3) determining (and being transparent about) the performance of the assay in the defined context of use. 11. Page 13 line 383: “The zone of uncertainty thus divides the continuous range of values into confidently normal, confidently abnormal, and indeterminant. In addition, incorporating a zone of uncertainty may lessen fluid/ PET discordances, particularly for A biomarkers.” We recommend rewording guidance on the reporting of indeterminant zones. Depending on the assay used, the lab’s informatics system, and whether the relevant data is provided by the manufacturer AND is relevant to the population the laboratory serves, reporting an “indeterminant zone” may not be feasible. Instead of advocating for reporting ‘indeterminant zones’ we recommend (1) advocating for physician education in the interpretation of AD biomarker findings, and (2) advocating for manufacturers and labs running LDTs to provide detailed performance data around their assays’ medical decision limits. 12. Page 17 line

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39: "The onset of abnormal ptau 181, 217 and 231 seems to occur around the time of amyloid PET and much earlier than neocortical tau PET abnormalities" Conflating established fluid biomarkers (pTau 181 and 217) and research stage biomarkers (pTau205, pTau231, MTBR) would imply that they perform similarly and that their use is supported by an equivalent level of scientific evidence. This is inaccurate. Research-grade biomarkers should not be found in clinical guideline, other than perhaps a "Future Directions" section where it should be noted that the biomarkers therein have not been rigorously evaluated for clinical use. 13. Page 19 line 574: "We have identified specific fluid biomarkers to denote the early, intermediate, and advanced fluid stages. However, these fluid biomarkers have not yet been widely tested" We recommend striking the use of "fluid" as terminology and instead specify CSF and/or blood for each instance where it is relevant. 14. Page 25, Section 8 "Treatment effects" We recommend simplifying this section to indicate that at this point there is not enough data to provide specific recommendations of the use of biomarkers for monitoring treatments effects. The numerous data points in the draft document on this topic without specific statement on readiness may lead to misinterpretation or potential misuse. 15. Text box 1: Change 'diagnostic of AD' to 'diagnostic of AD pathology' 16. Text box 2: "Development of plasma biomarkers with excellent diagnostic performance" Vague and overly simplistic language. There is promising data for plasma biomarkers but as stated in the appropriate use recommendations, there are issues specific to blood-based biomarkers that need to be addressed before these can be established as diagnostic biomarkers. 17. Text box 3: a. Change 'diagnosis of AD' to 'diagnosis of AD pathology' b. "Stringently validated biomarkers (fluid or PET)" is too broad i. Recommend differentiating between CSF and blood, and pointing out what is needed before blood tests are ready for clinical use (DOI: 10.1002/alz.12756, DOI: 10.1002/alz.13026). 18. Table 1 a. CSF and blood should have their own columns as the performance is not interchangeable. b. A caveat should be included that (1) performance varies greatly by assay when using blood, 2) most blood assays are not yet clinically validated, and (3) publicly available clinical validation studies for blood assays have focused on symptomatic patients in a specialist setting, yet the guidelines suggest these tests can be ordered by general practitioners, where prevalence of AD pathology will be lower. c. For CSF fluid, ptau181/ab42 and tTau/Ab42 need to be mentioned as they have similar performance to (in CSF) to Abeta42/40. The fact that they do not fit in nicely with the ATN acronym should not be part of the rationale for their exclusion from a clinical guideline. d. For pTau181 and tTau mentioning their strong correlation would be valuable, so as to be convey that one or the other could be used (but both may be unnecessary) in combination with Abeta42. 19. Table 2: a. CSF and blood should have their own columns. i. A caveat should be included that (1) performance varies greatly by assay when using blood and (2) blood assays are not yet clinically validated. b. There is no

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rationale provided for the inclusion of NfL and GFAP in this table. Under what context should they be used clinically for the diagnosis/prognosis/staging of AD? 20. Table 3: a. CSF and blood should have their own columns. b. Research grade biomarkers should be deleted – it is unclear why there is a mix of research use and clinical use assays as this is a clinical guideline and not a review paper of the status of AD biomarkers. 21. Table 4: a. Tau/abeta42 ratios should be added and Abeta 42 should also be listed as a biomarker like ptau181. b. CSF and blood should have their own rows.

62. "Dear NIA-AA draft clinical guidelines authors, Thank you for working to advance the Alzheimer's field. Biogen is grateful for the opportunity to provide our feedback and look forward to a continued dialogue on this important initiative. We are happy to discuss these recommendations further as needed. We acknowledge that the field is moving towards a biological understanding of disease, but appreciate that in the context of real-world practice, the full individual patient context, including clinical symptomatology, must be assessed to address their full needs. Our suggestions are as follows: The Biogen team is reviewing these draft guidelines under the following guiding principles: • Testing should be actionable and considered in the context of enabling access to treatment or individual patient management • We consider these guidelines in the context of potential disease modifying treatments being available within the next 5 years (including anti amyloid therapies, anti tau therapies, and preclinical AD anti amyloid therapies) • Risk / benefit (including expense and access to future care) to patients (must be considered for any testing that is performed NIA-AA Proposal: AD can be diagnosed by a core AD biomarker (AB PET, fluid AB42/40, fluid pTau, or neocortical Tau PET) • Biogen suggestion: AD can be identified by a core AD biomarker (AB PET, fluid AB42/40, CSF pTau/AB42, or neocortical Tau PET) but can only be diagnosed with the full clinical context of the patient considered, including but not limited to AD pathologic change o Fluid pTau biomarkers: ▪ CSF pTau is not sufficient alone to diagnose AD; the ratio with AB42 should be considered ▪ There is promise of plasma pTau markers, but the science is not yet sufficient to use as a sole diagnostic measure o In the context of a clinical trial or preclinical diagnosis, a single core biomarker may be sufficient. In a purely clinical context, patient/family considerations will involve a broader assessment of other biomarker or clinical symptoms o Clinicians are encouraged to refer to memory specialists to determine how to appropriately counsel patient and provide expertise in the context of all AD factors • Rationale: o Although it may be that non-symptomatic individuals with AD pathology are in presymptomatic stages of disease, there remains little benefit in identifying this population until treatments are available; likelihood and timeline of progression to

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disease should be considered o We recognize that recent research has reflected limited psychological impacts of identifying biomarkers in presymptomatic patients, but believe providers should consider the potential impacts on an individual family and patient basis; guidelines are strongly needed for sharing this information appropriately with patients & families NIA-AA Proposal: Symptoms are not necessary to diagnose AD; Symptoms are a result of the disease process, not its definition • Biogen suggestion: “Biomarker diagnosis of AD is possible in the absence of symptoms, but the risk / benefit of biomarker testing should be considered in the context of the indications of approved treatments and the need for further research to better understand the risks of testing to populations outside those indications.” • Rationale: o Through the lens of upcoming treatments, we agree that this is an important distinction o Although a recent meta-analysis (van der Schaar et al. 2023) showed limited impact on patient psychology, until there is available treatment, there may still be risk for patient well-being in diagnosing without symptoms o Additionally, exposing patients to information without actionable insights may lead to influx of “worried well” that lead to greater demand and extended wait time for neurologists, potentially delaying treatment for patients for whom treatments are currently available and indicated NIA-AA Proposal: pTau is a marker “T” in the ATN framework • Biogen suggestion: We recommend pTau be considered a marker of “A” pathology in the ATN framework o This may be updated at a future date when more is understood about the relationship between amyloid, tau, and pTau • Rationale o Although pTau is biologically linked to tau, fluid pTau correlates with both amyloid and tau pathology o The current use of fluid pTau is to help determine amyloid status, as seen in Table 4 NIA-AA Proposal: NfL is a clinically useful marker of N (neurodegeneration) • Biogen suggestion: NfL is a non-specific marker of N (neurodegeneration), but clinical relevance of NfL in AD has not yet been determined • Rationale: o Although NfL has been broadly linked to neurodegeneration, it has not been established that NfL is specific to Alzheimer's disease trajectory or treatment (see results from CLARITY-AD, TRAILBLAZER-ALZ 2, and ADNI) o Without additional information and clear context of use, there is not enough evidence to suggest clinical use o NfL may be useful for disease staging, prognosis (see DIAN results), or comorbidities, but further evidence is needed to determine exact context of use NIA-AA Proposal: Clinical value and use cases for other biomarkers deemed “suitable for use in clinical practice” (e.g., GFAP, asyn) – see Table 2 • Biogen suggestion: Although additional markers may be indicative of common comorbid pathology, there is not enough information to use biomarkers (aside from amyloid, tau, asyn, and vascular markers) in clinical practice • Rationale: o GFAP and imaging measures (aside from amyloid, tau PET, and standard MRI) are not yet suitable for this use due to limited understanding of appropriate context of use o Biomarkers should only be used in cases where we may expect meaningful changes to patient

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management (including: Asyn SAA and vascular markers) • Other biomarkers are not well enough understood and therapies are not available to lead to meaningful changes to patient management and should therefore be excluded NIA-AA Proposal: Table 3 biomarkers for research applications and associated pathology • Biogen suggestion: List core biomarkers as "Potential Core Biomarkers" • Rationale: o There is not yet enough known about these biomarkers to link to core biomarker categories NIA-AA Proposal: Definition of suitable biomarker tests: rigorous validation of tests as defined by "high standards of sensitivity, specificity, and precision" • Biogen suggestion: Definition of suitable biomarker tests: rigorous validation of tests as defined by "high standards of precision (including stability, robustness, and other measures for analytical validation), clinical validation (sensitivity, specificity and/or positive and negative predictive value that account for racial/ethnic differences and common comorbidities), and clear contexts of use / intended patient populations." • Rationale: o Diagnostic companies are often seeking guidance on what information is needed; providing a clear definition of "rigorous validation" will provide advice on a standardized path forward to align biopharma, diagnostic companies, regulatory bodies, and payers o Precision is only one measure of analytical validation; other elements should be included in the definition o Context of use (i.e., disease progression, treatment response) and populations tested will have dramatic impact on sensitivity and specificity • E.g., a test may have high sensitivity / specificity in detecting amyloid positive cognitive decline from amyloid negative healthy controls, but this is not a clinically relevant context of use / population NIA-AA Proposal: Fluid biomarker staging as proposed in Table 4 • Biogen suggestion: Denote biomarkers beyond AB42/40, fluid pTau 181 and 217 as conceptual with further clinical validation needed to support appropriate staging • Rationale: o These biomarkers are not yet clinically validated and assays are not viable for clinical use o Clinically relevant cutoffs and trajectories are not yet understood to determine staging appropriately"

63. Dear Dr. Jack and Committee Members: On behalf of our clinical and scientific teams at Alzheon we sincerely thank you for developing the biological definition and the 2018 research framework for Alzheimer's disease (AD). These clinical research criteria were simply transformational, paving the way for successful clinical trials and the approval of disease-modifying treatments. We also congratulate you on this timely update to the NIA-AA criteria that incorporates the use of blood- based biomarkers and advances a more comprehensive biological framework for AD, that recognizes the complexity of AD pathophysiology and the emergence of new informative plasma biomarkers. We also wish to highlight an aspect that requires further consideration and focus, namely the role of APOE

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genotyping in the proposed clinical diagnostic scheme. Please consider the following points: 1. Beyond being a lifetime risk factor for Alzheimer's pathology and onset of clinical disease, the APOE4 allele is the major risk factor for occurrence of amyloid-related imaging abnormalities with brain edema/effusion and microhemorrhage or hemosiderosis (ARIA-E and ARIA-H respectively) with the class of plaque-clearing anti-amyloid antibodies (Sperling, 2011). The two amyloid-targeting drugs currently approved by the US FDA carry a Black Box warning highlighting the increased risk of symptomatic and serious ARIA in APOE4/4 homozygotes. 2. The FDA now recommends APOE genotyping and urges prescribers to consider the APOE4/4 genotype when assessing the benefit-risk equation for treatment decisions with individual patients. Therefore, the new clinical diagnostic criteria should directly address this requirement and discuss the role of APOE4 as a "modulator" of AD pathology and its role in an individual's response to amyloid-targeting immunotherapies. 3. APOE4 is well known to be associated with decreased amyloid clearance and increased cerebral amyloid angiopathy, a well-known risk factor for occurrence of ARIA (Shinohara 2016, Greenberg, 2020). Furthermore, the APOE4 isoform has been reported to modulate microglial responses in AD brain, leading to heightened inflammatory responses (Wang 2021, Ferrari-Souza, 2023). This is supported by the published cases of fatal severe vasculitis with one of the approved amyloid antibodies, both of whom were APOE4/4 homozygote females (Piller 2022, Reish 2023). 2 111 Speen Street, Suite 306 Framingham, MA 01701 T: 508.861.7709 www.alzheon.com 4. A substantial body of literature has been accumulated in the last two decades that describes the distinct biology of APOE4 carriers in various ex-vivo cell systems and transgenic mouse models of AD (Shi 2017, Zhao 2020, Wang 2023). Distinct profiles of APOE4 carriers are also seen on volumetric-MRI, amyloid-PET, and tau-PET clinical imaging studies in individuals on the AD spectrum (Ossenkoppele 2015, Degenhardt 2016, Mishra 2018, Abushakra 2020, La Joie 2021). Furthermore, the new clinical findings with the approved anti-amyloid antibodies show that these biological differences are indeed clinically relevant since APOE4 allele shows a dose response on the risk of ARIA. 5. The argument in the draft document that APOE4 effects may vary in different ethnic groups does not lessen its importance for treatment decisions in clinical care, rather it underscores the need to gather more data in these groups. The inclusion of APOE genotyping in the proposed biomarker diagnostic scheme will encourage collection of these data by clinicians and facilitate studies in various under-represented and under-served groups. These data can begin to close the knowledge gap that exists today. 6. Of particular note is that the big majority of cohort studies evaluating the diagnostic utility of fluid biomarkers in AD include APOE4 genotype in their models, with APOE4 consistently increasing diagnostic accuracy in ROC analyses. This can be explained by the potentiating effects of APOE4 on the relationship between A and tau pathologies described

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from PET imaging and CSF p-tau analyses in ADNI and TRIAD cohorts (Therriault, 2021). Therefore, in cases where the core plasma biomarkers are in the borderline range, having an APOE4/4 genotype can facilitate a clinical diagnosis. 7. Indeed, the first commercial blood test used to aid AD diagnosis in the US, "PrecivityAD" by C2N diagnostics, includes plasma APOE proteotype (together with plasma A42/40) in determining the "amyloid positivity score", highlighting how the APOE4 allele improves the predictive value of other core AD biomarkers. We therefore respectfully urge the esteemed committee to add a dedicated section on the role of APOE4 genotyping in increasing the predictive value of core AD plasma biomarkers for AD diagnosis, and as a tool for risk stratification when considering the choice of an immunotherapy treatment.

64. Many thanks for your leadership in developing the framework for a biological definition of Alzheimer's disease. I thank you for your effort and want you to know that I appreciate how sometimes it can be exhausting. I applaud this progress in transforming Alzheimer's into a biological diagnosis. I look forward to the day when I can use this framework in the care of my patients at the Penn Memory Center. The purpose of this letter is to relay constructive comments. The focus of these comments is the concept of "amyloidosis." Amyloidosis is a key concept to define Alzheimer's disease: amyloid positive – or "A+" – is Alzheimer's disease: "People with amyloidosis, who by definition have AD..." from the presentation in Amsterdam. "Stage a (initial) – abnormal amyloid PET with no uptake on tau PET (A+T-)," from the draft document. My overall point is the field is not ready to adopt this into clinical practice. A+ is amyloidosis. Below, I explain why. The document needs a definition of amyloidosis that supports "A+ is Alzheimer's disease." I think the effort to compose this definition will result in a conclusion that, knowing what we know now, amyloidosis alone cannot define Alzheimer's disease. You may arrive at a different conclusion. Regardless, the end of this intellectual journey will, I submit, be worth the effort. The definition should answer whether amyloid is, in some state or states (monomers, oligomers, plaques), normal, meaning the state is part of physiology, like glycosylated hemoglobin (HbA1c)? Or, is amyloid inherently pathophysiologic, inherently abnormal, like cancer? This term "inherently pathologic" captures how some diseases are defined by measurable entities that are by definition "not normal." Cancer is a useful model. The presence of mitotic figures, uncontrolled cell growth that spreads beyond the basement membrane, with or without necrosis, describe an entity that is not part of physiology. Infectious diseases such as COVID-19 or ebola are other examples. In contrast, hemoglobin A1c (HbA1c, or glycosylated hemoglobin) is not inherently pathologic. Glycosylation of hemoglobin occurs as part of the physiology of glucose metabolism. All of us have some level of HbA1c. Medicine

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uses this level to define diabetes. A person has diabetes when her level is “too high,” that is, above a cut-off. The document adopts this model, not the inherently pathologic model, to explain why amyloidosis defines AD: “all biomarkers we discuss exist on a continuous scale and the definition of an abnormal test value requires creating a cut point in that continuous range. Cutpoints denoting normal vs abnormal values may be selected by various means and will vary with the assay platform, and for PET will depend on the specific ligand and details of the analytic pipeline.” At least three kinds of data validate a cut-off to signify a disease: actuarial, experimental and gold standard. Below, I review how the document addresses each. Actuarial data means the cut-off is set because overwhelming evidence shows that persons who are above (or below) a certain level are highly likely to experience future impairments in health and well-being. For these data alone to define a disease they need to be quite rigorous; in particular, they need to be quite predictive. Why? They’re essentially association or correlation data. Biases are therefore inherent. Epidemiology has all kinds of techniques to firm up a causal inference but even with them we can only infer causation. Second, the data need to be as compendious as possible. By compendious, I mean the data speak to the risk in all kinds of people who might experience the disease, not just the select few who have participated in longitudinal cohort studies. To be sure, there are a host of actuarial data that suggest amyloidosis increase a person’s years later risk of developing dementia (ADNI etc.), but I think we lack “Framingham quality data” to allow us to conclude “A+” defines a disease. By “Framingham quality data” I mean data from a large, diverse population followed for years and years. Notably, for example, there is evidence that amyloid is “not as predictive” of dementia in certain racialized minorities when compared to non-minority persons. Another source of data to inform the judgment that an entity that is not inherently pathologic is a measure of a disease are the results of an experiment done in humans who have the entity. These experiments are typically randomized and controlled trials. Such trials show the intervention manipulates the measure which in turn alters the occurrence of valuable clinical outcomes. For example, the drug hydrochlorothiazide lowers systolic blood pressure and, as a result, a person is less likely to experience a heart attack, stroke or congestive heart failure. A similar story has been told by experiments that manipulated low density lipoprotein using HMG coA reductase inhibitors. These experiments helped to establish that systolic hypertension and elevated LDL are, above some levels, pathologic. Unlike actuarial data, the experimental design allows more robust inferences of causation and so the data are more likely to be clear and convincing. Do we have similar experiments in persons with amyloidosis? We have at least two (donanemab and lecanemab) and possibly three (aducanumab). These experiments were performed in persons who had either MCI or mild stage dementia. One of them also required the persons to have at

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least “low to intermediate tau” as measured using a PET scan. The document recognizes the importance of these experiments in validating amyloidosis as disease defining: “Most importantly [*italics mine*], recent trials have demonstrated that anti A β immunotherapy, that reduces fibrillar amyloid levels measured on PET imaging, can slow the rate of cognitive decline in early symptomatic AD. There is consistency across both successful and failed immunotherapy agents that the amount of amyloid PET reduction is associated with the degree of clinical benefit. These findings linking biology to clinical manifestations, which have been replicated across independent therapeutic programs, provide solid empiric support for a biological definition of AD.” Taken together, do the results of these experiments validate that manipulating amyloid in a person produces valuable clinical outcomes? For persons with MCI and mild stage dementia they do, but what about persons with no evidence of cognitive impairment? Here we have mixed data. We have several studies showing drugs lowered amyloid but did not cause valuable outcomes for the persons who received them. Examples include solanezumab and bapinezumab. In these cases, drugs that lowered amyloid in persons with elevated amyloid and normal cognition failed to slow cognitive decline and even caused cognitive worsening. In sum, the existing experimental data is not sufficient to call A+ alone Alzheimer’s disease, especially in persons who are cognitively unimpaired. As an aside, I should note that both actuarial and experimental data introduce a clinical aspect to a non-clinical, or biological, definition of a disease. The former relies on accepting the anticipated future health state is not desired (for example, strokes are bad) and that the risk is sufficiently high to be concerned. Experimental data also rely on accepting the anticipated future health state, the outcome, is not desired and the magnitude of benefit, the reduction in risk, is sufficient. This is perhaps somewhat ironic as biological definitions are viewed as “preclinical.” It is, I think, the unspoken instigator of disagreements over actuarial and experimental based disease definitions. A third source of data is the use of a gold standard. This is the approach used for the definition of diabetes. The gold standard for the HgA1c cut-off is an abnormal glucose tolerance test. The HgA1c value that best predicts a person has an abnormal glucose tolerance test defines an abnormal HgA1c which in turn defines a person has diabetes. The document does not state what is the gold standard against which a cutoff of amyloid can be set. Notably, the document acknowledges setting cut-offs is at present uncertain because “Pathophysiologic mechanisms involved with aggregation and clearance of protein fragments may be involved very early in the disease process, but these are not yet well understood” and amyloidosis is distinct from the pathologic gold standard: “By defining AD as any abnormal core AD biomarker, as we have done in this update, the link between the pathologic gold standard and the in vivo definition will not always be consistent. Many individuals with only an abnormal amyloid PET, fluid A β 42/40 or ptau may not be at Braak. NFT stage 3 or higher

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neuropathologically and thus would not qualify for a pathological diagnosis of intermediate/high AD neuropathologic change.” To sum up, a cut-point based disease definition of an entity that is not inherently pathologic, that is part of pathophysiology, often blends all three sources of data: actuarial, experimental and gold standard cut-off. That is the case for HgA1c. In the case of amyloidosis, there are actuarial and experimental data. Based on my conversations with colleagues, they're not crossing a threshold of clear and convincing evidence to call amyloidosis alone – “A+” – a disease. It is instead in that liminal space called a “risk factor.” A final point on the definition based on a cut point is the state of measurement. This seem dull but it is in fact quite important because even if the actuarial, experimental and gold standard evidence are clear and convincing, if they cannot hold up to measurement, the definition of the disease stumbles into practice and so becomes contested. Diagnosing cancer requires obtaining a tissue sample, fixation, stains and a microscope. The methods to do this are generally well-accepted, reliable and reproducible. To be sure, pathologists can disagree on specifics of a tumor type but they don't dispute how they do this and that they are looking at cancer. HgA1c level is easily and reliably measured using standardized laboratory practices. It took several years of international efforts at standardization to achieve this. The Alzheimer's field is not there with amyloidosis. Not yet. At present competent professionals are not measuring amyloidosis using methods that are reliable, consistent and widely accepted among their fellow professionals. Amyloidosis can be measured using a variety of technologies (blood, CSF, PET images). Has the field arrived at a reliable, consistent and common approach? For example, what centiloid value defines amyloidosis, what cut-off have experts decided (akin to HgA1c or systolic blood pressure)? The document recognizes that the science of measurement is in development and so has not yet achieved methods that are reliable, consistent and widely accepted: “Third, research studies have demonstrated that imaging and fluid biomarkers within a category are not equivalent for many use cases. In the present document we have updated biomarker classification criteria to accommodate nonequivalence between fluid and imaging biomarkers within a category.” “Assay standardization and cutoffs are not yet established for many fluid biomarkers and therefore staging with fluid biomarkers is conceptual rather than firmly operationalized at present.” PET measures image amyloid plaques. Plaque however may not be the pathologic entity that the drugs affect, but, instead oligomers or perhaps monomers (Chris VanDyck observed in a debate on whether amyloid PET is worthy as a surrogate for FDA accelerated approval, the measure may well be a “surrogate of a surrogate”). Moreover, preliminary proposals lower the threshold for “pathologic fibrillar amyloid,” illustrating that replicated, agreed upon thresholds remain a moving target. In sum, the current state of measuring amyloidosis is in flux and under development. To return to the diabetes example, it is as if there are several ways to measure HgA1c, perhaps

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several species of HgA1c as well, and depending on the method, the cut point varies. The implications of the shortcomings of measurement are vast. They encompass spectacular risks to credibility and reputation and so trust in science. Labeling an individual with Alzheimer's engenders a disease experience. The label "Alzheimer's" is a mind transformative experience. People feel differently about who they are and their skills. Their feelings about the future change. So too their behaviors and relationships. If that disease label "depends on the cut point" and that cut-point is a contested work-in-progress, people will readily lose trust in the keepers of the cut point. They'll accuse the scientists of extra-scientific motives, such as profiting from selling tests and drugs. On a population level, when estimates of prevalence vary depending on the assay used and its cut-points, policy makers are readily frustrated. Policy makers complain the scientists are unable to clearly and consistently explain just how big is the problem and so we can't make coherent policies to help them tackle it. "Come back when you have one number, not several." In conclusion, as written, the document needs a definition of amyloidosis that supports "A+ is Alzheimer's disease." I think the effort to compose this definition should review the quality of actuarial, experimental and gold-standard data. I think this effort will result in a conclusion that, knowing what we know now, amyloidosis alone cannot define Alzheimer's disease. Someday it may, but not yet. Why? The actuarial data are provocative, but they're not clear and convincing. So too the experimental data. There's no gold standard. The measurement science is a work in progress. In sum, A+ is not Alzheimer's. It is, for now, what it is. Amyloidosis.