

CLAIMS

1. A method for measuring tau in a biological sample, the method comprising
 - (a) providing a biological sample selected from a blood sample or a CSF sample, wherein the biological sample (i) optionally comprises an isotope labeled internal standard of tau, and (ii) optionally is depleted of amyloid beta, N-terminal tau, mid-domain tau, or any combination thereof;
 - (b) removing proteins from the biological sample by protein precipitation and separation of the precipitated proteins to obtain a supernatant;
 - (c) purifying tau from the supernatant by solid phase extraction;
 - (d) cleaving the purified tau with a protease and then optionally desalting the resultant cleavage product by solid phase extraction to obtain a sample comprising proteolytic peptides of tau; and
 - (e) performing liquid chromatography - mass spectrometry with the sample comprising proteolytic peptides of tau to detect and measure the amount of at least one proteolytic peptide of tau.
2. The method of claim 1, wherein the biological sample is depleted of amyloid beta, N-terminal tau, mid-domain tau, or any combination thereof.
3. The method of claim 1, wherein the solid phase in step (c) comprises a reversed-phase sorbent that adsorbs tau.
4. The method of any one of the preceding claims, wherein:
 - step (b) comprises admixing an acid to precipitate proteins of the biological sample, optionally wherein the acid is perchloric acid; and/or
 - in step (e), the liquid chromatography - mass spectrometry is performed by a nano-LC/MS system.

5. A method for measuring tau in a biological sample, the method comprising

(a) decreasing in a biological sample by affinity depletion N-terminal tau, mid-domain tau, or N-terminal tau and mid-domain tau, and optionally decreasing by affinity depletion amyloid beta, wherein the biological sample is a blood sample or a CSF sample and the biological sample optionally comprises an isotope-labeled, tau internal standard;

(b) enriching MTBR tau by a method that comprises (i) removing additional proteins from the biological sample by protein precipitation and separation of the precipitated proteins to obtain a supernatant, and then purifying tau from the supernatant by solid phase extraction, or (ii) affinity purifying MTBR tau, thereby producing by either (i) or (ii) enriched MTBR tau;

(c) cleaving the enriched MTBR tau with a protease and then optionally desalting the resultant cleavage product by solid phase extraction to obtain a sample comprising proteolytic peptides of tau; and

(d) performing liquid chromatography-mass spectrometry (LC/MS) of the sample comprising proteolytic peptides of tau to detect and measure the amount of at least one proteolytic peptide of tau.

6. The method of claim 5, wherein the biological sample comprises human tau and wherein step (a) further comprises

contacting the biological sample with at least one epitope-binding agent that specifically binds to an epitope within amino acids 1 to 243 of tau-441, inclusive; or

contacting the biological sample with a first epitope-binding agent that specifically binds to an epitope within amino acids 1 to 103 of tau-441, inclusive, and a second epitope-binding agent that specifically binds to an epitope within amino acids 104 to 243 of tau-441, inclusive; or

contacting the biological sample with a first epitope-binding agent that specifically binds to an epitope within amino acids 1 to 103 of tau-441, inclusive, a second epitope-

binding agent that specifically binds to an epitope within amino acids 104 to 243 of tau-441, inclusive; and a third epitope binding agent that specifically binds to an epitope of amyloid beta; or

contacting the biological sample with a first epitope-binding agent that specifically binds to an epitope within amino acids 1 to 103 of tau-441, inclusive, a second epitope-binding agent that specifically binds to an epitope of amyloid beta; or

contacting the biological sample with a first epitope-binding agent that specifically binds to an epitope within amino acids 104 to 243 of tau-441, inclusive; and a third epitope binding agent that specifically binds to an epitope of amyloid beta.

7. The method of claim 6, wherein the epitope-binding agent that specifically binds to amyloid beta is HJ5.1, the epitope-binding agent that specifically binds to an epitope within amino acids 1 to 103 of tau-441, inclusive, is HJ8.5, and the epitope-binding agent that specifically binds to an epitope within amino acids 104 to 243 of tau-441, inclusive, is Tau1.

8. The method of claim 5, wherein the epitope binding agent of step (b) specifically binds to an epitope

within amino acids 221 to 441 (inclusive) of tau-441, or

within amino acids 235 to 441 (inclusive) of tau-441, or

within amino acids 235 to 368 (inclusive) of tau-441, or

within amino acids 244 to 368 (inclusive) of tau-441, or

within amino acids 244 to 299 (inclusive) of tau-441.

9. The method of claim 8, wherein the epitope-binding agent is an antibody selected from the group consisting of 77G7, RD3, RD4, UCB0107, PT76, E2814 and 7G6.

10. The method of claim 5, wherein step (d) comprises detecting and measuring the concentration at least one proteolytic peptide of MTBR tau, wherein the proteolytic

peptide of tau has an amino acid sequence chosen from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

11. A method for measuring Alzheimer disease (AD)–related pathology in a subject, the method comprising measuring tau in a CSF or blood sample according to a method of any one of claims 1 to 9,

wherein the tau measured are MTBR tau species comprising the amino sequence of SEQ ID NO: 3, MTBR tau species comprising the amino sequence of SEQ ID NO: 6, MTBR tau species comprising the amino sequence of SEQ ID NO: 7, MTBR tau species comprising the amino sequence of SEQ ID NO: 8, or a combination thereof, wherein the amount of the measured MTRB-tau species or their ratios is a representation of AD–related pathology in a brain of a subject.

12. A method for diagnosing Alzheimer’s disease, the method comprising

measuring tau in a CSF or blood sample according to a method of any one of claims 1 to 9, wherein the tau measured are MTBR tau species comprising the amino sequence of SEQ ID NO: 3, MTBR tau species comprising the amino sequence of SEQ ID NO: 6, MTBR tau species comprising the amino sequence of SEQ ID NO: 7, MTBR tau species comprising the amino sequence of SEQ ID NO: 8, or a combination thereof; and

diagnosing Alzheimer’s disease when the quantified MTBR tau species differs by about 1.5σ or more, where σ is the standard deviation defined by the normal distribution measured in a control population that is amyloid negative as measured by PET imaging and/or A β 42/40 measurement in CSF.

13. A method for measuring Alzheimer disease (AD) progression in a subject, the method comprising

measuring tau in a first and a second CSF or blood sample according to a method of any one of claims 1 to 9, wherein the tau measured are MTBR tau species comprising

the amino sequence of SEQ ID NO: 3, MTBR tau species comprising the amino sequence of SEQ ID NO: 6, MTBR tau species comprising the amino sequence of SEQ ID NO: 7, MTBR tau species comprising the amino sequence of SEQ ID NO: 8, or a combination thereof; and

calculating the difference between the quantified MTBR tau species in the second sample and the first sample, wherein a statistically significant increase in the quantified MTBR tau species in the second sample indicates progression of the subject's Alzheimer's disease.

14. The method of claim 11 or claim 13, wherein the subject is amyloid negative.

15. The method of claim 14, wherein the subject has no dementia.

16. The method of claim 14, wherein the subject has dementia.

17. The method of claim 11 or claim 13, wherein the subject is amyloid positive.

18. The method of claim 17, wherein the subject has no dementia.

19. The method of claim 17, wherein the subject has dementia.

20. The method of any one of claims 11 to 19, the method further comprising quantifying amyloid beta, quantifying N-terminal tau, quantifying mid-domain tau, quantifying post-translational modifications of tau, or identifying an ApoE isoform in the biological or CSF sample.

21. The method of claim 20, wherein quantifying amyloid beta includes quantifying an Ab40/42 value, and quantifying post-translational modifications of tau include quantifying one or more of phospho-tau217, phospho-tau205, phospho-tau181, phospho-tau153, phospho-tau111, and phospho-tau208

22. A method for measuring tau pathology in a brain of a subject, the method comprising measuring tau in a CSF or blood sample according to a method of any one of claims 1 to 9, wherein the tau measured are MTBR tau species comprising the amino

acid sequence of SEQ ID NO: 2, MTBR tau species comprising the amino sequence of SEQ ID NO: 3, MTBR tau species comprising the amino sequence of SEQ ID NO: 4, MTBR tau species comprising the amino sequence of SEQ ID NO: 5, MTBR tau species comprising the amino acid sequence of SEQ ID NO: 6, MTBR tau species comprising the amino sequence of SEQ ID NO: 7, MTBR tau species comprising the amino sequence of SEQ ID NO: 8, MTBR tau species comprising the amino sequence of SEQ ID NO: 9, or combinations thereof, wherein the amount of the quantified MTRB-tau species or their ratios is a representation of tau pathology in a brain of a subject.