CASE REPORT

Tau seeding in cases of multiple sclerosis

Michael S. LaCroix¹, Hilda Mirbaha¹,², Ping Shang², Stephanie Zandee³,⁶, Chan Foong², Alexandre Prat³,⁶, Charles L. White III², Olaf Stuve⁴,⁵ and Marc I. Diamond¹,⁴*

Abstract

Relapsing remitting multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system that in many cases leads to progressive MS, a neurodegenerative disease. Progressive MS is untreatable and relentless, and its cause is unknown. Prior studies of MS have documented neuronal accumulation of phosphorylated tau protein, which characterizes another heterogeneous group of neurodegenerative disorders, the tauopathies. Known causes of tauopathy are myriad, and include point mutations within the tau gene, amyloid beta accumulation, repeated head trauma, and viral infection. We and others have proposed that tau has essential features of a prion. It forms intracellular assemblies that can exit a cell, enter a secondary cell, and serve as templates for their own replication in a process termed “seeding.” We have previously developed specialized “biosensor” cell systems to detect and quantify tau seeds in brain tissues. We hypothesized that progressive MS is a tauopathy, potentially triggered by inflammation. We tested for and detected tau seeding in frozen brain tissue of 6/8 subjects with multiple sclerosis. We then evaluated multiple brain regions from a single subject for whom we had detailed clinical history. We observed seeding outside of MS plaques that was enriched by immunopurification with two anti-tau antibodies (HJ8.5 and MD3.1). Immunohistochemistry with AT8 and MD3.1 confirmed prior reports of tau accumulation in MS. Although larger studies are required, our data suggest that progressive MS may be considered a secondary tauopathy.

Keywords: Multiple sclerosis, Tauopathy, FRET biosensor, Tau seeding activity, tau, prion, propagation, neurodegeneration

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system that in many cases leads to neurodegeneration [1]. MS most commonly presents with a relapsing–remitting phenotype (RRMS), in which clinical findings often coincide with magnetic resonance imaging (MRI) abnormalities. RRMS responds to a variety of immunosuppressive treatments [2, 3]. However, a substantial fraction of RRMS patients transition to a secondary-progressive neurodegenerative phase that lacks signs of acute inflammation, and does not respond to immunosuppression. A minority of cases present with a chronic progressive course at the outset [2, 3]. Epidemiological evidence supports a primary neurodegenerative process shared in all cases of MS [4, 5]. The cause of progressive MS is unknown, but prior reports have documented accumulation of phosphorylated forms of the microtubule-associated protein tau [6, 7].

The intracellular accumulation of tau assemblies, or aggregates, underlies myriad disorders collectively known as “tauopathies” [8]. Many tauopathies feature detergent-insoluble filaments, which are composed of distinct, disease-associated structures [9, 10]. Considerable experimental evidence indicates that prion mechanisms underlie the progression of neurodegenerative tauopathies, whereby pathological assemblies that form in one cell exit to gain entry to connected neurons, and thereby propagate disease through specific brain...
networks in a process termed “seeding” [11]. Unique tau assembly structures, termed “strains,” propagate in vivo by serving as templates for their own replication, dictate rates of progression and neuronal vulnerability in mouse models, and thus likely account for phenotypic variability in humans [9, 12]. Seed detection has been facilitated by the development of specialized “biosensor” cell lines that express the tau repeat domain (which forms the core of amyloid assemblies) containing a single disease-associated mutation (P301S) fused to fluorescent proteins (Fig. 1A). When tau seeds enter biosensor cells, they initiate aggregation of tau, which is then quantified via flow cytometry based on fluorescence resonance energy transfer (FRET). Biosensor cells are highly sensitive and specific for tau pathology [13, 14], and have been used by our group and many others to quantify levels of pathological tau in a variety of disease states [15–18]. Recently, we have augmented the sensitivity of detection through development of conformation-specific antibodies that preferentially bind seed-competent forms of tau—revealing seeding otherwise undetectable by detergent fractionation or phospho-tau antibody staining [19, 20]. MD3.1, which was created by mimicking a neoepitope from seed-competent tau monomer [21], preferentially binds tau seeds after immunoprecipitation or immunostaining of tauopathy brain [19]. Several studies have described tau pathology in progressive MS, as measured by immunohistochemistry to detect phospho-tau [6, 7, 22]. Insoluble tau has been reported only in progressive MS, but not in relapsing MS. These findings may reflect the cause of neurodegeneration [6, 7], however no prior studies have evaluated MS brain for the presence of tau seeds. We hypothesized that if tau mediates neurodegeneration in MS, seeds should be present.

To address this question, we initially analyzed brain homogenates from 8 MS subjects, enriching for seeding by immunoprecipitation with MD3.1. We detected seeds in 6/8 cases (Fig. 1B). Information about the subjects and the regional source of samples is available (Additional file 1: Table 1). Both forms of MS had detectable seeds: 3/3 cases of RRMS, and 3/5 cases of SPMS.

To better understand the relationship between MS plaques and tau seeding, we prepared homogenates from plaque-bearing and adjacent brain tissues from a deceased 52 year old female subject with a 19 year history of RRMS that was well controlled until a rapid decline. Axial FLAIR MRI images indicated abnormalities in the periventricular white matter, corpus callosum, cortical gray matter, and brainstem (Fig. 2A).

Upon neuropathological examination, we observed characteristic findings of active phase demyelinated plaque formation in plaque-bearing regions (Fig. 2B). We also observed evidence of pathological tau accumulation based on immunoreactivity with anti-tau monoclonal antibodies AT8 and MD3.1 (Figs. 2C–J).

We next tested soluble brain homogenates for seeding using tau biosensor cells. We detected no seeding in homogenates from plaque-bearing tissue. By contrast, we easily detected seeding from soluble homogenates...
distinct conformation, but this will require further study. S1B–D). This could indicate that tau seeds in MS have a
from MS was relatively similar to AD, but one monoclo-
overall pattern of tau immunoprecipitation efficiency
transcellular propagation of pathology mediated by tau
expression, there is no effective treatment. In tauopathies,
contrast to RRMS, which is highly responsive to immunosup-
precipitate tau seeds (Additional file 2: Fig. S1A). The
tested a panel of antibodies for their ability to immuno-
differentially bind different seed conformers [20]. We
distinct epitopes, especially within the repeat domain,
mouse models [12]. Anti-tau antibodies directed against
missible pathology upon inoculation into experimental
parietal cortices (Fig. 2L).

In summary, we detected tau seeding activity and evi-
dence of pathological tau accumulation in tissues adja-
cent to MS plaques, whereas the plaques themselves had
no seeding activity and minimal evidence of phospho-tau
accumulation. This could be because the neuronal con-
ent of plaques was reduced due to gliosis. MD3.1 effi-
ciently enriched tau seeds from plaque-adjacent regions
and detected disease-associated tau accumulation based
on immunohistochemistry. Our findings in this regard
are consistent with prior reports of tau accumulation in
MS brain [6, 7, 22].

Distinct tau strains are associated with different
tauopathies [9, 10], and create unique patterns of trans-
missible pathology upon inoculation into experimental
mouse models [12]. Anti-tau antibodies directed against
distinct epitopes, especially within the repeat domain,
differentially bind different seed conformers [20]. We
tested a panel of antibodies for their ability to immuno-
cipitate tau seeds (Additional file 2: Fig. S1A). The
overall pattern of tau immunoprecipitation efficiency
from MS was relatively similar to AD, but one monoclo-
inal antibody (MD6.1) failed to bind seeds of the MS brain
homogenate, while it did so for AD (Additional file 2: Fig.
S1B–D). This could indicate that tau seeds in MS have a
distinct conformation, but this will require further study.

The cause of progressive MS is unknown, and in con-
trast to RRMS, which is highly responsive to immuno-
suppression, there is no effective treatment. In tauopathies,
transcellular propagation of pathology mediated by tau
seeds has been proposed as a cause of disease progres-
several findings provide evidence of an association with other inflammatory CNS diseases such as post-encephalitic Parkinsonism, Nodding syndrome, chronic traumatic encephalopathy, and subacute sclerosing panencephalitis [23–27]. Future studies will be required to test more definitively a causal relationship between inflammation and tauopathy.

**Methods**

**Biosensor cell line**

Sensitive second-generation tau biosensor cells termed v2H [13] were used for seeding assays. These cells are based on expression of tau repeat domain fragment (246–378) containing the disease-associated P301S mutation (tau-RD) fused to mCerulean3 or mClover3. The v2H line was selected for high expression with low background signal and high sensitivity. Seeding experiments used previously established protocols [14].

**Cell culture**

v2H biosensors were grown in Dulbecco’s Modified Eagle’s medium (Gibco) supplemented with 10% fetal bovine serum (HyClone), and 1% glutamax (Gibco). For terminal experiments, 1% penicillin/streptomycin (Gibco) was included. Cells were tested free of myco-
plasma (VenorGem, Sigma) and cultured at 37 °C with
5% CO2 in a humidified incubator. To avoid false posi-
tive signal from v2H biosensors, cells were passaged prior
to ~ 80% confluency.

**Human brain samples**

At the CRCHUM, fresh frozen human brain tissue was
obtained from patients diagnosed with clinical and
neuropathological MS according to the revised 2010
McDonald’s criteria [28]. Tissue samples were collected

---

**Fig. 2** Anatomic distribution of tau seeding in an MS subject. The brain of an MS subject was preserved frozen, and then dissected to the indicated regions. Indicated areas were fixed for immunohistochemistry. For the tau seeding assay, unfixed tissue was homogenized to create
total clarified lysate [10% (wt/vol)] followed by immunoprecipitation with MD3.1 to enrich for tau seeds. A Axial FLAIR MRI antemortem images showed extensive demyelination. B Periphery of an MS plaque was stained with Luxol fast blue-PAS-hematoxylin, showing preserved myelin in adjacent brain (left) and loss of myelin within the plaque (right). The plaque also contained abundant macrophages, and the interface (indicated with arrowheads) between plaque and adjacent brain contained many swollen axons. Scale bar = 1 mm. (C–J) Tau immunohistochemistry (AT8 and MD3.1) in plaque-adjacent brain regions. Scale bars = 50 µm. C, D temporal lobe, showing a collection of AT8-immunoreactive neurophil threads and MD3.1-immunoreactive tangle-like structures in 2 neurons; E, F parietal lobe, showing MD3.1-immunoreactive structures at the periphery of a plaque (consistent with swollen axons), but no AT8 immunoreactivity; G, H hippocampus, showing AT8-immunoreactive neurophil threads in entorhinal cortex, which are MD3.1-negative; and I, J substantia nigra, showing sparse AT8-immunoreactive neurophil threads, but no focal MD3.1
immunoreactivity. K Tau seeding in total clarified lysate from various regions of an MS brain. L Tau seeding in pellets after immunoprecipitation with MD3.1. Columns represent the mean % FRET positive cells from three technical replicates (dots). Statistical significance was determined by performing one-way ANOVA followed by Dunnett’s multiple comparisons testing of all samples vs. Lipofectamine treated negative controls, *** = p < 0.001, **** = p < 0.0001. Errors bars = S.D.
Fig. 2 (See legend on previous page.)
from MS patients with full ethical approval (BH07.001, Nagano 20.332-YP) and informed consent as approved by the local ethics committee. At UT Southwestern, human brain tissue was obtained from a 52 year old female subject with 19 year history of multiple sclerosis with Institutional Review Board approval at University of Texas Southwestern Medical Center. Informed written consent for donation of tissue was obtained from next of kin prior to collection. The brain was sectioned, tissue from each region was separated into two, with one half being flash frozen in liquid nitrogen for biochemical analysis and the other half fixed in formalin and processed to paraffin for histological analysis and immunohistochemical staining. Fresh frozen tissue was used to prepare total soluble protein lysates for further experiments.

**Human sample preparation**

Fresh frozen pulverized tissue was suspended in tris-buffered saline (TBS) containing complete mini protease inhibitor tablet (Roche) at 10% w/vol. Samples were then dounce homogenized, followed by pulsing probe sonication at 75 watts for 10 min (Q700, Qsonica) on ice in a hood. The sonication probe was washed with a sequence of ethanol, bleach, and distilled water to prevent cross-contamination of seeds. Lysates were then centrifuged at 23,000 × g for 30 min and the supernatant was retained as the total soluble protein lysate. Protein concentration was measured with the BCA assay (Pierce). Fractions were aliquoted and stored at −80 °C prior to immunoprecipitation and seeding experiments.

**Immunoprecipitation**

Immunoprecipitations were performed as described previously [29]. 50 µL of magnetic Protein A Dynabead slurry (Thermofisher) was washed twice with immunoprecipitation (IP) wash buffer (0.05% Triton-X100 in PBS), followed by a 1 h room temperature incubation with 20 µg of anti-tau antibody. Beads were washed three times in IP wash buffer, 1000 µg of total protein lysate was added to the Protein A/anti-tau antibody complexes on the beads and rotated overnight at 4 °C. Supernatant was then removed as the tau-depleted fraction and the beads were washed three times in IP wash buffer, and then moved to clean tubes for elution. IP wash buffer was removed and beads were then incubated in 65 µL of IgG Elution Buffer (Pierce) for 7 min to elute tau. The elution buffer was collected in a separate microcentrifuge tube and a second elution step in 35 µL of IP elution buffer was performed for 5 min, and pooled with the initial elution. The tau-enriched IP pellet was then neutralized with 10 µL of Tris–HCl pH 8.4.

**Transduction of biosensor cell lines, flow cytometry and seeding analyses**

The seeding assay was conducted as previously described [14] with the following changes: v2H cells were plated 20 h before seed transduction at a density of 16,000 cells/well in a 96-well plate in a media volume of 180 µL per well. Mouse and human total protein lysates were thawed on ice, while tau-depleted IP supernatants and tau-enriched IP pellets were isolated just prior to seeding. For total protein lysates, 10 µg of protein was used per well. For tau-enriched pellets, 10 µL of elution was used per well. Samples were incubated for 30 min with 0.5 µL Lipofectamine 2000 (Invitrogen) and OptiMEM such that the total treatment volume was 20 µL. For each experiment, cells treated with OptiMEM alone and Lipofectamine 2000 in OptiMEM as negative controls. The v2H line, which expresses high levels of tau RD, can show false-positive FRET signal when treated with Lipofectamine 2000, which is mitigated by passing prior to ~80% confluency. Recombinant tau fibrils at 1 pM and 100 fM (monomer equivalent) were used for positive controls. Cells were incubated for an additional 48 h after treatment prior to harvesting. Cells were harvested with 0.25% trypsin and fixed in 4% PFA for 10 min, then resuspended in flow cytometry buffer (HBSS plus 1% FBS and 1 mM EDTA). The LSRFortessa SORB (BD Biosciences) was used to perform FRET flow cytometry. Single cells double-positive for mCerulean and mClover were identified and the % FRET positive cells within this population was quantified following a gating strategy previously described [14]. For each experiment 10,000 cells were analyzed in triplicate. Flow data analysis was performed using FlowJo v10 software (Treestar).

**Light microscopy and immunohistochemistry**

Histopathologic processing, staining, and analysis were performed in the UT Southwestern Neuropathology Research Laboratory. Sections for light microscopy were cut at 4 µm on a Microm HM355S rotary microtome (ThermoScientific, Rockford, IL) and mounted on Fisherbrand Superfrost Plus positive charged slides (Fisher Scientific, Pittsburgh, PA). Adjacent sections were stained with hematoxylin and eosin, Luxol fast blue-PAS-hematoylin, AT8 monoclonal antibody (ThermoScientific) 1:200 dilution, and MD3.1 antibody (1:16,000 dilution). AT8 and MD3.1 immunohistochemistry was performed at room temperature on a Leica Bond-III automated immunostaining platform (Leica Biosystems Inc., Buffalo Grove, IL), using the proprietary Leica Polymer Refine detection system, which includes H2O2 block, EDTA-based epitope retrieval solution, rabbit anti-mouse IgG secondary antibody, anti-rabbit poly-HRP-IgG,
DAB, and hematoxylin counterstain. Stained sections were reviewed on an Eclipse NiU brightfield microscope (Nikon Instruments, Melville, NY), and virtual whole slide images for illustrations were created on an Aperio ScanScope CS2 robotic slide scanner (Leica) with 20× objective and selected fields captured using Aperio ImageScope v.12 software.

Statistical analyses
Fresh frozen brain regions were obtained by M.S.L. from H.M. M.S.L. remained blinded for all seeding analyses. Flow cytometry gating and analysis of seeding activity was completed prior to decoding and interpreting the results. All statistical analysis was performed using GraphPad Prism v9.2.0 for Mac OS and Excel v16.52 (Microsoft).

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s40478-022-01444-2.

Acknowledgements
This work was supported by the Chan Zuckerberg Initiative and NIH/NIA 1R01AG059689 (M.I.D., C.L.W.); the Winspear Family Center for Research on the Neuropathology of Alzheimer Disease; the McCune Foundation (C.L.W.). This work was supported by the Chan Zuckerberg Initiative and NIH/NIA 1R01AG059689 (M.I.D., C.L.W.); the Winspear Family Center for Research on the Neuropathology of Alzheimer Disease; the McCune Foundation (C.L.W.).

Author contributions
MD6.1 conceived experiments and wrote the manuscript. All authors read and approved the final manuscript.

References
1. Compston A, Coles A (2008) Multiple sclerosis. Lancet 372:1502–1517. https://doi.org/10.1016/S0140-6736(08)61620-7
2. Bar-Or A, Li R (2021) Cellular immunology of relapsing multiple sclerosis: interactions, checks, and balances. Lancet Neurol 20:470–483. https://doi.org/10.1016/S1474-4422(21)00063-6
3. Lassmann H, van Horsen J, Mahad D (2012) Progressive multiple sclerosis: pathology and pathogenesis. Nat Rev Neurol 8:647–656. https://doi.org/10.1038/nrneurol.2012.168
4. Milo R, Koriczyn AD, Manouchehri N, Stuve O (2020) The temporal and causal relationship between inflammation and neurodegeneration in multiple sclerosis. Mult Scler 26:876–886. https://doi.org/10.1177/1352458519866943
5. Trapp BD, Nave KA (2008) Multiple sclerosis: An immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269. https://doi.org/10.1146/annurev.neuro.30.051606.094313
6. Anderson JM, Patani R, Reynolds R, Nichols R, Compston A, Spillantini MG, Chandran S (2010) Abnormal tau phosphorylation in primary progressive multiple sclerosis. Acta Neuropathol 119:591–600. https://doi.org/10.1007/s00401-010-0671-4
7. Anderson JM, Patani R, Reynolds R, Nichols R, Compston A, Spillantini MG, Chandran S (2009) Evidence for abnormal tau phosphorylation in early aggressive multiple sclerosis. Acta Neuropathol 117:583–589. https://doi.org/10.1007/s00401-008-0515-2
8. Lee VM, Trojanowski JQ (1999) Neurodegenerative tauopathies: human disease and transgenic mouse models. Neuron 24:507–510. https://doi.org/10.1016/s0896-6273(00)81106-x
9. Sanders DW, Kaufman SK, Devos SL, Sharma AM, Mirbaha H, Li A, Barker SJ, Foley AC, Thorpe JA, Serpel LC et al (2014) Distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron 82:1271–1288. https://doi.org/10.1016/j.neuron.2014.04.047
10. Shi Y, Zhang W, Yang Y, Murzin AG, Falcon B, Kotecha A, van Beers M, Tarutani A, Kametani F, Garringer HJ et al (2021) Structure-based classification of tauopathies. Nature 598:359–363. https://doi.org/10.1038/s41586-021-03911-7
11. Vaquer-Alicea J, Diamond MI, Joachimaki LA (2021) Tau strains shape disease. Acta Neuropathol 142:57–71. https://doi.org/10.1007/s00401-021-02301-7
12. Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, Sharma AM, Miller TM, Diamond MI (2016) Tau prion strains dictate patterns of cell pathology, progression rate, and regional vulnerability in vivo. Neuron 92:796–812. https://doi.org/10.1016/j.neuron.2016.09.055
13. Hitt BD, Vaquer-Alicea J, Manon VA, Beaver JD, Kashmer OM, Garcia JN, Diamond MI (2021) Ultraresilient tau biosensor cells detect no seeding in Alzheimer’s disease CSF. Acta Neuropathol Commun 9:99. https://doi.org/10.1007/s40478-021-01185-8
14. Holmes BB, Furman JL, Mahan TE, Yamasaki TR, Mirbaha H, Eades WC, Belaygord L, Cains NJ, Holtzman DM, Diamond MI (2014) Proteopathic tau seeding predicts tauopathy in vivo. Proc Natl Acad Sci U S A 111:E4376-4385. https://doi.org/10.1073/pnas.1411649111
15. Blaudeau de F, Lassus B, Schaler AW, Fowler SL, Goulbourn CN, Jeggo R, Mannoury la Cour C, Millan MJ, Duff KE (2021) PS2 accumulates through neuroanatomical circuits in response to tauopathy propagation. Acta Neuropathol Commun 9:177. https://doi.org/10.1186/s40478-021-01280-w
16. Danis C, Dupre E, Zejnulli O, Cailliere R, Arrial A, Regard S, Mortelette J, Eddarkaoue S, Lovenis A, Cantrelle FX et al (2022) Inhibition of tau seeding by targeting tau nucleation core within neurons with a single domain antibody fragment. Mol Ther 30:1484–1499. https://doi.org/10.1016/j.ymthe.2022.01.009
17. Devos SL, Corjuc BT, Oakley DH, Nobuhara CK, Bannon RN, Chase A, Commins C, Gonzalez IA, Dooley PM, Froesch MP et al (2018) Synaptic tau seeding precedes tau pathology in human Alzheimer’s disease brain. Front Neurosci 12:267. https://doi.org/10.3389/fnins.2018.00267
18. Woerman AL, Aoyagi A, Patel S, Kazmi SA, Lobach I, Grinberg LT, McKee AC, Seeley WW, Olson SH, Prusiner SB (2016) Tau prions from Alzheimer’s disease and chronic traumatic encephalopathy patients propagate in cultured cells. Proc Natl Acad Sci U S A 113:E8187–E8196. https://doi.org/10.1073/pnas.1616344113
19. Hitt BD (2022) In Prep
20. LaCroix MS, Hitt BD, Beaver JD, Estill-Terpack S, Gleason K, Tamminga CA, Evers BM, White CL, 3rd, Diamond MI (2022) Tau seeding without tauopathy
21. Mirbaha H, Chen D, Morazova OA, Ruff KM, Sharma AM, Liu X, Goodarzi M, Pappu RV, Colby DW, Mirzaei H et al (2018) Inert and seed-competent tau monomers suggest structural origins of aggregation. Elife. https://doi.org/10.7554/eLife.36584
22. Anderson JM, Hampton DW, Patani R, Pryce G, Crowther RA, Reynolds R, Franklin RJ, Giovannoni G, Compston DA, Baker D et al (2008) Abnormally phosphorylated tau is associated with neuronal and axonal loss in experimental autoimmune encephalomyelitis and multiple sclerosis. Brain 131:1736–1748. https://doi.org/10.1093/brain/awn119
23. Beece-Scherrer V, Buee L, Leveugle B, Perl DP, Vermersch P, Hof PR, Delacourte A (1997) Pathological tau proteins in postencephalitic parkinsonism: comparison with Alzheimer’s disease and other neurodegenerative disorders. Ann Neurol 42:356–359. https://doi.org/10.1002/ana.410420312
24. McKee AC, Stein TD, Kiernan PT, Alvarez VE (2015) The neuropathology of chronic traumatic encephalopathy. Brain Pathol 25:350–364. https://doi.org/10.1111/bpa.12248
25. McQuaid S, Allen IV, McMahon J, Kirk J (1994) Association of measles virus with neurofibrillary tangles in subacute sclerosing panencephalitis: a combined in situ hybridization and immunocytochemical investigation. Neuropathol Appl Neurobiol 20:103–110. https://doi.org/10.1111/j.1365-2990.1994.tb01168.x
26. Pollanen MS, Onzivua S, Robertson J, McKeever PM, Olawa F, Kitara DL, Fong A (2018) Nodding syndrome in Uganda is a tauopathy. Acta Neuropathol 136:691–697. https://doi.org/10.1007/s00401-018-1909-9
27. Wong KT, Allen IV, McQuaid S, McConnell R (1996) An immunohistochemical study of neurofibrillary tangle formation in post-encephalitic Parkinsonism. Clin Neuropathol 15:22–25
28. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O’Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS (2011) Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol 69(2):292–302. https://doi.org/10.1002/ana.22366
29. LaCroix MS, Hitt BD, Beaver JD, Estill-Terpack S-J, Gleason K, Tamminga CA, Evers BM, White CL, Diamond MI (2022) Tau seeding without tauopathy. bioRxiv: 2022.02.03.479049, https://doi.org/10.1101/2022.02.03.479049

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.