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What is T+? A Gordian Knot of Tracers, Thresholds & Topographies

Victor L Villemagne,[1,2,3] Brian J Lopresti,[4] Vincent Doré,[5,6] Dana Tudorascu,[1] Milos D. Ikonomovic,[1,7] Samantha Burnham,[6,8] Davneet Minhas,[4] Tharick A Pascoal,[1] N Scott Mason,[4] Beth Snitz,[1] Howard Aizenstein,[1] Chester A Mathis,[4] Oscar Lopez,[1] Christopher C Rowe,[2,5] William E Klunk[1,7] and Ann D Cohen.[1]

1. Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
2. Department of Medicine, The University of Melbourne, Melbourne, VIC, Australia.
3. School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia
4. Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
5. Department of Molecular Imaging & Therapy, Austin Health, Melbourne, VIC, Australia.
6. CSIRO Health & Biosecurity, Melbourne, Victoria, Australia.
7. Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
8. Center for Alzheimer Research and Treatment, Brigham and Women's Hospital Massachusetts General Hospital, Boston, MA, USA.

Corresponding author: Victor L Villemagne
Address: Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara St. Pittsburgh PA, 15213, USA.
Tel: +1 412-246-6010
Email: victor.villemagne@pitt.edu

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ABSTRACT

In this review we examine, in the context of the AT(N) framework, the available evidence as well as the potential alternatives on how to establish tau-positivity (T+) for multiple tau imaging tracers in order to come to a consensus for "normal" and "abnormal" tau imaging values that can be universally implemented in clinical research and therapeutic trials.

INTRODUCTION

Aggregates of amyloid-β (Aβ) and tau contribute to hallmark pathologies of Alzheimer's disease (AD), which start to accumulate in the brain long before clinical symptom onset and can be assessed and quantified non-invasively with positron emission tomography (PET). The introduction of radioligands for Aβ and tau has allowed for detection and monitoring of AD pathology progression, while providing critical diagnostic and prognostic information. As is the case for Aβ imaging, multiple PET tracers exist for tau imaging having different pharmacokinetic characteristics, as well as different specific, non-specific and "off-target" binding. (Supplemental Table 1) These differences lead to difficulties in interpreting imaging results, comparing between different tracers and use sites, and defining standard criteria for considering a certain tau PET level "abnormal".

Imaging and fluid tau biomarkers have been incorporated to the new Amyloid, Tau and Neurodegeneration (AT(N)) framework (*1*) that requires all the continuous biological variables to be expressed in a binary categorical classification. A requisite for this transformation from continuous to categorical variables is the establishment of a cut-off demarcating normal from abnormal values. Setting thresholds of tau abnormality has been relatively easier when dealing with biofluids, especially when using fully automated platforms (*2*). Tau imaging poses several challenges because location of the tau aggregates is crucial, so it is important not only to establish the level of pathology and/or the rates of change over time, but also to determine *where* this is happening, in terms of regional and potentially cellular localization (intracellular –neuronal, glial– or extracellular – neuropil threads, ghost tangles–), and interpret the results with full knowledge of the strengths and limitations of the tools (tracers and scanners) being employed.

For a categorical classification to be applied in a similar way across multiple sites, especially if this classification is going to be used for inclusion in therapeutic trials, requires standardization and a certain consensus on both the target and reference region brain areas to be sampled and the threshold(s) to be used. The establishment of these thresholds raises epistemological concerns in regards to tau, primarily based on what role we believe tau pathology plays in AD. Considerations include, among other things, disease progression, brain volumetrics and region-specific neuronal circuits subserving cognitive function, as well as where and at what stage of the AD continuum tau exerts such effects,

both by itself and/or intertwined with potential synergistic, potentiating or antagonistic interactions with other coexisting factors (e.g. Aβ, neuroinflammation, etc.). The selection of thresholds will be strongly swayed or coloured by these beliefs.

In the conduct of biomedical research, it is typically advantageous to record observations using continuous variables in order to better understand the sometimes nuanced pathophysiological interactions, or lack thereof, between the different variables, but as with any other clinical biomarkers (cholesterol, blood pressure, plasma glucose, etc.) it is crucial to establish a threshold of "abnormality" in order to classify or characterize the individual patient, because this has therapeutic and prognostic implications. In that sense the AT(N) framework is not different from the TNM (Tumor, Node, and Metastasis) classification used in oncology (*3*). As important as establishing area(s)-specific threshold(s), it is also important to define the purpose for which that categorization will be used. The use of categorical values requires pondering ways to first harmonize PET imaging data that will allow the generation of highly robust, compatible, and reproducible imaging measurements across multiple sites. Once basic agreement is achieved in regards to area(s) to be sampled, universal or tracer-specific levels of "normality/abnormality" can be established. In the context of the AT(N) framework, we aim to examine the available evidence, as well as potential alternatives on how to establish tau-positivity (T+) for multiple tracers in order to come to a consensus for "normal" and "abnormal" values that can be implemented in clinical research and therapeutic trials. That is the objective of this review.

TOPOGRAPHY

The topographical distribution of pathologic tau deposits in the brain is crucial: (*4*) it defines pathological subtypes (*5*), (Supplemental Figure 1) it is correlated with and mediates cognitive decline (*6,7*), as well as clinical phenotype (*8*) and patterns of brain atrophy (*9*). Tau deposits are also a robust predictor of neurodegeneration as assessed by glucose metabolism and/or grey matter atrophy (*10,11*).

Achieving a consensus on defining T+ will require, as has been done with Centiloids (*12*), the use of a "universal" mask either focusing on one or two key regions or a composite of several regions that allows the robust and reproducible identification of *early* tau deposition. The same applies to the reference region and most research centers agree in using a cerebellar cortex mask, that avoids the still

unexplained tracer retention often observed at the head of the cerebellar vermis and spill over from the occipital lobe, but one that should also avoid the most inferior aspects of the cerebellum in order to minimize partial volume effects as well as the problems associated with being close to the edge of the field of view (fewer coincidence events, body scatter, scatter correction, etc.) (*13*). There are several aspects related longitudinal changes that should also be taken into account when selecting the topographical sampling, especially if tau imaging is used as predictor of: 1. future tau accumulation, and/or 2. clinical progression and cognitive decline. For example, the region(s) selected should be able to reliably detect longitudinal tau accumulation. While entorhinal cortex (EC) and the posterior cingulate show early tau deposition, accumulation in the EC slows down at as disease progresses, while accumulation in the posterior cingulate continues and in the lower aspects of the temporal lobe increases (*14*).

Early brain tau deposition that occurs in the brain before the onset of cognitive symptoms associated with AD, is often found in the medial temporal lobe (MTL). A logical corollary of this observation would seem to be that the MTL should be the region selected for determining T+ (*15*). The problem is that, while MTL tau is high in AD, there is also a slow Aβ-independent and age-related accumulation of tau in the MTL known as primary age-related tauopathy (PART) (*16*). Therefore, selecting exclusively the MTL might lead to an overclassification as T+ of normal, elderly individuals who might not be at risk of tau neocortical spreading and developing AD.

While the distribution of tau deposits in AD as measured by PET usually follows the stereotypical distribution described by Braak and Braak (*17*), or in much more lobar detail by Delacourte (*18*), (Figure 1B) the cortical signal from tau imaging studies, especially at the early stages, tends to be quite asymmetric and less stereotypical (*19*). (Figure 1A) Furthermore, the volume of the regions that define Braak stages I-II are relatively small, and more susceptible to partial volume effects (PVE) as well as signal spill-in from adjacent structures such as the optic nerve and from "off-target" binding observed with some tracers in the choroid plexus (*13,19*). In contrast, the regions that define Braak stages V-VI are relatively large, "diluting" any small, focal, cortical signal and less reliably capturing the inter-subject variability observed at the AD stage (*19*). Moreover, the Braak and Delacourte parcellations usually tend to over- or under-represent regions that are clinically relevant in predicting disease progression, cognitive decline or grey matter atrophy.

In contrast to the approach used with Centiloids, no tau tracer can be used as gold standard because all of them differ in the degree of specific and non-specific binding, have different regional "off-target" retention, or have not yet been fully characterized (*20*). (Supplemental Table 1) In order to circumvent this, a "universal" tau mask reflecting the areas that are different between Aβ+ AD and Aβ- elderly controls and common to ALL tau tracers is being constructed (*21*), over which different region of interests (ROI) or a composite ROI could be sampled. The tau mask derived from those areas that are high in Aβ+ AD *and* common to all tracers, will minimize dilution of the signal and allow referral to the same exact region(s) in the brain irrespective of tracer and use site (*21*).

For example, for the detection of early tau deposition, the temporal meta-region (TMR) proposed by Jack and colleagues (*14*) provides an optimal balance by including both MTL (amygdala, parahippocampal gyrus and entorhinal cortex) and neocortical regions (fusiform, inferior and middle temporal gyri) and has been shown to yield the highest discriminatory accuracy between AD and non-AD neurodegenerative conditions, outperforming the Braak parcellation (*22*). A modified version of the TMR might include the hippocampus proper, while also adding the supramarginal gyrus which is not only an area of early neocortical tau deposition, but that has been very recently reported as an area of early Aβ deposition (*23*). The TMR is well-suited to document the transition from PART to AD, while also capturing the distinctive regional patterns described for the different tau pathological subtypes in AD (*5,24*). (Supplemental Figure 1) However, the TMR might not fully capture some atypical presentations such as posterior cortical atrophy (PCA) (*8*). The TMR is also a composite well-suited for the detection of longitudinal changes in tau. That said, the use of this TMR does not preclude its decomposition into MTL and neocortical indices, or the use of other regions to examine their effect on different cognitive domains or neurodegeneration (*7,11*). Optimally, the region(s) selected for definition of one or more T+ cut-offs should capture both PART as well as the earliest evidence of neocortical tau deposition that can be clearly associated with the pathological process of AD.

TRACERS

Currently several selective tau PET tracers have advanced to investigational human studies (see Supplemental Table 1) and one, ^{18}F-flortaucipir (FTP), has been approved by the FDA for diagnostic use. Although most of these tracers were designed for 3R/4R paired helical filaments –the most

prevalent isoform conformation in AD–, differences in their molecular structures lead to different tau binding affinities, in vivo kinetic behaviour, levels of non-specific binding, different patterns of "off-target" binding and hence, inconsistencies in PET-derived measurements. Additionally, variations in scanning protocols and quantification pipelines, further increases inconsistencies, decreasing reproducibility. While a better characterization of the tau tracers will allow optimization of scanning protocols and injected radioactivity dosing regimens, there are other factors that are more relevant for the establishment of T+. For the purposes of this commentary, we set aside the important issue that most of these tau tracers do not reach apparent steady state during the scanning period and focus on the use of tissue ratios.

When assessing tau images visually, and more specifically at the AD stage, there are no significant differences in the relative regional distribution of the "tau-specific" signal among tau tracers. However, there are substantial differences in the signal-to-noise ratios, dynamic range, non-specific binding, as well as differences in the patterns of "off-target" binding. These non-tau signals are very noticeable in visual reads and can make the relatively consistent tau-specific signal more or less apparent across tracers.

Not all tau tracers are created equal, but they are all affected by different degrees of "off-target" binding in areas such as the choroid plexus, basal ganglia, longitudinal sinuses or meninges (*25-27*), and some of them showed strong binding to MAO-B (*28*). (Supplemental Table 1) Moreover, some of these tracers present high non-specific binding likely precluding detection of low levels of tau deposition (*29*), which might explain why FTP has very high accuracy in detecting Braak stages V-VI (*30*), but appears to be much less accurate in detecting earlier Braak stages. It is therefore important to determine how much of the *in vivo* signal is attributable to non-specific binding, as was done with FTP (*29*).

THRESHOLDS

Like any categorical variable, T+ requires and is dependent on a particular threshold. All categorical variables in medicine can be reduced to either an abnormal level (higher or lower), or an abnormal rate of change (faster or slower). Thresholds are somewhat arbitrary and can be based on

several imperfect sources of "ground truth," such as their relation to pathology or clinical outcomes (for example prognostic and/or staging), or both. Probably the most important aspect for AD biomarkers is their ability to predict the risk of clinical progression or cognitive decline, and their use for trial inclusion, target engagement or as outcome measure in clinical trials.

Thresholds may be conservative or liberal (*31*), and in the case of tau imaging, as the signal is usually so elevated at the later stages of the disease, a more liberal threshold might be more capable of capturing early cortical tau deposition. Thresholds will also depend on the application, so thresholds might not be the same for clinical diagnosis, detection of the earliest stages of presymptomatic pathology, or to determine entry into clinical trials. Thresholds will also be influenced by the relationship between Aβ and tau, like for example the presence or absence of high Aβ, since existing imaging data appears to tell us that detectable neocortical Aβ precedes detectable neocortical tau (*6,32*). Furthermore, given that these proteinopathies are strongly associated with cognitive impairment and neurodegeneration leading to dementia, the kind of threshold selected will determine staging and therefore, timing of treatments when such treatments become available (*33,34*). Considering the aforementioned factors, it seems logical that interventions early in the disease (e.g. subthreshold Aβ and pre-T+) would have the highest chance to delay or prevent onset of AD.

Several methods for establishing thresholds have been applied to imaging biomarker studies, including cluster analysis, ROC analysis, iterative outlier, z-scores, Gaussian mixture modelling, percentiles or standard deviation of a control group, reliable worsening, etc. (*35,36*). Unfortunately, there have been only a few head-to-head comparison studies, approach that might also help to establish a "universal" threshold (*27,37*). (Figure 2) Like Centiloids (*12*), tau imaging would benefit from the use of a single standardized scale (e.g. CenTauR) in which to apply a certain threshold so the use of T+ means the same in all contexts. Z-scores (*38,39*) have the advantage of being easily generated and incorporate the variance of the tracer, especially if the control group are young adults. It is also easier to agree on z-scored thresholds, and the threshold should theoretically be the same for all tau tracers. The main issue is, what kind of controls should be used to generate these Z-scores? There are two main options: use either young controls or Aβ- elderly controls. If the objective is *early* detection, the use of young controls will likely yield more sensitive thresholds (*40*). Also, specific binding is likely affected by the type of tau pathology (e.g., neurofibrillary tangles, neuropil threads, dystrophic neurites) and

tangle maturity (*25*). While each center could have its own controls, we will get closer to a universal definition of T+ if a freely accessible repository (GAAIN?) of tau imaging studies of well-characterized, gender balanced and ethnically diverse young controls or Aβ- elderly controls utilizing each tracer, acquired with different scanners, different attenuation correction and reconstruction algorithms at different centers, is created, where the larger sample size will allow to account for the differences yielding a more robust Z-score, or any other method (from percentiles to machine learning). Also, a confidence interval around the threshold might be needed to account for the differences in scanners, reconstruction and attenuation correction algorithms when implemented at different sites.

Other proposed ways to establish thresholds are to use fluid biomarkers or visual readouts. The problem of using biofluids (e.g. cerebrospinal fluid -CSF-) to establish tau -or for that matter amyloid (*41*)- imaging thresholds is that, while they both reflect the same process of protein accumulation, they represent distinct biochemical pools of that protein, one being soluble and diffusible while the other being an insoluble aggregate. This, added to the intrinsic limitations of PET (sensitivity, PVE, etc.), explains why changes in biofluids tend to precede changes in imaging (*42*). Furthermore, the correlation between tau imaging and CSF p-tau181 seems not to be very high when assessing different clinical groups separately, reinforcing the concept that they might be capturing different aspects or different biochemical pools of tau (*43*). Visual examination of the images at the late stages of the disease are usually easy to interpret because the regional distribution of tracer retention in the brain follows a quite stereotypical pattern involving MTL, the temporoparietal, lateral occipital and posterior cingulate cortices as well as the frontal lobe, predominantly in the dorsolateral prefrontal area (*26,32*). (Figure 1A) At this point we need to note that at these late stages of the disease the cortical brain distribution of the different tau tracers is quite similar. (Figure 2) While this represents the typical pathological form of AD, it is also important to remember that there are two other pathological variants: hippocampal sparing and limbic predominant (*5*). (Supplemental Figure 1) Also, tau deposits in the brain correspond to the phenotypical AD presentation, so for example PCA will present with high tracer retention in the occipital cortex (*8*). Things are more nuanced at the early stages because tracer retention tends to be more focal and asymmetric. (Figure 1A) Visually derived thresholds also tend to be subjective, easily affected by the training and expertise of the readers and can even be affected by how images are displayed. Thus, an expert's visual examination, while more sensitive and likely valid for

clinical use or, more importantly, to confirm high tau in an atypical location (PCA), it is less objective than quantification and might not be an ideal approach to *establish* an "universal" threshold.

We believe that the best approach to establish thresholds is by combining neuropathology and clinical information, always keeping in mind that neuropathology only represents a small sample of a whole brain region and could vary depending on the sampling, the type of stain or antibody used, which affect what is visible under the light microscope. Given that the purpose is early detection, it would be useful to determine what is the lowest level of pathology detectable with PET by combining neuropathology with biochemical methods in brain tissue from post-mortem studies with ante-mortem PET imaging studies. This may allow the prediction of disease progression and cognitive decline – always accounting for the presence of covariates such as Aβ, neuroinflammation and other comorbidities–, allowing the early identification of those at risk of "progression at follow up".

CODA: THE GORDIAN KNOT

While several issues discussed in this review remain to be resolved, what is clear is that we need to be able to robustly and consistently detect early tau deposition. The issue of defining T+ depends on how the above parameters are weighted. What regions of early tau deposition are more likely predictive of cognitive decline and clinical progression? Should we consider MTL and other temporal regions apart or together? Can we use a single scale for all tracers that account for the intrinsic pharmacokinetic differences they have? What is the optimal threshold? And for what purpose is T+ to be used, a trial, an observational study, clinical diagnosis?

We should take advantage of the fact that we do have a sensitive biomarker in Aβ imaging, and a very specific one in tau imaging. Tau imaging has been shown to be exquisitely specific for AD dementia (*22,44*) to the point that high tau in a severely cognitively impaired individual confirms the differential diagnosis of AD likely without the need of an Aβ scan. But there is a caveat. If the tau scan is negative it will require an Aβ scan because 15-25% of Aβ+ AD patients present subthreshold levels of tau (*22,44*) and therefore tau imaging alone cannot rule out AD. Being so specific, and associated with cognition, it might be necessary to increase the sensitivity of tau imaging in order to capture the earliest cortical tau deposition in order to prevent, when therapy becomes available, further tau accumulation.

We also need to remember that variables such as Aβ and neurodegeneration are closely intertwined and their interplay is affected by or associated with the topographical distribution of tau deposits in the brain, which in turn affects cognition (*6*). Accounting for all these variables will inform not only diagnosis and prognosis but also clinical trials.

Nowadays, we can confirm AD in vivo in those with both high Aβ and tau (*1*). But the challenge is at the other end of the spectrum, (Figure 1A) in those individuals with preclinical and prodromal AD where therapy should be started before irreversible neuronal loss ensues and thus, provide the greatest benefit. This is probably where the greatest value of a consensus on T+ lays.

T+ is also important for disease staging, participant selection and as outcome measure of anti-tau (and anti-Aβ) therapy. Instead of striving for a monotherapy, maybe – like the treatment of HIV– a combinational staggered AD therapy should aim at addressing all the variables associated with cognitive impairment: Aβ, tau, neuroinflammation, vascular, etc. once we can confirm an individual patient is at/entering that stage.

We have attempted to weigh the pros and cons of several alternatives, tried to balance the different variables in order to optimize the outcome, and then proposed an outline for a potential solution. Of course, there will be issues that either by ignorance, space or neglect were not considered, but the main function of this review is to start a conversation that will lead to a consensus on what T+ is, and how to implement it.

Aldous Huxley said: "*Good is that which makes for unity. Evil is that which makes for separateness*."[*] Paradoxically, it is imperative to find consensus around the T(+/-) dichotomy to avoid the polarizations so common these days. We need to find the common ground that leads to the maximization of what the technique(s) can deliver, both as categorical as well as continuous variables in order to translate them into clinical outcomes that will ameliorate the emotional and economic burden that AD imposes on patients, their care providers, and society.

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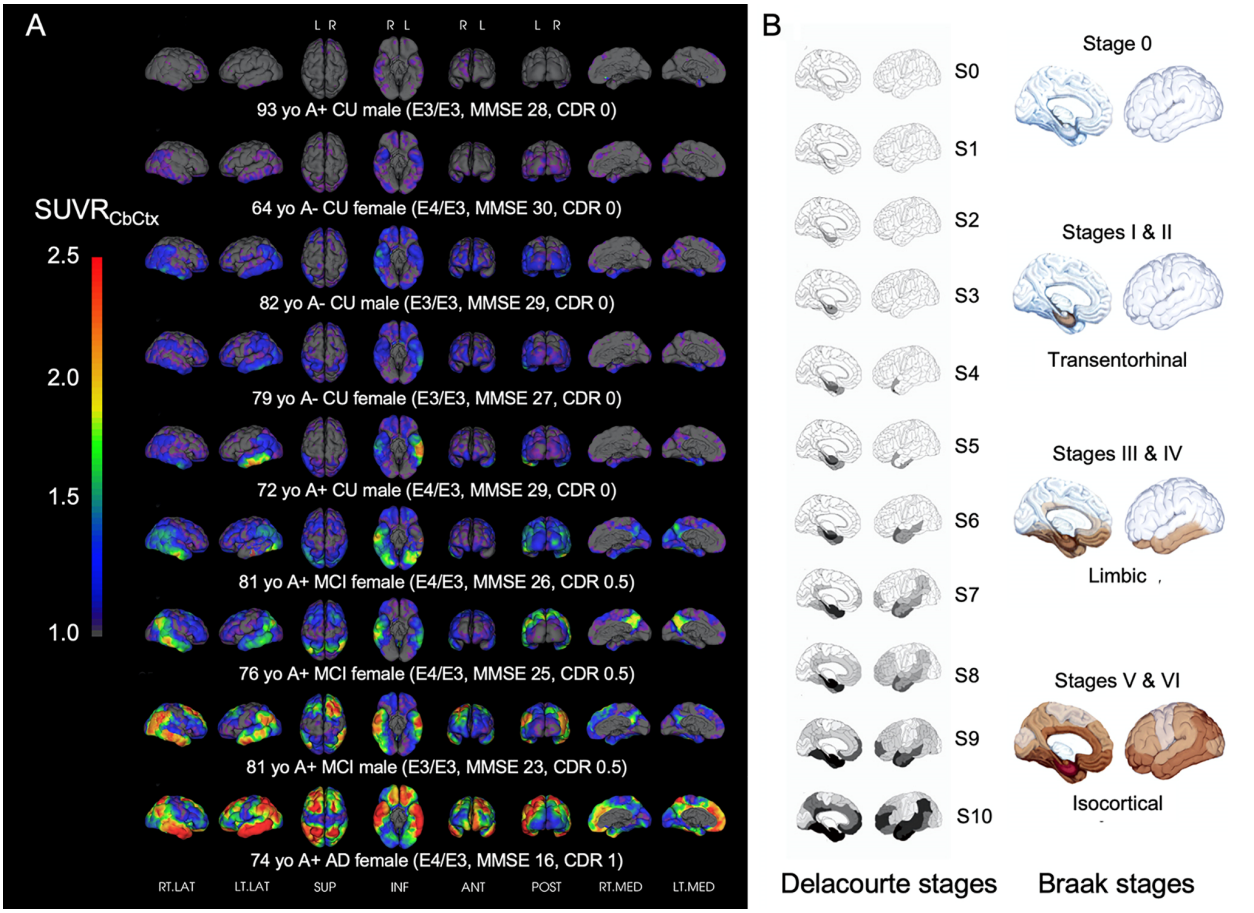
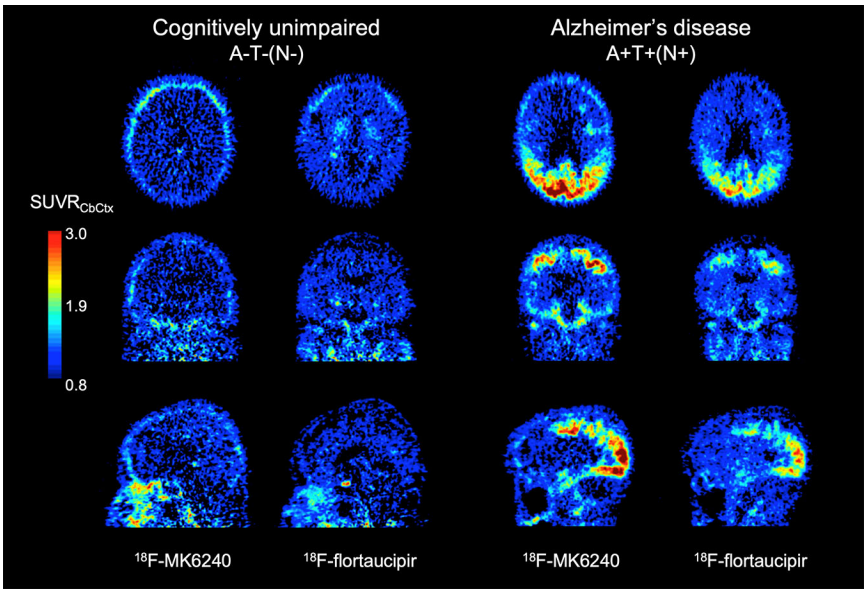
Figure 1.



A. Representative surface projections of tau imaging studies with ^{18}F-flortaucipir (FTP) along a spectrum of cases with increasing tau burdens, comprising –from top to bottom- cognitively unimpaired (CU) controls, mild cognitive impaired (MCI) subjects and Alzheimer's disease (AD) patients, along with their respective Aβ status (A- or A+), Mini Mental State Examination (MMSE), and Clinical Dementia Rating (CDR). It is unlikely to observe cortical tau in A- cases. *Images generated through CapAIBL® (capaibl-milxcloud.csiro.au) CSIRO Biomedical Imaging Group. Australia.*

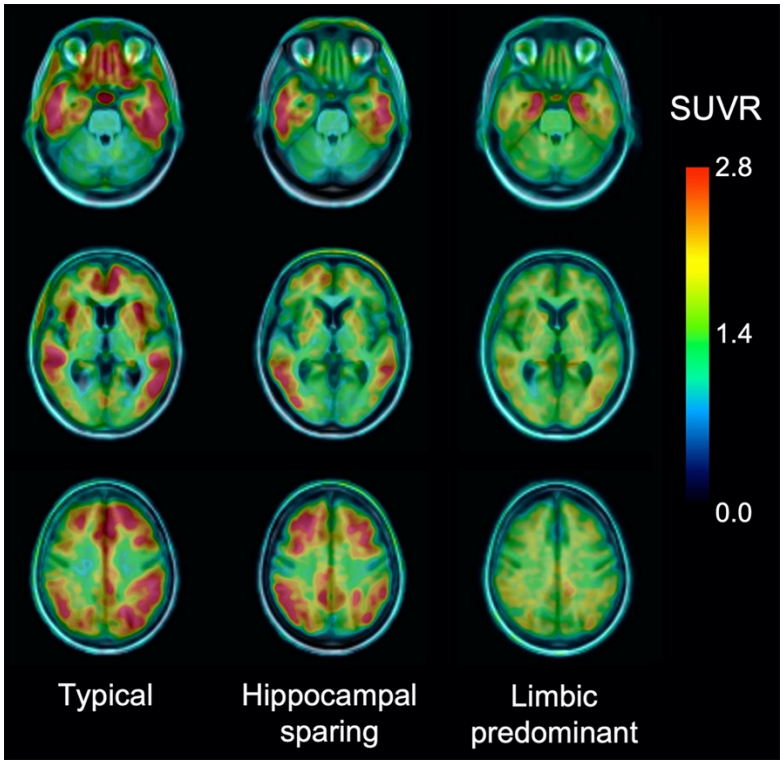
B. Stages of tau pathology according to Delacourte (Stages S0-S10) (*18*) and Braak and Braak (Stages 0-VI) (*17*). These neuropathology-based parcellations might not be suitable to accurately capture early tau deposition *in vivo*, either because the regions are too small (subject to the technical limitations of PET such as partial volume effects), or too large ("diluting" the PET signal and/or not focusing in areas of relatively early cortical tau deposition like the temporooccipital region or supramarginal gyrus)

Figure 2.



Head-to-head comparison of ^{18}F-flortaucipir (FTP) and ^{18}F-MK6240 (MK) in a cognitively unimpaired control (75 y.o. A-T-(N-) female) and an Alzheimer's disease patient (64 y.o. A+T+(N+) female). In the low tau case, there are clear differences in the off-target binding of the tracers, with MK showing tracer retention in the meninges and also some degree of defluorination where the pineal gland becomes visible; and FTP showing tracer retention in the choroid plexus, the basal ganglia and also a small amount in the meninges around te frontal lobes. In the high tau case, the regional brain distribution of the tracers is very similar, with MK showing a larger dynamic range and better contrast than FTP. More head-to-head comparisons between tau tracers will aid in developing a more robust "universal" threshold. *Images courtesy of Brian Lopresti, UPMC PET Center, Pittsburgh, PA, USA*.

SUPPLEMENTARY MATERIALS



Supplemental Figure 1.

Tau imaging studies with ^{18}F-flortaucipir PET images in severely cognitively impaired patients, two AD, one MCI, showing the three different pathological subtypes of AD: the typical presentation (left column, 76 y.o. female Aβ+ AD, MMSE 16) with widespread retention in both MTL and neocortical areas, limbic sparing (center column, 81 y.o. male Aβ+ MCI, MMSE 23) where tracer retention is primarily in the neocortex, and limbic predominant subtype (right column, 69 y.o. male Aβ+ AD, MMSE 20) with tracer retention mostly restricted to the mesial temporal cortex.

Supplemental Table 1. Tau imaging tracers used in clinical studies

Tracer	Off target binding	Non-specific/non-tau binding Other issues
^{18}F-THK523[1,2]	Basal ganglia	low signal to noise
^{18}F-T807[3-6] (a.k.a. AV1451, Flortaucipir, TAUVID)	Choroid plexus, anterior midbrain, basal ganglia (meninges)	~60% non-specific binding in A-CU
^{18}F-T808[7]	Basal ganglia, choroid plexus, anterior midbrain	Defluorination
^{11}C-PBB3[8-10@]	Longitudinal sinus, basal ganglia (choroid plexus)	@radiolabeled lipophilic metabolite (binding to other non-tau targets?)
^{18}F-THK5105/^{18}F-THK5137[11-13]	Basal ganglia, anterior midbrain	(binding to MAO-B?)
^{18}F-RO948[14-16]	Calvarium (choroid plexus, basal ganglia) anterior midbrain	(defluorination)
^{18}F-GTP1[17,18]	Choroid plexus (basal ganglia) anterior midbrain	
^{18}F-THK5351[19-21]*	Basal ganglia, anterior midbrain	*mainly binding to MAO-B
^{18}F-PI2620[22-24]	Longitudinal sinus (scalp)	
^{18}F-MK6240[25-28]	Meninges (anterior midbrain) Calvarium	(defluorination)
^{18}F-Lanzoprazole[29,30 #]		#low binding to tau in vivo
^{18}F-PM-PBB3[31]	Choroid plexus, anterior midbrain (basal ganglia)	
^{18}F- JNJ-64326067[32,33]		

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