Text box 1: Fundamental principles

The NIA-AA convened 3 separate work groups in 2011 and a single work group in 2018 to create recommendations for the diagnosis and characterization of Alzheimer's disease (AD). The NIA-AA also convened a workgroup that published a consensus document on the neuropathologic diagnosis of AD in 2012. Fundamental principles that emerged from these efforts are listed below.

It is necessary to separate syndrome (clinically identified impairment) from biology (etiology)

AD is defined by its biology with the following implications

The disease is first evident with the appearance of β-amyloid plaques, and later neocortical tau tangles, while people are asymptomatic. Pathophysiologic mechanisms involved with processing and clearance of protein fragments may be involved very early in the disease process, but these are not yet well understood.

In living people the disease is diagnosed by disease specific core biomarkers

Unimpaired individuals with abnormal biomarker testing are at risk for symptoms due to AD. They are not at risk for a disease they already have.

Symptoms are a result of the disease process and are not necessary to diagnose AD

AD exists on a continuum not as discrete clinically defined entities

Clinical syndromes commonly seen with AD may also be caused by disorders other than AD and therefore clinical presentation alone is not diagnostic of AD

The same AD biology may result in different phenotypic presentations

Text box 2. Diagnosis of Alzheimer's disease: Core 1 and Core 2 AD Biomarkers

The diagnosis of Alzheimer's disease can be established by abnormality on a single Core 1 biomarker (see Table 2); however, not all available Core 1 biomarker tests have sufficient diagnostic accuracy to be suitable for clinical use. At the present time, we regard the following CSF, plasma, or imaging biomarkers to be diagnostic of AD: amyloid PET; CSF Aβ42/40, CSF p-tau181/Aβ42, CSF t-tau/Aβ42; or, "accurate" plasma assays (defined below). Core 1 biomarkers are useful for: (1) the early detection of AD in people without symptoms (2) the confirmation that AD is an underlying pathology in someone with symptoms.

Core 2 biomarkers are not typically considered standalone tests for the diagnosis of AD. Core 2 biomarkers are those in the T_2 category in Tables 1, 2 and include tau PET, pT205, MTBR-423 and non-phosphorylated tau. Core 2 biomarkers can be combined with Core 1 to stage biological disease severity and, (1) provide information on the likelihood that symptoms are associated with AD, 2) inform on the risk of progression in people without symptoms, 3) inform on the likely rate of progression in symptomatic individuals.

<u>Below we list important qualifiers around the biological diagnosis of AD:</u>

Only biomarkers (fluid or PET) that have been proven to be accurate with respect to an accepted reference standard should be used for clinical diagnostic purposes. We recommend as a minimum requirement, an accuracy of approximately 90% for the identification of intermediate/high AD neuropathologic change at autopsy (or an approved amyloid PET or CSF surrogate) in the intended use population. For plasma assays this translates to accuracy equivalent to that of approved CSF assays. We focus on accuracy (True positive + True negative)/(True positive + True negative + False positive + False negative) as the most concise performance metric because it is equally important that a test used for clinical diagnosis is correct when the test result is positive and correct when it is negative. The specification of accurate "in the intended use population" addresses positive and negative predictive value which depend on the prior probability of AD in the population of interest.

Clinical judgement is always required when employing or interpreting biomarker tests clinically. The judgement of the clinician is paramount, 1) in situations where a biomarker test result seems discordant with the clinical presentation, 2) when assessing the likely contribution

of AD vs other pathologies to clinical symptoms, particularly when the clinical presentation suggests copathology is present, 3) to assess potential effects of confounding medical conditions on biomarker results. The committee strongly recommends that clinicians should not be restricted by payers in pursuing further testing when this is indicated in the judgement of the clinician. Finally, we recommend that biomarkers testing should only be performed under the supervision of a physician.

At present the population in which a rule in or rule out diagnosis of AD would provide medically actionable information for clinical care is symptomatic persons. In the absence of approved interventions for unimpaired individuals, we do not advocate AD biomarker testing in this population currently, although this may change in the future. In addition, we do not advocate initiating treatments targeting core AD pathology in all symptomatic persons with biologically confirmed AD without regard to clinical context. Rather we explicitly state that treatment in symptomatic individuals with AD should be based on clinical assessment of risk/benefit at the individual patient level.

Text Box 3: Limitations of biomarkers

1) Lack of certified reference methods and materials (except for CSF Aβ42/40, where these are available)
2) Biomarkers (fluid and PET) are less sensitive than neuropathologic examination for detection of early/mild Alzheimer's disease pathologic change. While the inherent sensitivity limits of biomarkers could be viewed as a weakness, this could also be viewed as a strength when using Core 1 biomarkers for diagnosis because very mild levels of ADNPC that lie below the limits of detection may not be clinically relevant.
3) Thoroughly studied biomarkers are not available for all relevant diseases therefore it cannot be known with certainty in vivo what diseases in addition to AD are present in any individual, or what the proportional disease-specific burden is among various pathologic entities. This leads to (#4).
4) Because of the above, the proportion of the cognitive deficit observed in any individual that is attributable to AD vs other neuropathologic entities cannot be known with certainty; probabilistic estimates based on clinical judgment are the best one can do.

Text Box 4: Clinical vignette

The scenario is a patient with a diagnosis of mild dementia who is being evaluated for treatment with an approved anti Aβ monoclonal antibody. Amyloid PET was abnormal thus establishing the diagnosis of AD. Safety evaluations included MR which demonstrated severe bilateral hippocampal atrophy. Because of uncertainty in risk/benefit, the patient wished to proceed to tau PET for more intensive evaluation. Little uptake was present on tau PET. Thus, the patient was in biological AD stage A and the ATN profile was A+T-N+. The absence of significant tauopathy in the presence of severe hippocampal atrophy indicates that the patient almost certainly has copathology with AD, most likely LATE given the MR atrophy pattern.

Some clinicians may recommend treatment based on the assumption that although AD is unlikely the only cause of the patient's impairment, removing amyloid may slow cognitive decline - i.e., a clinical impression of favorable risk/benefit. Other clinicians may recommend not treating based on an impression of unfavorable risk/benefit. Scenarios like this will inevitably arise as treatments targeting core disease pathology are introduced into clinical practise. The recommendation to treat or not will always require clinical judgement.

Table 1. Categorization of fluid analyte and imaging biomarkers

Biomarker category	CSF or plasma analytes	Imaging
Core Biomarkers		
Core 1		
A (Aβ proteinopathy)	Aβ42	Amyloid PET
T_1: (phosphorylated and secreted AD tau)	p-tau 217, p-tau 181, p-tau 231	
Core 2		
T_2 (AD tau proteinopathy)	pT205, MTBR-243, non-phosphorylated tau fragments	Tau PET
Biomarkers of non-specific processes involved in AD pathophysiology		
N (injury, dysfunction, or degeneration of neuropil)	NfL	Anatomic MR or CT, FDG PET
I (inflammation) Astrocytic activation	GFAP	
Biomarkers of non-AD co-pathology		
V vascular brain injury		Anatomic infarction, WMH
S α-synuclein	αSyn-SAA*	

Core 1 imaging and individual fluid analyte biomarkers are those in the A and T_1 categories. The Core 1 biomarker category addresses the conceptual difficulty with appropriate classification of plasma ptau 217, 181 and 231. Although theses become

abnormal around the same time as amyloid PET, they are tau fragments, and it is therefore difficult to reconcile these analytes as biomarkers of the Aβ proteinopathy pathway.

The T_2 fluid biomarkers belong in the Core 2 category and correlate more strongly with tau PET than amyloid PET.

P-tau 231, pT205, MTBR-243, and non-phosphorylated tau fragments are listed in this table because they are discussed in the text; however, these analytes have not undergone the same level of validation testing as other biomarkers in the T_1 or T_2 category.

If a fluid analyte is presently informative only when measured in CSF this is denoted by (*), if informative with plasma or CSF then no specific notation added.

Biomarkers are categorized in this table based on four criteria. First, we identify three broad mechanistic groupings. Second, we subclassify based on the proteinopathy or pathophysiologic pathway that each biomarker measures (e.g. A,T,N etc). Third, within the Core category we distinguish between Core 1 and Core 2 biomarkers. Fourth, imaging and fluid analyte biomarkers are listed separately within each category.

Table 2. Intended uses for imaging and fluid biomarker assays

Intended Use	CSF	Plasma	Imaging
Diagnosis			
A: (Aβ proteinopathy)			Amyloid PET
T_1: (phosphorylated and secreted AD tau)		p-tau 217	
Hybrid ratios	p-tau181/Aβ42, t-tau/Aβ42, Aβ42/40	p-tau217/np-tau 217	
Staging, prognosis, as an indicator of biological treatment effect			
A: (Aβ proteinopathy)			Amyloid PET
T_1: (phosphorylated and secreted AD tau)		p-tau 217	
Hybrid ratios	p-tau181/Aβ42, t-tau/Aβ42, Aβ42/40	p-tau217/np-tau 217	
T_2: (AD tau proteinopathy)	pT205, MTBR-243, non-phosphorylated tau fragments	pT205	Tau PET
N (injury to or degeneration of neuropil)	NfL	NfL	Anatomic MR, FDG PET
I (inflammation) Astrocytic activation	GFAP	GFAP	
Identification of co-pathology			
N (injury, dysfunction, or degeneration of neuropil)	NfL	NfL	Anatomic MR, FDG PET
V vascular brain injury			Anatomic infarction, WMH, abundant dilated perivascular spaces
S α-synuclein	αSyn-SAA *		

Plasma p-tau 231, ptau 181, and Aβ42/40 are not included in the diagnosis or staging sections of this table because these assays have not yet demonstrated diagnostic accuracy equivalent to approved CSF assays.

pT205, MTBR-243, and non-phosphorylated tau fragments have not undergone the same level of validation testing as tau PET and therefore are only considered for a "conceptual" staging scheme outlined in Table 4.

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156 **Supplementary Table 1. Core biomarkers currently with regulatory approval**

Biomarker	**type**	**Regulatory approval** 157
Amyvid (florbetapir)	Amyloid PET	FDA
Vizamil (flutemetamol)	Amyloid PET	FDA
Neuraceq (Florbetaben)	Amyloid PET	FDA
Tauvid (Flortaucipir)	Tau PET	FDA
p-tau181/Aβ42 (Roche Elecsys)	CSF	FDA and CE mark
t-tau/Aβ42 (Roche Elecsys)	CSF	FDA and CE mark
Aβ42/40 (Fujirebio Lumipulse)	CSF	FDA and CE mark

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159 **Table 3a. Biological staging**

	Initial stage biomarkers	**Early stage biomarkers**	**Intermediate stage biomarkers**	**Advanced stage biomarkers**
	(A)	**(B)**	**(C)**	**(D)**
PET	amyloid PET	tau PET medial temporal region	tau PET moderate neocortical uptake	tau PET high neocortical uptake
	A+T-	$A+T_{MTL}+$	$A+T_{MOD}+$	$A+T_{HIGH}+$
Core 1 fluid	CSF Aβ42/40, p-tau181/Aβ42, t-tau/Ab42, and accurate* plasma assays can establish that an individual is in biological stage A or higher, but cannot discriminate among PET stages A-D at present			

160 Staging may be accomplished by 1) a combination amyloid PET and tau PET, or 2) a
161 combination of a Core 1 fluid biomarker (which would establish biological stage A or higher),
162 plus tau PET (which would be used to discriminate among stages).

163 *Accurate is defined in the text and in text box 2

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166 **Table 3b. Operationalization of biological staging by PET**

	Amyloid PET	**Tau PET medial temporal**	**Tau PET moderate neocortical uptake**	**Tau PET high neocortical uptake**	**AT notation**
Stage A	+	-	-	-	A+T-
Stage B	+	+	-	-	$A+T_{MTL}+$
Stage C	+	+	+	-	$A+T_{MOD}+$
Stage D	+	+	+	+	$A+T_{HIGH}+$

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Table 4: Conceptual Biological Staging with Fluid Biomarkers

	Initial stage biomarkers	**Early stage biomarkers**	**Intermediate stage biomarkers**	**Advanced stage biomarkers**
	(A)	**(B)**	**(C)**	**(D)**
Fluid staging	CSF Aβ42/40, p-tau181/Aβ42, t-tau/Aβ42, and accurate** plasma assays	pT205*	MTBR-243*	Non phosphorylated tau*

PET and fluid measures are not equivalent and hence stages A-D with PET are not equivalent to stages a-d for fluid biomarkers.

*Validation of pT205, MTBR-243 and non-phosphorylated tau as early, intermediate and advanced stage fluid markers respectively is conceptual for now, awaiting further studies.

** Accurate is defined in the text and in text box 2

Table 5: Clinical staging for individuals on the Alzheimer's disease continuum

Stage 0 Asymptomatic, deterministic gene

No evidence of clinical change. Biomarkers still in normal range

Stage 1 Asymptomatic, biomarker evidence only

Performance within expected range on objective cognitive tests.

No evidence of recent cognitive decline or new symptoms

Stage 2 Transitional decline: Mild detectable change, but minimal impact on daily function

Normal performance within expected range on objective cognitive tests.

Decline from previous level of cognitive or neurobehavioral function, that represents a change from individual baseline within past 1-3 years, and has been persistent for at least 6 months.

May be documented by evidence of subtle decline on longitudinal cognitive testing which may involve memory or other cognitive domains but performance still within normal range

May be documented through subjective report of cognitive decline (SCD)

May be documented with recent onset change in mood, anxiety, motivation not explained by life events

Remains fully independent with no or minimal functional impact on daily life activities (ADL)

Stage 3 Cognitive impairment with early functional impact

Performance in the impaired/abnormal range on objective cognitive tests

Evidence of decline from baseline, documented by the individual's report or by observer (e.g. study partner) report or by change on longitudinal cognitive testing or neurobehavioral behavioral assessments.

Performs daily life activities independently but cognitive difficulty may result in detectable functional impact on complex activities of daily life, i.e., may take more time or be less efficient but still can complete, either self-reported or corroborated by observer.

Stage 4 Dementia with mild functional impairment

Progressive cognitive and mild functional impairment on instrumental ADL with independence in basic ADL

Stage 5 Dementia with moderate functional impairment

Progressive cognitive and moderate functional impairment on basic ADLs requiring assistance

Stage 6 Dementia with severe functional impairment

Progressive cognitive and severe functional impairment on dependence for basic ADLs

* Individuals with Down Syndrome may not be fully independent even in stage 0 because of underlying intellectual disability. In these individuals decline in functional independence from baseline may be a more appropriate indicator of stage

Table 6. Integrated biological and clinical staging

	Stage 0	**clinical Stage 1**	**clinical Stage 2**	**clinical Stage 3**	**clinical Stages 4-6**
Initial biological stage (A)	X	1A	2A	3A	4-6A
Early biological stage (B)	X	1B	2B	3B	4-6B
Intermediate biological stage (C)	X	1C	2C	3C	4-6C
Advanced biological stage (D)	X	1D	2D	3D	4-6D

The typical expected progression trajectory is along the diagonal shaded cells from cell 1A to 4-6D. However, considerable individual variability exists in the population. Deviations above the diagonal (i.e., worse clinical stage than expected for biological stage) will often be due to co morbid pathology. Deviations below the diagonal (i.e., better clinical stage than expected for biological stage) will often be due to exceptional cognitive reserve or resilience.

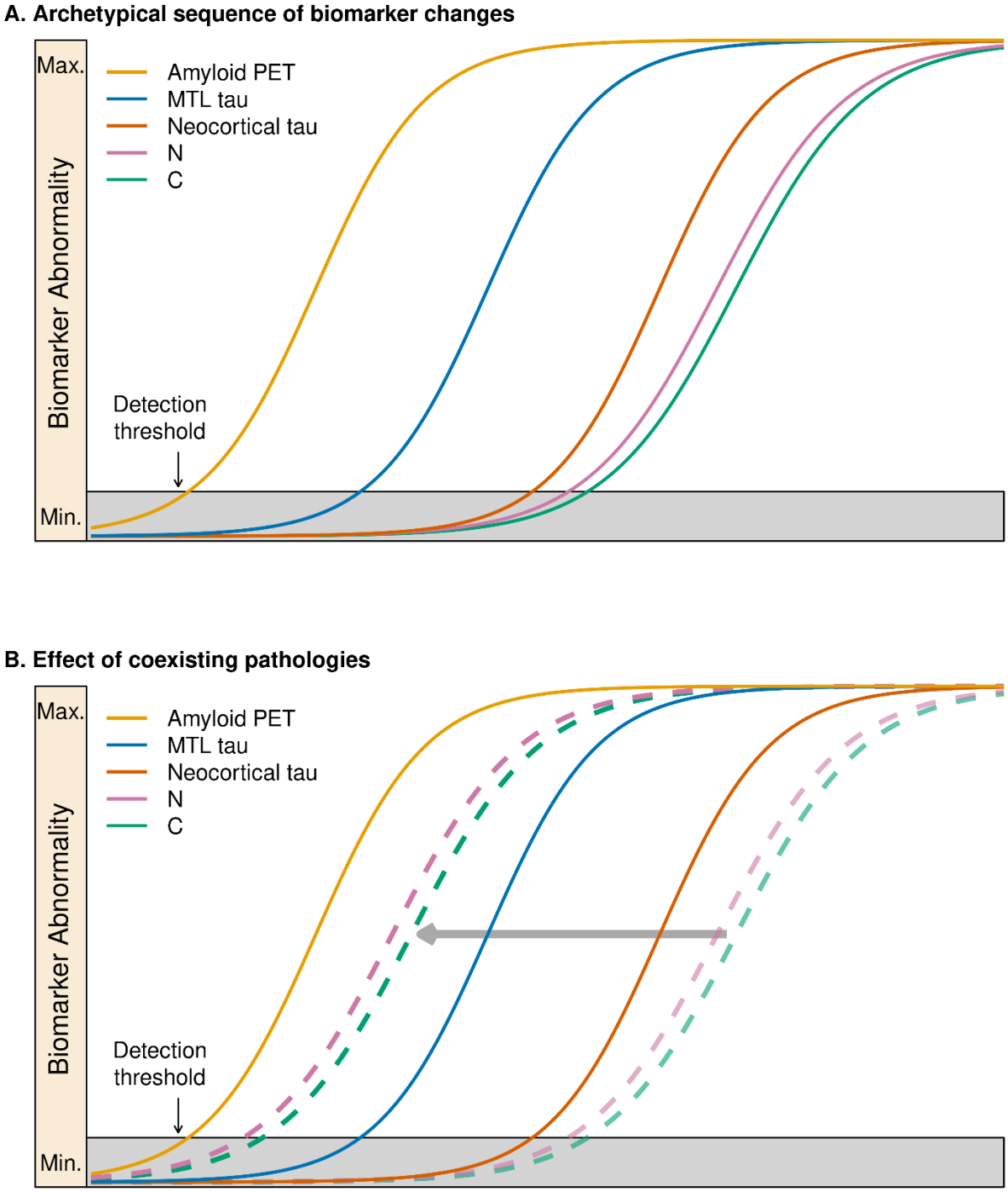

Figure 1. Staging and copathology effects. Figure 1a illustrates the prototypical temporal evolution of pure AD: sequential evolution of amyloid and tau PET followed later by neurodegeneration and clinical symptoms. Time is on the x axis and magnitude of biomarker or clinical abnormality on the y axis. Time dependent trajectories of amyloid and tau PET are

plotted and the point where a biomarker trajectory crosses the detection threshold denotes successive stages. Figure 1a illustrates an idealized evolution of AD staging biomarkers in an individual with only AD pathologic change. Figure 1b illustrates the effect of neurodegenerative co pathology in a person with biological AD stage A (i.e. A+T-) but severe neurodegeneration and clinical symptoms that are out of proportion for the degree of tauopathy. This is denoted by a leftward shift (horizontal grey arrow) of neurodegeneration and clinical symptoms relative to the pure AD temporal sequence. It is entirely possible that an individual may initially present as in Figure 1b but then later exhibit significant tauopathy due to interim progression of AD.

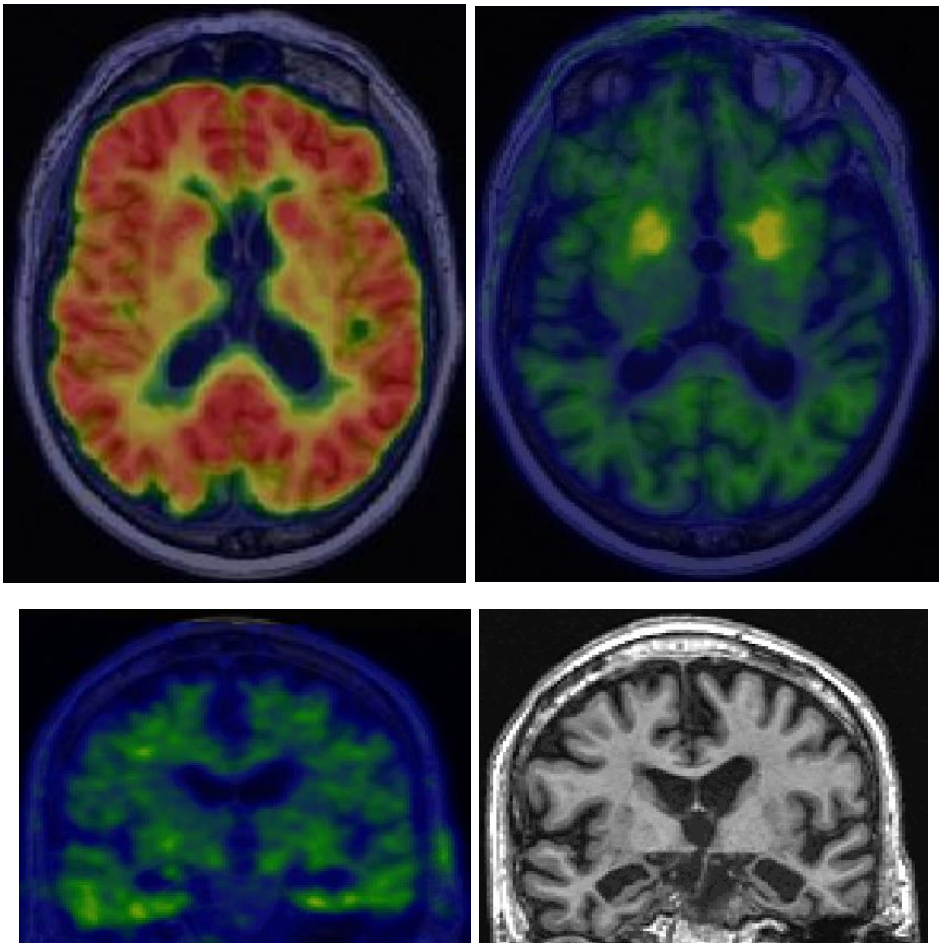

Figure 2. Co pathology and TN mismatch

89 yo man with slowly progressive amnestic dementia. He carried a clinical diagnosis of probable AD for several years and was receiving symptomatic treatment. When ATN imaging was done, however, this revealed an abnormal amyloid PET scan (top left panel) but an unremarkable tau PET scan (top right and bottom left) that was insufficiently abnormal to explain the degree of atrophy or cognitive impairment. (Tau PET color scale reference is provided visually by the off-target uptake in the basal ganglia, top right). The MR scan (bottom right) showed marked bilateral hippocampal atrophy that was consistent with the cognitive impairment but inconsistent with the level of tauopathy (i.e., TN mismatch). The A+T-N+ biomarker profile along with the atrophy pattern on MR suggested that the patient should have comorbid AD and LATE disease.